nacalai tesque
The quality for certainty.

Biochemical Reagents

8th Edition

NACALAI TESQUE, INC.

Contents

Nucleic Acid Isolation/ Electrophoresis

Cell Culture

Cell Extraction / Protein Assay

Contents

Total RNA Isolation Reagent: Sepasol®-RNA I Super G

» **Easy to identify interphase**
» **Less than 1hr for RNA isolation**
» **Enables to extract DNA and proteins along with RNA from a single sample**

Phenol phase color

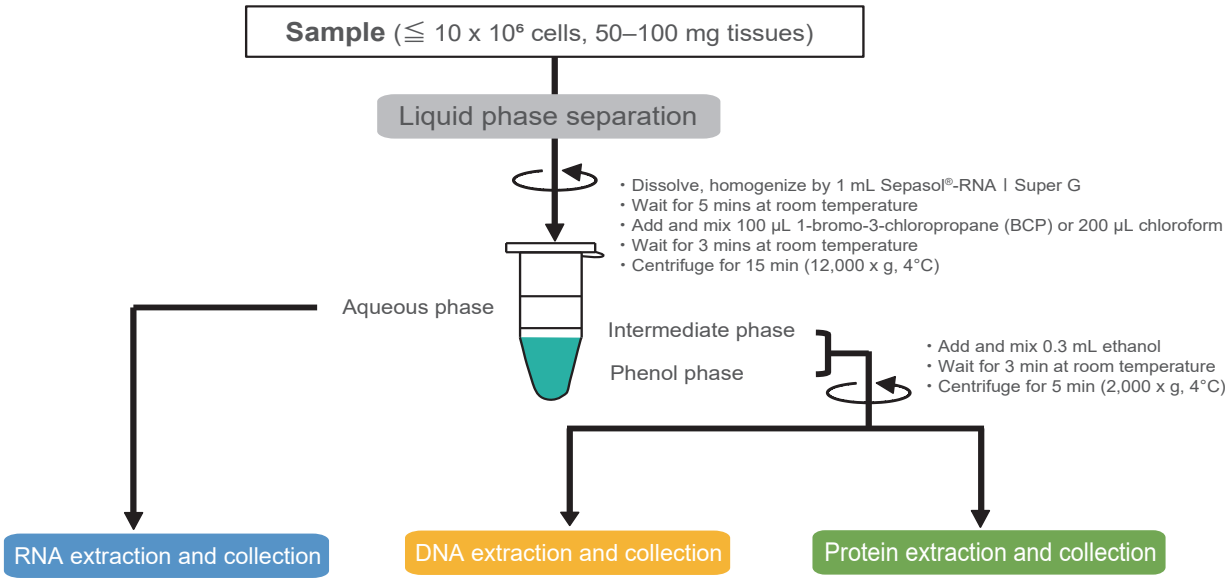
Sepasol-RNA I Super G

Aqueous phase

Phenol phase

Easy to identify interphase

Procedure



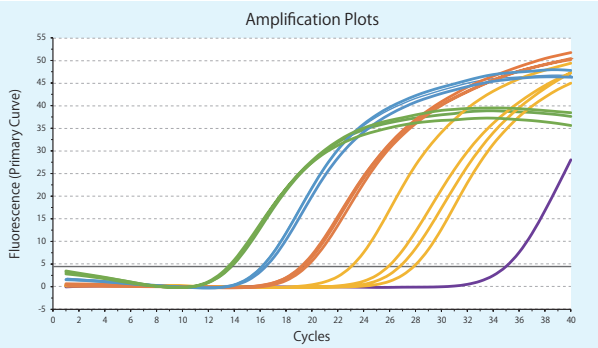
For details, refer to the instruction manual.

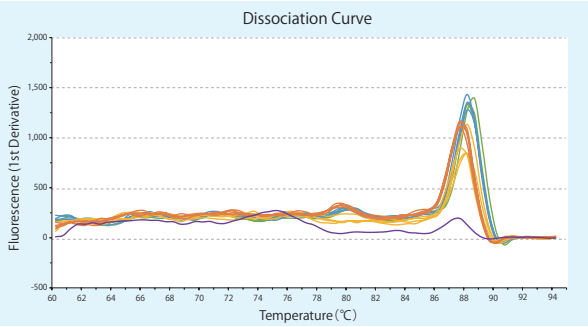
Performance evaluation

Total RNA was extracted from HL-60 cells at 8 x 10^6 cells, 1 x 10^6 cells, and 0.125 x 10^6 cells using this product. Additionally, for the 0.125 x 10^6 cells of HL-60 cells, samples were prepared with Gene-Packman Coprecipitant (#12680-30) as well as general alcohol precipitation. The extracted total RNA was treated with DNase according to the instruction manual attached and proceed to RT-qPCR. Please note that throughout the entire process, all cell number samples were treated under identical conditions, including the volume of the dissolution solution.

Result (RT-qPCR)

Using this product, it was possible to extract mRNA in a cell number-dependent manner. Moreover, although RNA yield decreased and greater variability was observed (yellow lines) when the cell number was low, such as 0.125 x 10^6 cells, Gene-Packman co-precipitant (#12680-30) increased the yield and reduced variability (orange lines).





Sample	Ct value
8 x 10^6 cells	13.55 - 13.8
1 x 10^6 cells	16.12 - 16.35
0.125 x 10^6 cells	23.04 - 27.87
0.125 x 10^6 cells (co-precipitation)	19.17 - 19.59
Non-template control	34.99

*Threshold: 4.47

(Conditions)

Primer:
[Gene target] β-Actin
[Forward primer] 5'-AAGAGAGGCATCCTCACCCT-3'
[Reverse primer] 5'-TACATGGCTGGGGTGTTGAA-3'
[PCR product] 218 bp

PCR enzyme (reverse transcriptase enzyme):
One Step TB Green PrimeScript RT-PCR Kit II
(Perfect Real Time) (Takara Bio #RR086A)

PCR condition:
1.Hold (reverse transcription) 42°C (5 min) → 95°C (10 s), 1 cycle
2. 2 Step PCR 95°C (5 sec.) → 60°C (30 s), 40 cycles
3.Dissociation 95°C (15 sec.) → 60°C (30 s) → 95°C (5 s), 1 cycle

Real-time PCR equipment
Thermal Cycler Dice Real Time System III

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Ordering Information

Product Name	Storage	Product No.	PKG Size
Sepasol®-RNA I Super G (for biological samples [cell, tissue, etc.])	R	09379-26	10 mL
		09379-84	100 mL
		09379-97	200 mL
		09379-55	500 mL
Sepasol®-RNA II Super (for liquid samples [blood, etc.])	R	30487-46	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

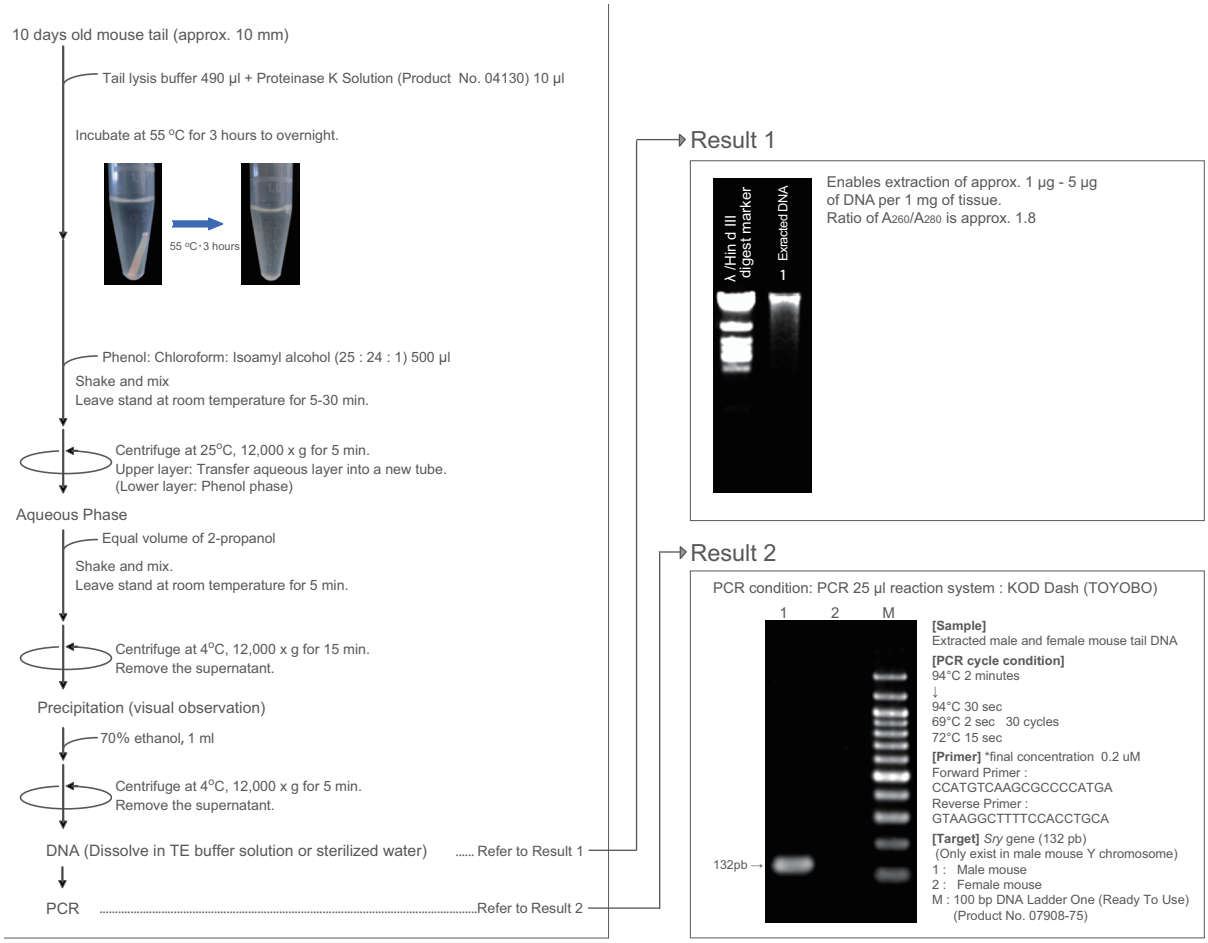
Tail Lysis Buffer

Tail Lysis Buffer is ready-to-use solution that enables simple genotyping procedure.

» **Ready-to-use solution**
» **DNase, RNase free**

Application: Genotyping of mouse tail

Procedure



Sry gene (132 bp), which only exists in male mouse Y-chromosomes was increased in male derived DNA, but was not increased in female derived DNA. The result shows that PCR operates correctly.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Tail Lysis Buffer	RT	06169-95	500 mL

Related Product

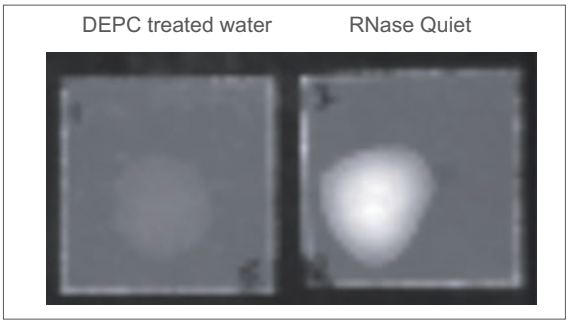
Product Name	Storage	Product No.	PKG Size
Proteinase K from Tritirachium album	R	29442-14	100 mg
		29442-85	500 mg
Proteinase K(Recombinant) Solution	R	15679-06	2 mL
		15679-64	10 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Nuclease Decontamination: RNase Quiet

» **Free from contamination** : removes nucleases and nucleic acids
» **Easy to wipe** : no detergent contained
» **Easy to use** : spray type
» **Non-carcinogenic** : no DEPC contained

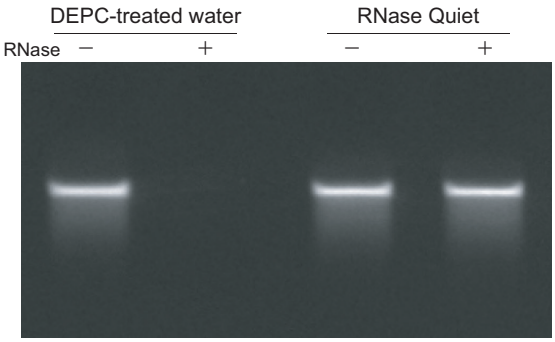
Application 1: Decontamination of cover glass



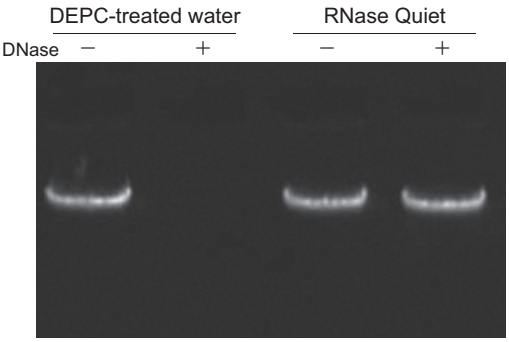
Condition
1. Apply 100 µl of RNase A solution (1 mg/mL) to cover glasses and dry them.
2. Spray with DEPC-treated water or RNase Quiet and wait for 1 minute. Wipe thoroughly with a clean paper towel and then rinse with RNase-free sterile water.
3. Apply 50 µl of RNA solution (40 µg/mL) on the cover glasses and incubate them at 37°C for 30 minutes.
4. Apply 1 µl ethidium bromide solution (20 µg/mL) to the cover glasses.
5. Observe with UV detector.

Application 2: RNase and DNase decontamination of 1.5 mL micro-tubes

RNase decontamination test



DNase decontamination test



Condition
① Add the following solutions and dry them up.
RNase decontamination test
(+): Add 10 µl of RNase A solution (10 mg/mL) to a 1.5 mL micro-tube.
(−): Add 10 µl of only the solution used to dissolve RNase A to a 1.5 mL micro-tube as a control.
DNase decontamination test
(+): Add 10 µl of DNase I (27.3 U/10 µl) to a 1.5 mL micro-tube, and dry it up.
(−): Add 10 µl of only the solution used to dissolve DNase I to a 1.5 mL micro-tube as a control.
② Add 1 mL of DEPC-treated water or RNase Quiet and wait for 1 minute.
③ Remove the solution from the tubes and rinse those with 1 mL of DEPC-treated water.
④ Remove the DEPC-treated water from the tubes and add 25 µl of RNA solution (40 µg/mL) for RNase decontamination test; 25 µl of DNA solution (40 µg/mL) and 1 µl of 50 mmol/L magnesium chloride for DNase decontamination test, and incubate them at 37°C for 30 minutes.
⑤ Reacted samples are electrophoresed on agarose gel and detected using EtBr.

Ordering Information

Product Name	Storage	Product No.	PKG Size
RNase Quiet (with spray nozzle)	RT	09147-14	475 mL
RNase Quiet Refill	RT	09477-94	475 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

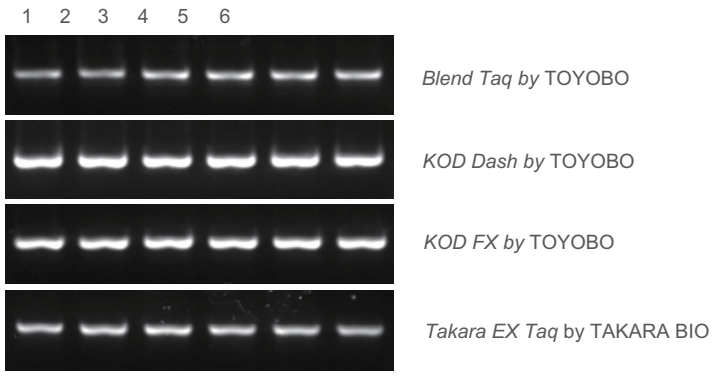
Coprecipitant of DNA/RNA: Gene-Packman Coprecipitant

- **Almost complete nucleic acid recovery**
- **No requirement of low-temperature incubation**
- **Endotoxin, DNase and RNase tested**
- **High nucleic acid pellet visibility**

No effect of Gene-Packman Coprecipitant to PCR

In order to figure out that Gene-Packman Coprecipitant does not effect PCR performance, Gene-Packman Coprecipitant was consciously added to PCR reaction solutions which amplify 1,000 bp DNA fragments. This data shows that Gene-Packman does not affect PCR performance.

Amount of Gene-Packman Coprecipitant to PCR reactin solution:
Lane 1: 0 µl, Lane 2: 0.2 µl, Lane 3: 0.5 µl, Lane 4: 1.0 µl, Lane 5: 3.0 µl and Lane 6: 5.0 µl.



PCR condition

Reaction Volume	: 25 µl reaction
Tempate DNA	: 15 ng
Denaturation	: 94ºC for 0.5 min.
Annealing	: 55ºC for 0.5 min.
Extension	: 72ºC for 1 min.
Cycle	: 25 times

The agarose gel image above shows no interference with Gene-Packman Coprecipitant to PCR performance.

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Ordering Information

Product Name	Storage	Product No.	PKG Size
Gene-Packman Coprecipitant	R	12680-30	1 KIT

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

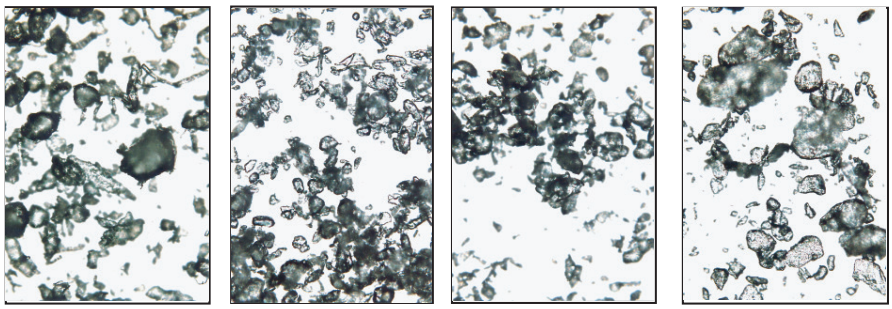
Agarose for Nucleic Acid Electrophoresis

Fine-powdered Agarose

» **High solubility** : Smaller average particle size for easy disolution
» **Simple** : Easy-to-weigh
» **High sharpness** : Sharp bands observable

Solubility comparison (particle size and solubility)

Solubility tests have been done using microwave oven (400W).
Photo image: microscope (x25)



Agarose:	Conventional	**Fine-powdered**	A Company	B Company
Solubility (speed):	Fast	Very fast	Very fast	Normal
Time:	2 min 50 sec	1 min 53 sec	1 min 55 sec	3 min 10 sec

Specification

Type	: ≧ 1kbp
Sulfate (%)	: ≦ 0.2
Gel Strength	: ≧ 2,500 g/cm^2 (at 1.5%)
Gel Point (°C)	: 36 ± 1.5
Electroendosmosis (-mr)	: 0.09-0.13

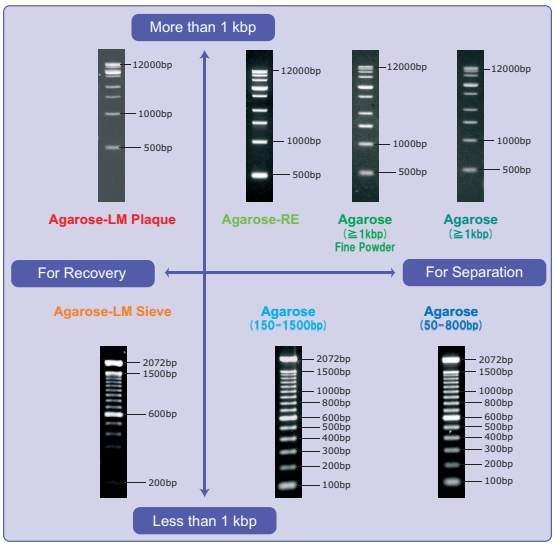
Ordering Information

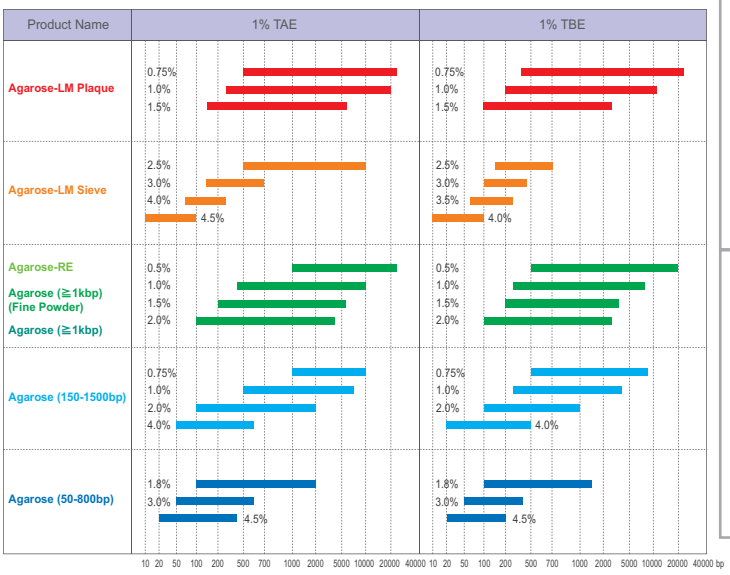
Product Name	Storage	Product No.	PKG Size
		02468-24	10 g
Agarose for ≧ 1kbp fragment (Fine Powder)	RT	02468-66	100 g
		02468-95	500 g

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Other Agaroses

Agarose gel selection guide





Ordering Information

Product Name	Storage	Product No.	PKG Size
Agarose-LM Plaque for ≧ 1kbp fragment Type : Low Melting Agarose ≧ 1kbp fragment Sulfate (%) : ≦ 0.5 Gel Strength : ≧ 250 g/cm^2 (at 1.5%) Gel Point (°C) : ≦ 30 Melting Temp. (°C) : ≦ 65.5 Electroendosmosis (-mr) : ≦ 0.12 DNase, RNase tested	RT	01650-02 01650-86	25 g 100 g
Agarose-LM Sieve for ≦ 1kbp fragment Type : Low Melting Agarose ≦ 1kbp fragment Sulfate (%) : ≦ 0.5 Gel Strength : ≧ 1,000 g/cm^2 (at 4%) Gel Point (°C) : ≦ 35 Melting Temp. (°C) : ≦ 65 Electroendosmosis (-mr) : ≦ 0.12 DNase, RNase tested	RT	01651-92 01651-76	25 g 100 g
Agarose-RE for ≧ 1kbp fragment, for Restriction and Ligation Type : ≧ 1kbp Sulfate (%) : ≦ 0.2 Gel Strength : ≧ 2,500 g/cm^2 (at 1.5%) Gel Point (°C) : 36 ± 1.5 Electroendosmosis (-mr) : 0.09 - 0.13 Tested for Silver staining, Dnase, RNase and Enzyme reaction	RT	01149-92 01149-76 01149-05	25 g 100 g 500 g
Agarose for ≧ 1kbp fragment (Fine Powder) refer to pxx Type : ≧ 1kbp Sulfate (%) : ≦ 0.5 Gel Strength : ≧ 2,500 g/cm^2 (at 1.5%) Gel Point (°C) : 36 ± 1.5 Electroendosmosis (-mr) : 0.09 - 0.13	RT	02468-24 02468-66 02468-95	10 g 100 g 500 g
Agarose for ≧ 1kbp fragment Type : ≧ 1kbp Sulfate (%) : ≦ 0.2 Gel Strength : ≧ 2,500 g/cm^2 (at 1.5%) Gel Point (°C) : 36 ± 1.5 Electroendosmosis (-mr) : 0.09 - 0.13	RT	01163-92 01163-76 01163-05	25 g 100 g 500 g
Agarose for 150-1,500bp fragment Type : 150-1,500bp Sulfate (%) : ≦ 0.1 Gel Strength : ≧ 2,000 g/cm^2 (at 1.5%) Gel Point (°C) : ≦ 36.5 Electroendosmosis (-mr) : ≦ 0.12 DNase, RNase tested	RT	01153-22 01153-64	25 g 100 g
Agarose for 50-800bp fragment Type : 50-800bp Sulfate (%) : ≦ 0.1 Gel Strength : ≧ 750 g/cm^2 (at 1.5%) Gel Point (°C) : 30 Electroendosmosis (-mr) : ≦ 0.12 DNase, RNase tested	RT	01147-12 01147-96	25 g 100 g
Agarose-LE, Classic type Type : LE, Classic Sulfate (%) : ≦ 0.2 Gel Strength : ≧ 2,500 g/cm^2 (at 1.5%) Gel Point (°C) : 36 ± 1.5 Electroendosmosis (-mr) : 0.09 - 0.13	RT	01157-82 01157-66 01157-95	25 g 100 g 500 g
Agarose-ME, Classic type Type : ME, Classic Sulfate (%) : ≦ 0.25 Gel Strength : ≧ 2,000 g/cm^2 (at 1.5%) Gel Point (°C) : 36 ± 1.5 Electroendosmosis (-mr) : 0.16 - 0.19	RT	01158-72 01158-56 01158-85	25 g 100 g 500 g
Agarose-LM (melting temperature ≦ 65°C) Type : Low Melting Agarose Sulfate (%) : ≦ 0.2 Gel Strength : ≧ 550 g/cm^2 (at 1.5%) Gel Point (°C) : 26 ± 2 Melting Temp. (°C) : ≦ 65 Electroendosmosis (-mr) : ≦ 0.12	RT	01161-12 01161-54	25 g 100 g

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

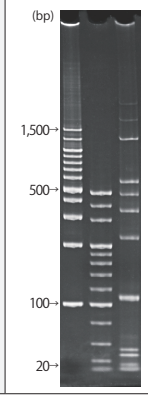
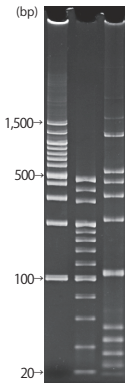
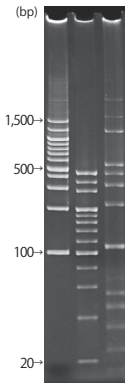
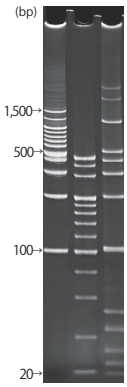
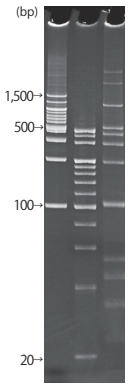
Bullet PAGE Plus Precast Gel

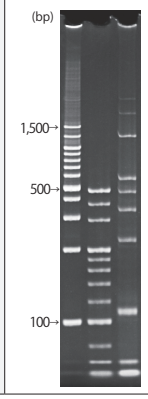
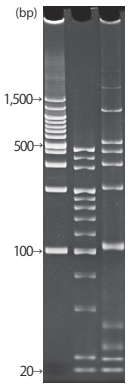
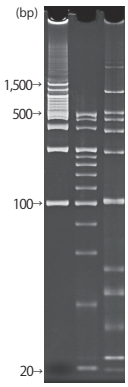
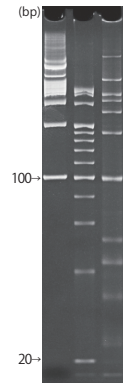
Bullet PAGE Plus is a precast gel for high-speed electrophoresis. (For protein electrophoresis, refer to p54.)

» **Only 12 minutes electrophoresis time for nucleic acid with 400 V**

» **Surfactant-free, so can be used for nucleic acid analysis.**

*Please use 25 mM Tris and 192 mM glycine buffer for nucleic acid electrophoresis.

Gel		Gradient gel				
Gel concentration		5-10 %	5-15 %	5-20 %	7.5-15 %	10-20 %
Product number	13 wells	21789-34	21791-84	21793-64	21795-44	21797-24
	17 wells	21790-94	21792-74	21794-54	21796-34	21798-14
Images of nucleic acids (PAGE)						

Gel		Homogeneous gel			
Gel concentration		7.5%	10%	12.5%	15%
Product number	13 wells	21799-04	21801-44	21807-84	21853-74
	17 wells	21800-54	21806-94	21811-14	21854-64
Images of nucleic acids (PAGE)					

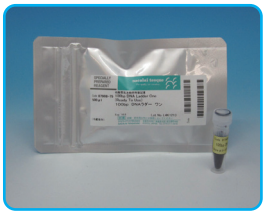
Ordering Information

Product Name	Storage	Product No.	PKG Size
Bullet PAGE Plus Precast Gel, 5-10%, 13wells	R	21789-34	10 Sheets
Bullet PAGE Plus Precast Gel, 5-10%, 17wells	R	21790-94	10 Sheets
Bullet PAGE Plus Precast Gel, 5-15%, 13wells	R	21791-84	10 Sheets
Bullet PAGE Plus Precast Gel, 5-15%, 17wells	R	21792-74	10 Sheets
Bullet PAGE Plus Precast Gel, 5-20%, 13wells	R	21793-64	10 Sheets
Bullet PAGE Plus Precast Gel, 5-20%, 17wells	R	21794-54	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5-15%, 13wells	R	21795-44	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5-15%, 17wells	R	21796-34	10 Sheets
Bullet PAGE Plus Precast Gel, 10-20%, 13wells	R	21797-24	10 Sheets
Bullet PAGE Plus Precast Gel, 10-20%, 17wells	R	21798-14	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5%, 13wells	R	21799-04	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5%, 17wells	R	21800-54	10 Sheets
Bullet PAGE Plus Precast Gel, 10%, 13wells	R	21801-44	10 Sheets
Bullet PAGE Plus Precast Gel, 10%, 17wells	R	21806-94	10 Sheets
Bullet PAGE Plus Precast Gel, 12.5%, 13wells	R	21807-84	10 Sheets
Bullet PAGE Plus Precast Gel, 12.5%, 17wells	R	21811-14	10 Sheets
Bullet PAGE Plus Precast Gel, 15%, 13wells	R	21853-74	10 Sheets
Bullet PAGE Plus Precast Gel, 15%, 17wells	R	21854-64	10 Sheets

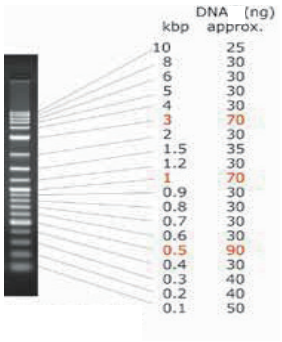
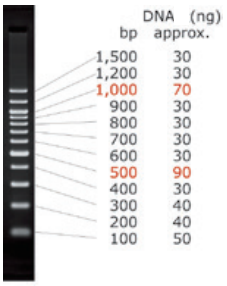
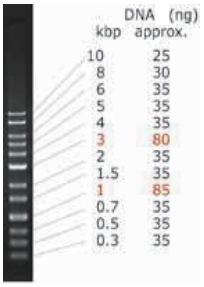
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

DNA Ladder Markers

» **Covers wide range from 0.1 kbp to 10 kbp**
» **Emphasis of 0.5, 1 and 3 kbp bands**
» **Ready-to-use markers containing 2 loading dyes**



Product Contents

Broad range	100bp	1kbp
		
2% Agarose gel/TAE DNA marker 5 µl	2.5% Agarose gel/TBE DNA marker 5 µl	0.8% Agarose gel/TAE DNA marker 5 µl
Concentration 0.15 µg/µL	Concentration 0.1 µg/µL	

Ordering Information

Product Name	Storage	Product No.	PKG Size
DNA Ladder One (Broad Range) (Ready-to-use)	R	08362-85	500 µL
100bp DNA Ladder One (Ready-to-use)	R	07908-75	500 µL
1kbp DNA Ladder One (Ready-to-use)	R	08232-85	500 µL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

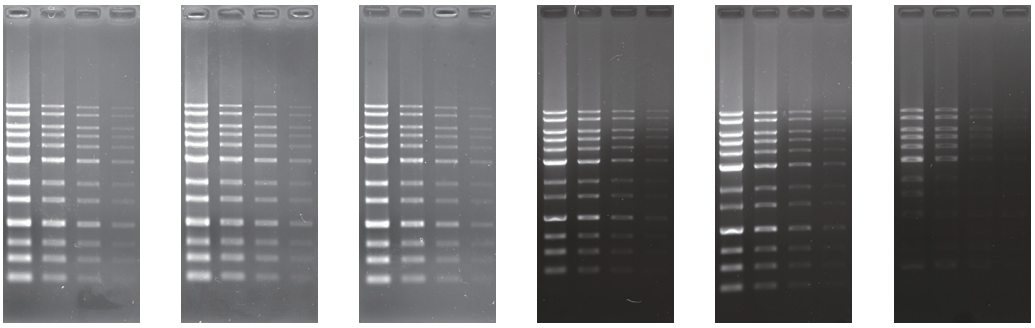
Dual Green Nucleic Acid Stain

» **Can be detected by UV excitation**
» **Can be applied in both pre-stain and post-stain procedures**
» **Can be used with agarose and polyacrylamide gels**
» **All double-stranded DNA, single-stranded DNA and RNA can be detected.**

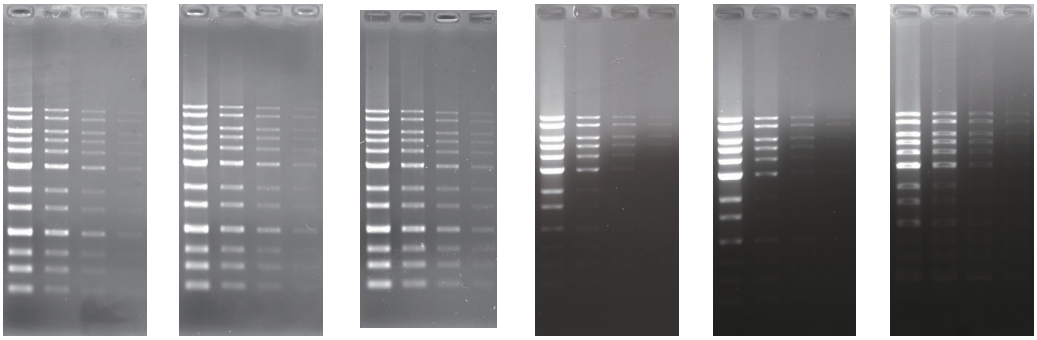
Comparison

In agarose gel, DNA detection was performed by using post-staining and pre-staining methods. This product has equivalent or superior performance compared to products from other companies.

Detect by UV excitation

Staining method	Post-staining			Pre-staining		
Staining reagents	This product	Company A	Company B	This product	Company A	Company B
Staining image						

Detect by Bule LED

Staining method	Post-staining			Pre-staining		
Staining reagents	This product	Company A	Company B	This product	Company A	Company B
Staining image						

(Conditions)
Sample : 1kbp DNA Ladder One(Ready to Use) (#08232-85) Total nucleic acid quantity: 600 ng, 200 ng, 67 ng, 20 ng from left
Gel : 1% Agarose gel
Electrophoresis buffer : Prepare Tris-Acetate-EDTA Buffer (#35430) at 1x concentration (dye-free)
Electrophoresis : Run at 100 V until BPB reaches about 3/4 of the gel.
Staining : [Pre-staining] After dissolving the agarose, each dye was added to make a 20,000x dilution, and then the gel was solidified.
[Post-staining] TAE (1x) to make a 10,000x dilution, then place the gel after electrophoresis and shake for 30 minutes (without destaining).
Detection : ChemiDoc Touch MP(Bio-Rad laboratories) The exposure time is detested automatically.
[UV excitation] SYBR Green mode (UV transmission, 590 / 110 nm detect on filter)
[Blue LED excitation] Alexa Fluor 488 mode (Blue incident light, 532 / 28 nm detect on filter)

All product names, trademarks, and registered trademarks are the property of their respective owners. Use of these names does not imply any affiliation or endorsement.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Dual Green Nucleic Acid Stain	RT	20599-41	1 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

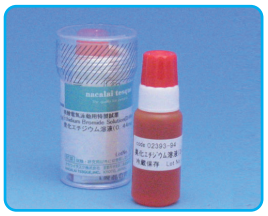
Ethidium Bromide Solution (0.44 mg/mL)

Ethidium Bromide Solution (0.44 mg/mL) is easy and safe to use because of its eye-drop bottle. It is used in adjustment of nucleic acid staining after electrophoresis or gel containing ethidium bromide.

How to use

Adjust the concentration of Ethidium Bromide Solution as follows

Concentration of ethidium bromide	Adjusting solution	Ethidium Bromide Solution (0.44 mg/mL)
0.1 µg/mL	200 mL	1 drop
0.2 µg/mL	100 mL	1 drop
0.5 µg/mL	40 mL	1 drop



Note:

1. 1 drop of Ethidium Bromide Solution (0.44 mg/mL) is 45 µl.
2. In situations where Ethidium Bromide Solution (0.44 mg/mL) is used in concentrations other than shown in the above table, remove the nozzle, collect the appropriate amount with a micropipette and dilute accordingly.
3. For adjustments of even greater ethidium bromide solution volumes, use Ethidium Bromide Solution (10 mg/mL) (Product no. 14631-94).

Ordering Information

Product Name	Storage	Product No.	PKG Size
Ethidium Bromide Solution (0.44 mg/mL) eye-drop-bottle	R	02393-94	10 mL
Ethidium Bromide Solution (10 mg/mL)	R	14631-94	10 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Phenol Solution for DNA/RNA Extraction

Ordering Information

Product Name	Details	Storage	Product No.	PKG Size
For DNA				
Phenol, Saturated with TE Buffer	Buffer-saturated phenol at pH 6.6. Easily adjusted to pH 7.9 adding the attached buffer before use.	R	25969-54 25969-96	100 mL 400 mL
Phenol, Saturated with TE Buffer	It contains 8-quinolinol as a stabilizer. The phenol layer (lower layer) is yellow in color and can be easily distinguished from the clear water layer (upper layer). pH 8 already adjusted.	R	26829-54 26829-96	100 mL 400 mL
Phenol:Chloroform:Isoamyl Alcohol 25:24:1 Mixed, pH6.7	Proteins and lipids can be removed. Easily adjusted to pH 8 adding the attached buffer before use.	R	25967-74 25967-16	100 mL 400 mL
Phenol:Chloroform:Isoamyl Alcohol 25:24:1 Mixed, pH7.9	It contains 8-quinolinol as a stabilizer. The phenol layer (lower layer) is yellow in color and can be easily distinguished from the clear water layer (upper layer). pH 8 already adjusted.	R	25970-14 25970-56	100 mL 400 mL
For RNA				
Phenol, Saturated with Citrate Buffer	Buffer-saturated phenol at pH4.3.	R	25968-64	100 mL
Phenol:Chloroform:Isoamyl Alcohol 25:24:1 Mixed, pH5.2	Proteins and lipids can be removed. Good for recovery of RNA with long poly(A).	R	26058-54 26058-96	100 mL 400 mL

Related Products

Product Name	Storage	Product No.	PKG Size
Reagent for lysation			
Proteinase K from Tritirachium album	R	29442-14 29442-85	100 mg 500 mg
Proteinase K(Recombinant) Solution	R	15679-06 15679-64	2 mL 10 mL
8mol/l-Guanidine Hydrochloride Solution	RT	17356-24	100 mL
6mol/l-Guanidine Thiocyanate Solution	RT	16689-04	100 mL
100g/l-Hexadecyltrimetylammonium Bromide Solution (10% (w/v) CTAB Solution)	RT	17472-94	100 mL
Zymolyase™ 20T	R	07663-91	1 g
Zymolyase™ 100T	R	07665-55	500 mg
Buffer for electrophoresis			
Tris-Acetate-EDTA Buffer (10x) [TAE Buffer]	RT	35430-61 35430-74	1 L 5 L
Tris-Acetate-EDTA Buffer (50x) [TAE Buffer]	RT	32666-81	1 L
Tris-Borate-EDTA Buffer (5x) [TBE Buffer]	RT	35432-41	1 L
Tris-Borate-EDTA Buffer (10x) [TBE Buffer]	RT	35440-31 35440-44	1 L 5 L
Running Buffer Solution(10x) for PAGE	RT	30340-91	1 L
MOPS Buffer Stock Solution (10x) (pH 7.0)	RT	22089-45	500 mL
Reagent for polyacrilamid gel			
40(w/v)%-Acrylamide/Bis Mixed Solution(19:1), Nuclease tested	R	06140-45	500 mL
30(w/v)%-Acrylamide/Bis Mixed Solution(19:1), Nuclease tested	R	07175-75	500 mL
10(w/v)%-Ammonium Peroxodisulfate Solution	F	02634-34	10 mL
N,N,N',N'-Tetramethylethylenediamine	RT	33401-72	25 g
Denaturing reagent			
Formaldehyde Solution	RT	16223-55	500 g
Urea	RT	35940-65	500 g
Formamide	F	02020-64	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Cell Culture Medium

» **Animal origin-free**
» **Sterility tested for bacteria, fungus and mycoplasma**
» **Endotoxin tested**
» **pH tested**

Product List

Media Name	Product No.	Glucose	L-Gln	Phenol Red	HEPES	Sodium Pyruvate	Other	GIBCO	Sigma
DMEM	08459	High	○	○	-	-		11965-092	D5796
	08458		○	○	-	○		11995-065	D6429
	11584		-	○	-	○		10313-021	D6546
	08457		○	○	○	-		12430-054	
	11585		-	○	○	-			D6171
	08488		-	○	-	-		11960-044	D5671
	08489		-	-	-	-		31053-028	D1145
	16971		○	○	-	○	with 1,500 mg/L sodium hydrogen carbonate		
	16972		-	○	-	-	without calcium	21068-028	
	08456	Low	○	○	-	○		11885-084	D6046
	08490		-	-	-	○		11054-020	
	09891	No	○	○	-	-		11966-025	
DMEM/ Ham's F-12	11581	○	○	○	-	○		11320-033	D8062
	08460	○	○	○	○	○		11330-032	D8437
	11583	○	-	○	○	○			D6421
	05177	○	○	-	○	○		11039-021	
	11582	○	○	-	-	○			
	09893	No	○	○	-	○			
Ham's F-12	17458	○	○	○	-	○		11765-054	N6658
G-MEM	12965	○	○	○	-	-		11710-035	
MEM	21442	○	○	○	-	-		11095-080	M4655
	21443	○	○	○	-	-	with non-essential amino acids		
	09848	No	○	○	-	-	with non-essential amino acids		
α-MEM	21445	○	○	○	-	○		12561-056	
	21444	○	○	○	-	○	with nucleosides	12571-063	
IMDM	11506	○	○	○	○	○		12440-053	I6529
RPMI 1640	30264	○	○	○	-	-		11875-093	R8758
	30263	○	○	○	○	-		22400-089	
	05176	○	-	○	-	-		21870-076	R0883
	06261	○	○	-	-	-		11835-030	R1780
	16970	○	○	○	○	○	with 4,500 mg/L glucose	A1049101	
	09892	No	○	○	-	-		11879-020	

Compositions of each product are available on online catalog, "e-Nacalai Search Version" at www.nacalai.com

For media additives, refer to p45.

Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
DMEM(4.5g/l Glucose) with L-Gln, without Sodium Pyruvate, liquid	SP (For TC)	R	08459-35	500 mL
			08459-64	10 x 500 mL
DMEM(4.5g/l Glucose) with L-Gln and Sodium Pyruvate, liquid	SP (For TC)	R	08458-45	500 mL
			08458-16	10 x 500 mL
DMEM(4.5g/l Glucose) with Sodium Pyruvate, without L-Gln, liquid	SP (For TC)	R	11584-85	500 mL
DMEM(4.5g/l Glucose) with L-Gln and HEPES, without Sodium Pyruvate, liquid	SP (For TC)	R	08457-55	500 mL
DMEM(4.5g/l Glucose) with HEPES, without L-Gln and Sodium Pyruvate, liquid	SP (For TC)	R	11585-75	500 mL
DMEM(4.5g/l Glucose) without L-Gln and Sodium Pyruvate, liquid	SP (For TC)	R	08488-55	500 mL
DMEM(4.5g/l Glucose) without L-Gln, Sodium Pyruvate and Phenol Red, liquid	SP (For TC)	R	08489-45	500 mL
DMEM(4.5g/l Glucose) with L-Gln, Sodium Pyruvate and 1.5g/l Sodium Hydrogen Carbonate, liquid	SP (For TC)	R	16971-55	500 mL
DMEM(4.5g/l Glucose) Calcium free without L-Gln and Sodium Pyruvate, liquid	SP (For TC)	R	16972-45	500 mL
DMEM(1.0g/l Glucose) with L-Gln and Sodium Pyruvate, liquid	SP (For TC)	R	08456-65	500 mL
			08456-36	10 x 500 mL
DMEM(1.0g/l Glucose) with Sodium Pyruvate, without L-Gln and Phenol Red, liquid	SP (For TC)	R	08490-05	500 mL
DMEM(No Glucose) with L-Gln, without Sodium Pyruvate, liquid	SP (For TC)	R	09891-25	500 mL
DMEM/Ham's F-12 with L-Gln and Sodium Pyruvate, without HEPES, liquid	SP (For TC)	R	11581-15	500 mL
DMEM/Ham's F-12 with L-Gln, Sodium Pyruvate and HEPES, liquid	SP (For TC)	R	08460-95	500 mL
DMEM/Ham's F-12 with Sodium Pyruvate and HEPES, without L-Gln, liquid	SP (For TC)	R	11583-95	500 mL
DMEM/Ham's F-12 with L-Gln, Sodium Pyruvate and HEPES, without Phenol Red, liquid	SP (For TC)	R	05177-15	500 mL
DMEM/Ham's F-12 with L-Gln and Sodium Pyruvate, without HEPES and Phenol Red, liquid	SP (For TC)	R	11582-05	500 mL
DMEM/Ham's F-12(No Glucose) with L-Gln and Sodium Pyruvate, liquid	SP (For TC)	R	09893-05	500 mL
Ham's F-12 with L-Gln, liquid	SP (For TC)	R	17458-65	500 mL
G-MEM with L-Gln, liquid	SP (For TC)	R	12965-65	500 mL
MEM with Earle's Salts and L-Gln, liquid	SP (For TC)	R	21442-25	500 mL
MEM with Earle's Salts, L-Gln and Non-Essential Amino Acids, liquid	SP (For TC)	R	21443-15	500 mL
MEM(No Glucose) with Earle's Salts, L-Gln and Non-Essential Amino Acids, liquid	SP (For TC)	R	09848-05	500 mL
α-MEM with L-Gln, without Ribonucleosides and Deoxyribonucleosides, liquid	SP (For TC)	R	21445-95	500 mL
α-MEM with L-Gln, Ribonucleosides and Deoxyribonucleosides, liquid	SP (For TC)	R	21444-05	500 mL
IMDM with L-Gln and HEPES, liquid	SP (For TC)	R	11506-05	500 mL
RPMI 1640 with L-Gln, liquid	SP (For TC)	R	30264-85	500 mL
			30264-56	10 x 500 mL
RPMI 1640 with L-Gln and HEPES, liquid	SP (For TC)	R	30263-95	500 mL
RPMI 1640 without L-Gln, liquid	SP (For TC)	R	05176-25	500 mL
RPMI 1640 with L-Gln, without Phenol Red, liquid	SP (For TC)	R	06261-65	500 mL
RPMI 1640(4.5g/l Glucose) with L-Gln, Sodium Pyruvate and HEPES	SP (For TC)	R	16970-65	500 mL
RPMI 1640(No Glucose) with L-Gln, liquid	SP (For TC)	R	09892-15	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Custom Cell Culture Media

For researchers who want

- Specific compositions that appeared in the literature
- To modify the composition of commercially available cell culture media
- To get rid of phenol red due to its estrogenic effect

Specification

Product Form	Liquid
Minimum PKG Size	500 ml
Guaranteed items	pH, Osmotic pressure, Sterilized, Endotoxin tested, Mycoplasma tested
Lead terms	8-10 weeks

How to order

Please visit our website at http://www.nacalai.co.jp/global/reagent/custom/Custom_Services.html and fill out the request form.

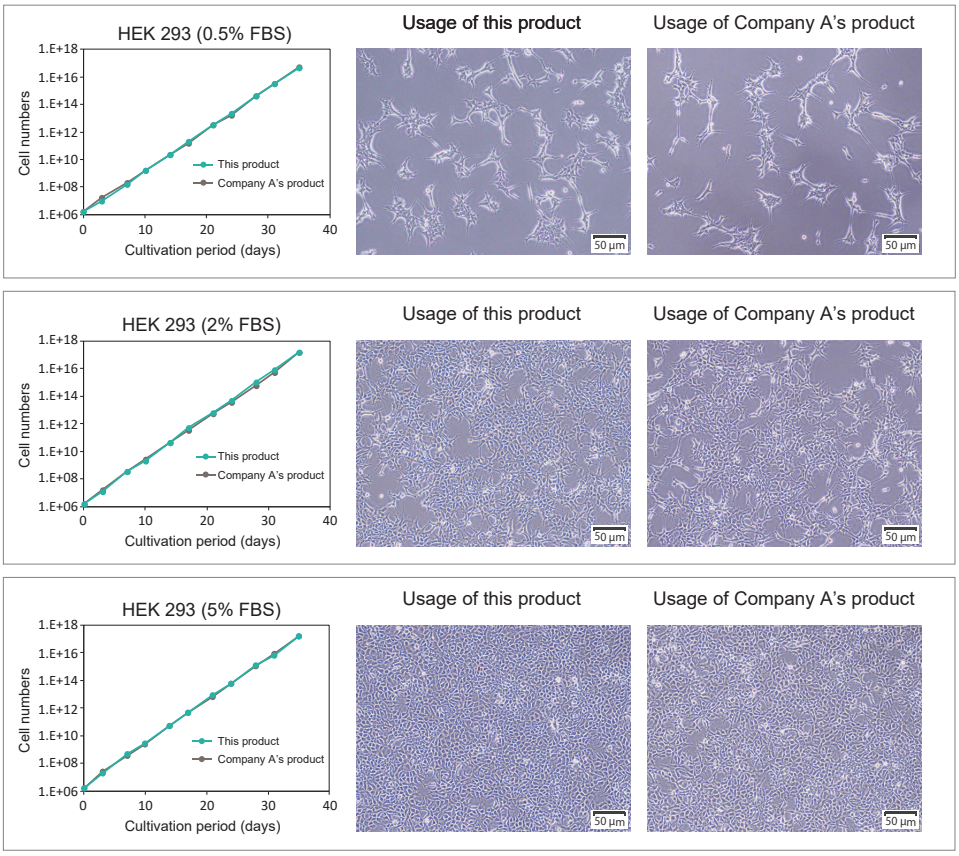
Reduced Serum DMEM/Ham's F-12 Medium

» **Cultivable under low-serum conditions**
» **Suitable as a basal medium for serum-free cultivation**
» **Reduce costs associated with serum usage**
» **Minimize variations between batches, reducing serum-related effects**

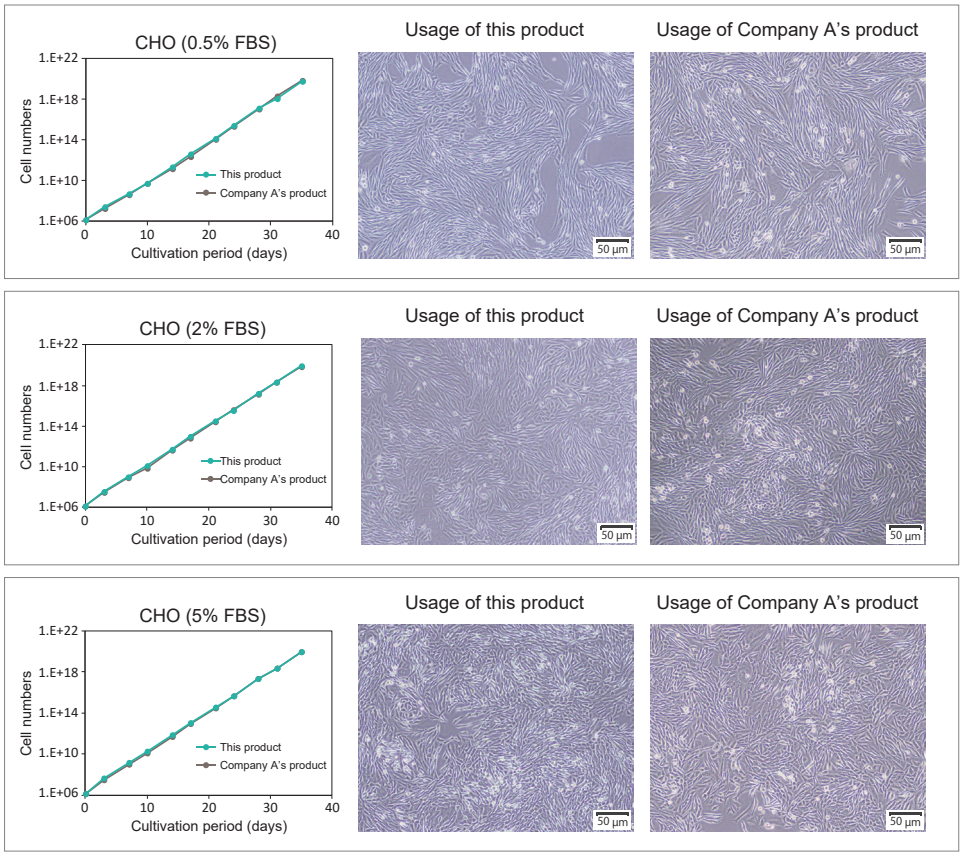
Comparison of cell proliferation rate and cell morphology

HEK 293 and CHO cells were cultured using both this product and Company A's serum-reduced medium. Similar to the medium from Company A, our product demonstrated that cultivation under low-serum conditions is feasible without affecting either cell proliferation rate or morphology

Proliferation curves and cell morphology of HEK 293 cells cultured under various serum concentrations (day 35)



Proliferation curves and cell morphology of CHO cells cultured under various serum concentrations (day 35)



Application

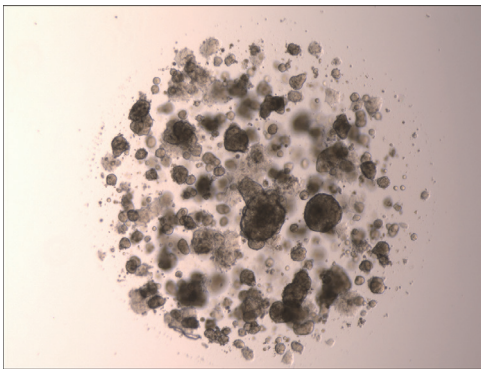
Cultivation of colorectal cancer patient-derived organoids (under serum-free conditions)

We prepared droplets (n=4) embedding dispersed colon cancer patient-derived organoid cells using Corning Matrigel Basement Membrane Matrix Growth Factor Reduced and TrypLE Express. Then, 1 ml of medium *was added on top, and the cultures were incubated.

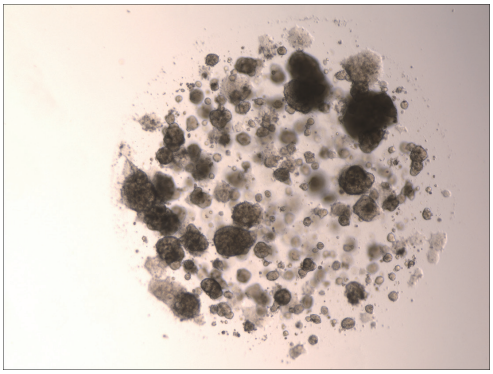
Visual assessment of proliferation did not reveal significant differences between the two conditions.

*2 mM L-Alanyl-L-Glutamine was added to both of this product and Company A's product.

Usage of this product



Usage of Company A's product



Data courtesy of Associate Professor Junpei Kondo, Department of Molecular Biochemistry, Division of Health Sciences, Graduate School of Medicine, Osaka University

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Ordering Information

Product Name	Storage	Product No.	PKG Size
Reduced Serum DMEM/Ham's F-12 Medium	R	21906-55	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

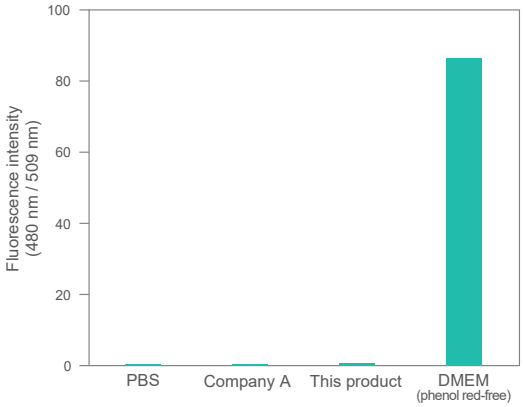
FluoroClear DMEM

This product is a culture medium with lower background fluorescence than phenol red-free DMEM. Since its background fluorescence is comparable to PBS, it is effective for detecting weak signals. In addition, because its composition is DMEM-based, it can also be used for maintenance culture of cells by adding fetal bovine serum and either L-glutamine or L-alanyl-L-glutamine.

» **Reduces background fluorescence**
» **Can also be used for maintenance culture of cells**

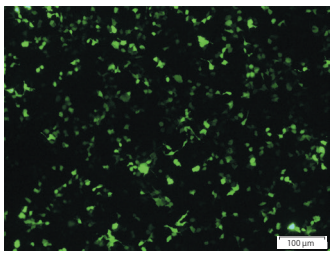
Comparison of background fluorescence

Background fluorescence of each product was measured using an F-2700 spectrofluorometer (HITACHI). lower fluorescence intensity than DMEM (phenol red-free) (#08489-45), and exhibited fluorescence intensity equivalent to PBS and Company A's fluorescence imaging medium.

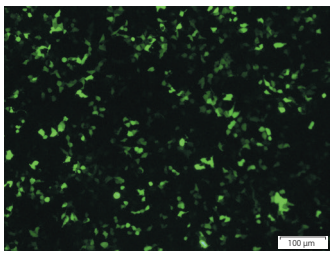


Fluorescence microscopy observation

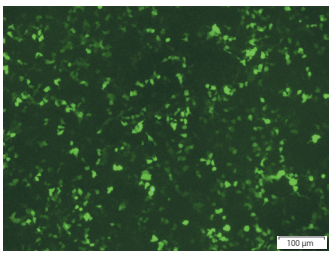
The pAcGFP-N1 vector was introduced into HEK293 cells, and GFP expression was observed using a fuorescence microscope. Similar to Company A's product, this product allowed observation with lower background than DMEM (phenol red-free).



This product



Company A

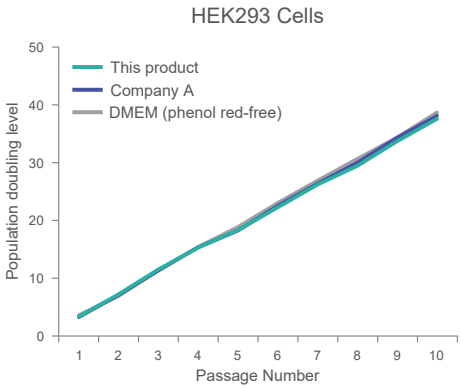


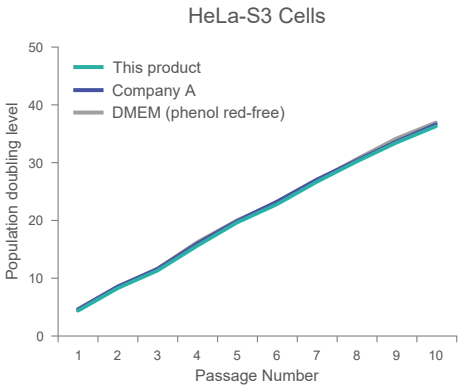
DMEM
(phenol red-free)

Culture of HEK293 Cells and HeLa-S3 Cells

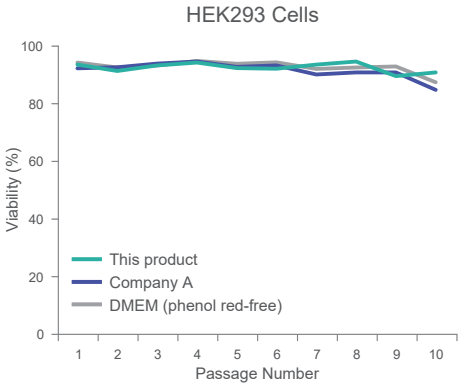
Cultures were performed using this product, Company A's product, and DMEM (phenol red-free), each supplemented with 10% FBS and 4 mM L-alanyl-L-glutamine, and cell proliferation ability as well as cell viability were evaluated. This product showed performance comparable to Company A's product and DMEM (phenol red-free) for maintenance culture of cells.

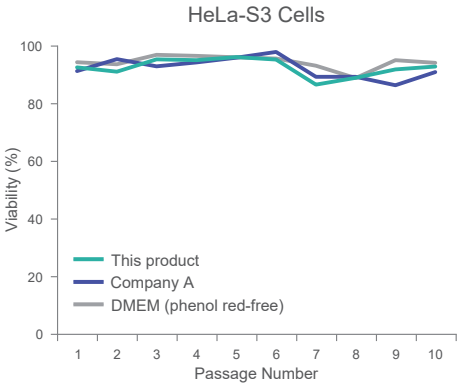
Cell proliferative capacity





Cell viability





Ordering Information

Product Name	Storage	Product No.	PKG Size
FluoroClear DMEM	R	22966-45	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Modified-MEM for Transfection

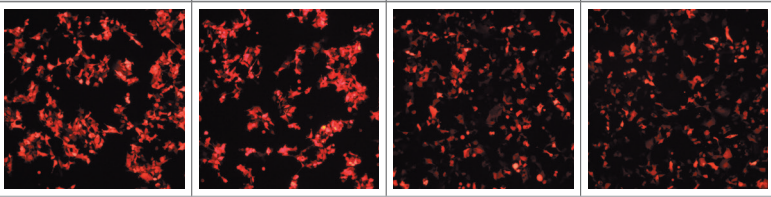
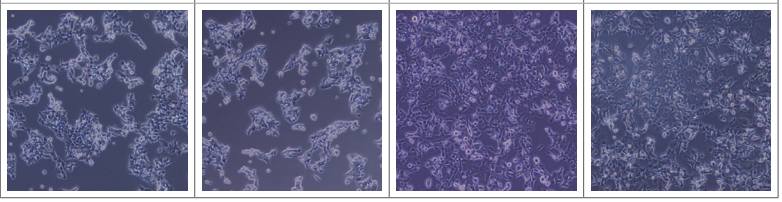
Modified-MEM for Transfection is an MEM-based medium designed for gene transfection.

» **Medium optimized for transfection**
» **Supports low-serum culture**
» **Medium composition disclosed**

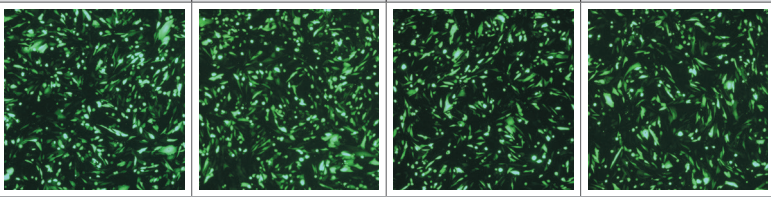
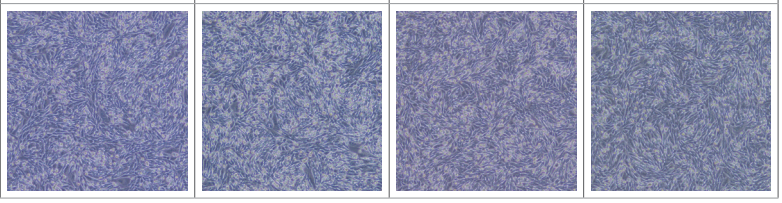
Transfection using this product or competitor media

Cells were transfected with a plasmid vector using this product or Company A's reduced-serum medium, which is commonly used for transfection, together with various transfection reagents. This product showed performance comparable to that of Company A's reduced-serum medium.

● Transfection of HEK293 cells with the pmCherry-N1 vector

Reagent	Company A's transfection reagent (1)		Company A's transfection reagent (2)	
Medium	This product	Company A	This product	Company A
Fluorescence microscope image				
Bright-field image				

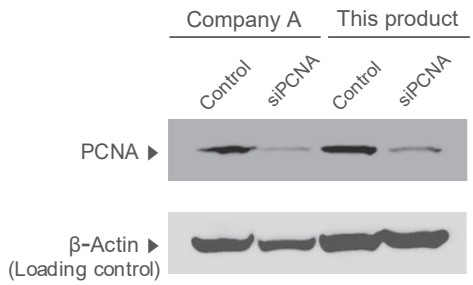
● Transfection of CHO-K1 cells with the pAcGFP1-N1 vector

Reagent	Company B's transfection reagent		Company C's transfection reagent	
Medium	This product	Company A	This product	Company A
Fluorescence microscope image				
Bright-field image				

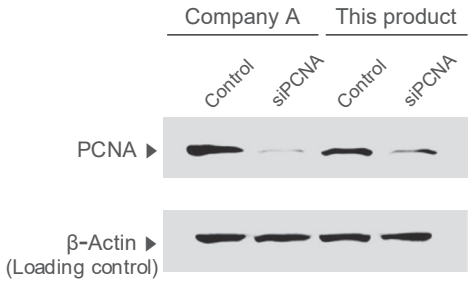
siRNA transfection using this product or competitor media

siRNA was introduced into HEK293 cells using Company A's transfection reagent together with either this product or Company A's reduced-serum medium, and the results were evaluated by Western blotting.

● Transfection using Company A's transfection reagent (1)

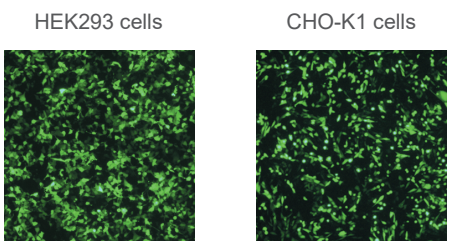


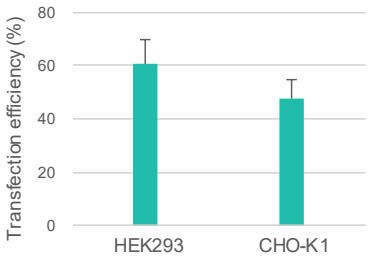
● Transfection using Company A's transfection reagent (2)



Transfection using this product and DailyFect Transfection Reagent

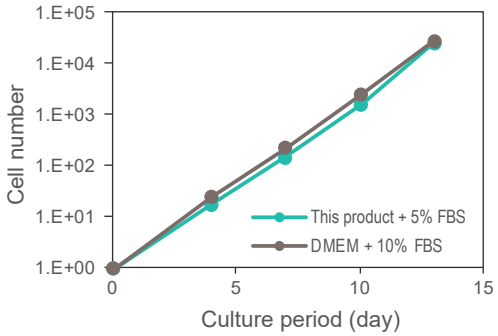
Using this product and DailyFect Transfection Reagent, 1 µg of the pAcGFP1-N1 vector was introduced into two types of adherent cells. Transfection efficiency was evaluated 48 hours after transfection by microscopy and flow cytometry.

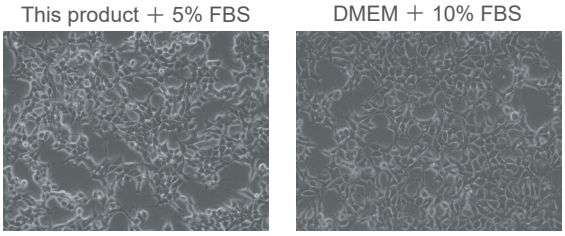




Cell proliferation rate and morphology under reduced-serum conditions

HEK293 cells were cultured using this product containing 5% FBS. Cell proliferation rate and morphology comparable to those observed with DMEM containing 10% FBS were obtained. These results confirmed that cells can be cultured under low-serum conditions using this product.





Ordering Information

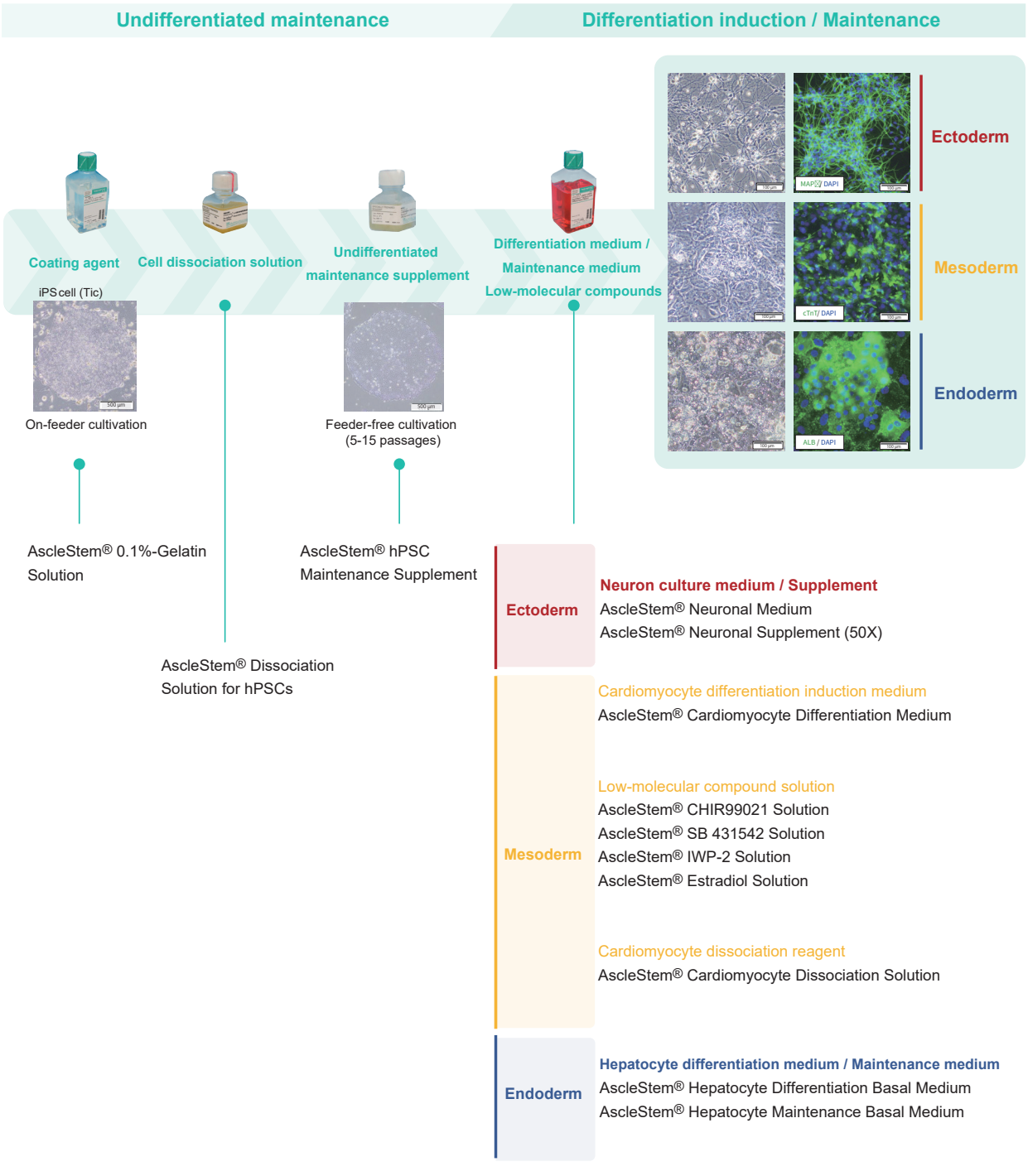
Product Name	Storage	Product No.	PKG Size
Modified-MEM for Transfection	R	22297-15	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

AscleStem® Series

Line-up and application of AscleStem®

The AscleStem® series offers a wide range of products, from undifferentiated maintenance to differentiation induction-related reagents. Using AscleStem® series products, we performed undifferentiated maintenance and differentiation induction to the trilineage in Tic line of human iPS cells.



Ectoderm

Neuron culture medium / Supplement
AscleStem® Neuronal Medium
AscleStem® Neuronal Supplement (50X)

Mesoderm

Cardiomyocyte differentiation induction medium
AscleStem® Cardiomyocyte Differentiation Medium

Low-molecular compound solution
AscleStem® CHIR99021 Solution
AscleStem® SB 431542 Solution
AscleStem® IWP-2 Solution
AscleStem® Estradiol Solution

Cardiomyocyte dissociation reagent
AscleStem® Cardiomyocyte Dissociation Solution

Endoderm

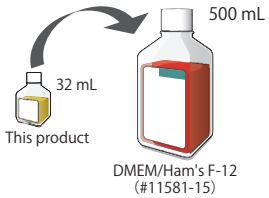
Hepatocyte differentiation medium / Maintenance medium
AscleStem® Hepatocyte Differentiation Basal Medium
AscleStem® Hepatocyte Maintenance Basal Medium

AscleStem® hPSC Maintenance Supplement

AscleStem® hPSC Maintenance Supplement (#21979-44), is a feeder-free culture supplement for maintaining undifferentiated human pluripotent stem cells (hPSCs). It can be easily prepared by simply adding it to DMEM/Ham's F-12 medium. This product is based on the composition of a clinical grade undifferentiated maintenance medium developed through collaborative research with Osaka University. Please refer to the provided references for more information.

» **Simply add it into DMEM/Ham's F-12 (#11581-15)**
» **Available to reduce the cost**
» **Applicable for the weekend skip protocol**
» **Medium components are disclosed** *** Concentration is confidential**

Protocol



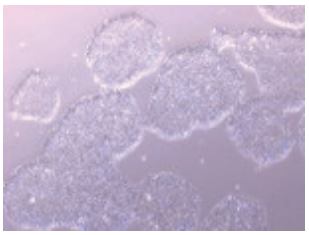
After thawed this product (stored in a frozen state) in a refrigerator, add the entire contents to 500 mL of DMEM/Ham's F-12. Please use it after thorough stirring.

Application

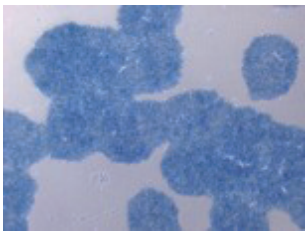
Cell morphology and alkaline phosphatase activity

The cell morphology of the 253G1 hiPSCs was confirmed after 2 weeks of culture with this product (prototype). Alkaline phosphatase staining was also performed to confirm that undifferentiated ability was not affected.

Phase contrast observation



Alkaline phosphatase staining



Reference

Hua Y, Yoshimochi K, Li J, Takekita K, Shimotsuma M, Li L, et al. Development and evaluation of a novel xeno-free culture medium for human-induced pluripotent stem cells. Stem Cell Res Ther. 2022;13:223.
doi:10.1186/s13287-022-02879-z https://pubmed.ncbi.nlm.nih.gov/35658933/

Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® hPSC Maintenance Supplement	F	21979-44	32 mL

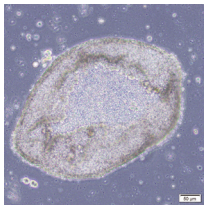
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

AscleStem® is a registered trademark of Nacalai Tesque, Inc.

AscleStem® Dissociation Solution for hPSCs

AscleStem® Dissociation Solution for hPSCs (#21777-84) is a cell dissociation solution for human pluripotent stem cells (hPSCs) composed of collagenase, trypsin, and serum substitutes. It allows for gentle dissociation of colonies during passaging of hPSCs without making single cells.

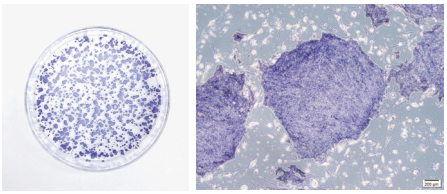
» **Allows for gentle passaging with pipetting only**
» **Mycoplasma tested**



<Cell detachment during passaging>
Observed passaging of human iPS cells in on-feeder culture with this product.

Application

We confirmed the undifferentiated potential of human iPS cells, which were passaged ten times using this product, by performing alkaline phosphatase staining.



201B7 on SNL76/7 feeder cells

Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® Dissociation Solution for hPSCs	F	21777-84	30 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

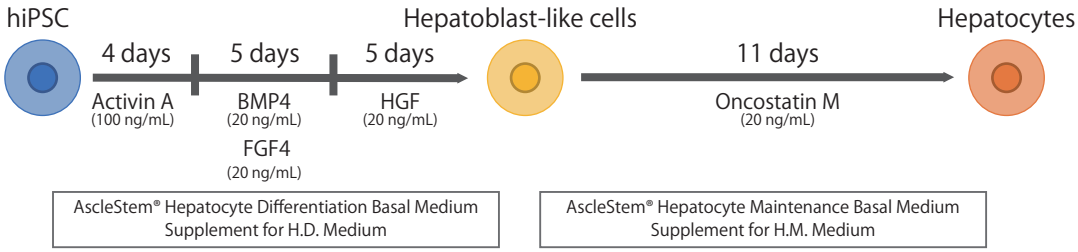
AscleStem® is a registered trademark of Nacalai Tesque, Inc.

AscleStem® Hepatocyte Differentiation & Maintenance Basal Medium

» **Enables human pluripotent stem cells to differentiate into hepatocytes**

» **Maintenance Basal Medium is suitable for drug screening applications**

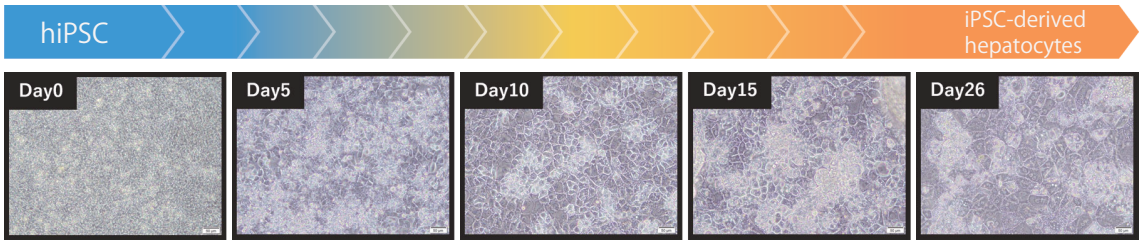
Protocol for hiPSC differentiation to hepatocytes



Application

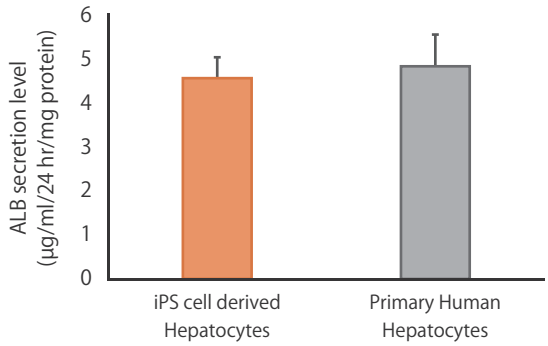
Using AscleStem® Hepatocyte Differentiation/Maintenance medium, the Tic hiPSCs was induced to differentiate into hepatocytes, and albumin production was compared with that of primary cultured human hepatocytes.

Imaging: Phase microscopy



Comparison of albumin production levels

This figure shows similar albumin production levels in hepatocytes differentiated from Tic hiPSCs using AscleStem® Hepatocyte Differentiation/Maintenance medium and primary human hepatocytes cultivated 24 hours after thawing.



Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® Hepatocyte Differentiation Basal Medium	R	20607-05	500 mL
Supplement for H.D. Medium	F	20612-54	5 mL
AscleStem® Hepatocyte Maintenance Basal Medium	R	20683-05	500 mL
Supplement for H.M. Medium	F	20615-24	30 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

AscleStem® is a registered trademark of Nacalai Tesque, Inc.

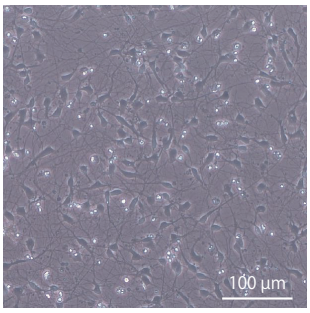
AscleStem® Neuronal Medium & Supplement(50x)

» **Serum-free media**
» **Suitable for iPSC-derived neuronal cell culture**

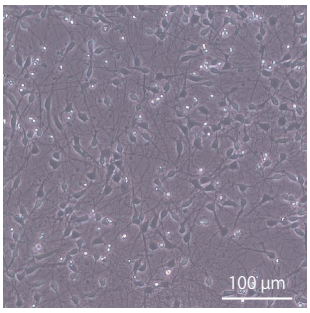
Differentiation from neural progenitor cells to neurons

hiPSCs-derived neural progenitor cells (day 17 after differentiation induction) were seeded at 500,000 cells/cm^2 and induced to differentiate into neurons by adding GlutaMAX and other inducers to our medium/ our supplement or Company A's medium / their supplement. Immunostaining was performed to confirm the expression of MAP2, a marker of mature neurons.
We confirmed that hiPSC-derived neurons cultured in AscleStem® Neuronal Medium and AscleStem® Neuronal Supplement(50x) were comparable in hiPSC-derived neurons cultured in Thermo's system.

Observation by phase microscopy 26 days after differentiation

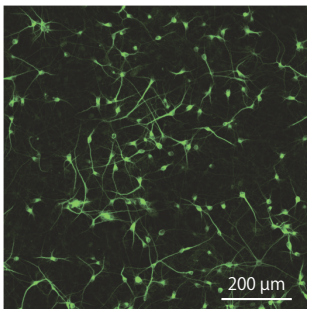


Company A's medium / their supplement

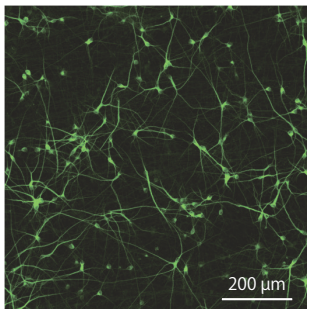


AscleStem® Neuronal Medium / AscleStem® Neuronal Supplement

Imaging by immunohistochemistry 56 days after differentiation



Company A's medium / their supplement



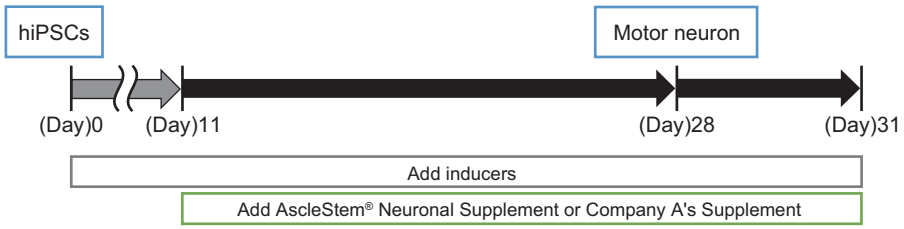
AscleStem® Neuronal Medium / AscleStem® Neuronal Supplement

Data courtesy of Assistant Prof. Kaneyasu Nishimura from Division of Integrated Pharmaceutical Sciences, Kyoto Pharmaceutical University

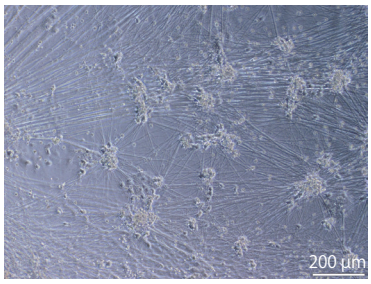
Differentiating motor neurons from hiPSCs

Using our supplement or Company A's supplement, human iPS cells were induced to differentiate into motor neurons. Immunostaining was performed to confirm the induction of motor neurons by choline acetyltransferase (ChAT) and the elongation of nerve axons by β III tubulin.

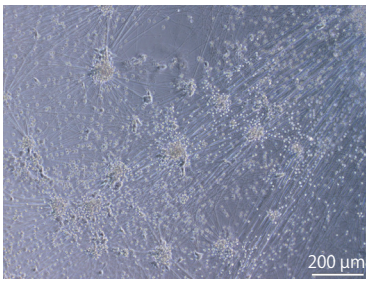
Protocol



Observed cell morphology and cell number and survival rate at detachment (28 days after start of differentiation induction)

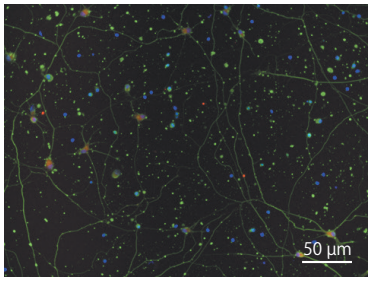


Company A
Survival rate: 41.3%
Cell number: 4.63 x 10^4 cells/cm^2

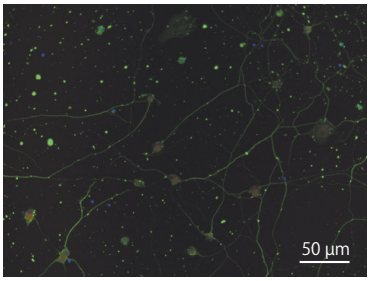


AscleStem® Neuronal Supplement
Survival rate: 50.2%
Cell number: 4.87 x 10^4 cells/cm^2

IHC (β III tubulin / ChAT / DAPI : 31 days after differentiation)



Company A



AscleStem® Neuronal Supplement

Morphology, degree of axon elongation, and ChAT expression levels were comparable using AscleStem® Neuronal Supplement compared to Company A's.

Data courtesy of Stem Cell & Device Laboratory, Inc. (SCAD)

Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® Neuronal Medium	R	21168-35	500 mL
AscleStem® Neuronal Supplement (50x)	F	21169-54	10 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

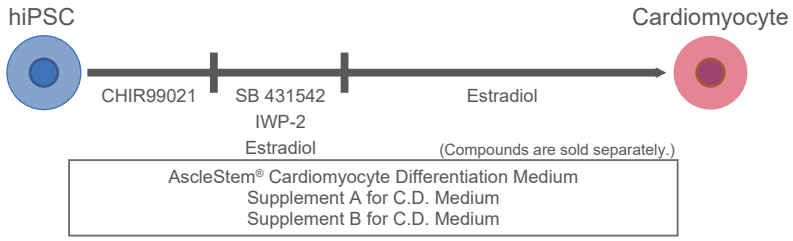
AscleStem® is a registered trademark of Nacalai Tesque, Inc.

AscleStem® Cardiomyocyte Differentiation Medium

AscleStem® Cardiomyocyte Differentiation Medium (#13166-05), Supplement A for C.D. Medium (#15345-24), and Supplement B for C.D. Medium (#15346-14) are serum-free medium and serum-free supplements for inducing differentiation of human pluripotent stem cells into cardiomyocytes. By replacing cytokines with small-molecule compounds, this protocol is expected to reduce costs, and it can be applied to both the monolayer culture method (adherent culture) and the embryoid body formation method (suspension culture).

» **Differentiates human pluripotent stem cells into cardiomyocytes**
» **Lower cost with cytokine-free protocol**
» **Compatible with monolayer and embryoid body methods**

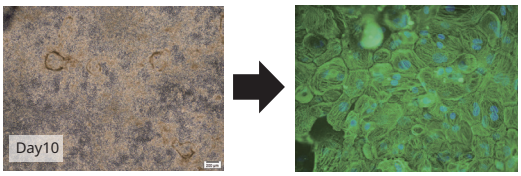
Example protocol for human iPSC-derived cardiomyocyte differentiation using this medium/ supplement



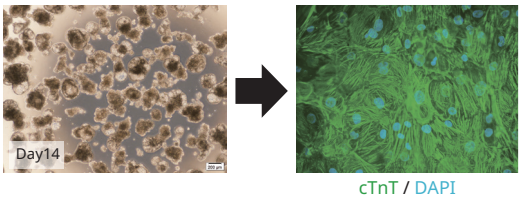
Cell morphology and immunostaining results after differentiation using two culture methods

Human iPSC cell lines were differentiated into cardiomyocytes using two culture methods: the monolayer culture method and the embryoid body formation method. Expression of the cardiomyocyte marker cTnT was confirmed by immunostaining using purified cells.

Cardiomyocytes differentiated by the monolayer culture method

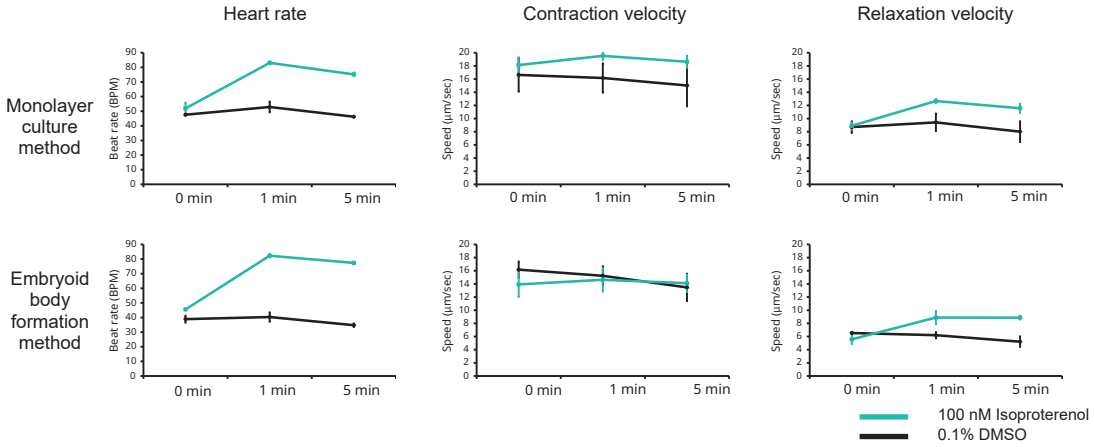


Cardiomyocytes differentiated by the embryoid body formation method



Response of cardiomyocytes differentiated by two culture methods to isoproterenol

The responsiveness of cardiomyocytes differentiated from human iPS cells by two culture methods (monolayer culture and embryoid body formation) to isoproterenol was evaluated using the SI8000 Cell Motion Imaging System (Sony). Similar results were obtained with both methods.



Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® Cardiomyocyte Differentiation Medium	R	13166-05	500 mL
Supplement A for C.D. Medium	F	15345-24	11 mL
Supplement B for C.D. Medium	F	15346-14	5 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

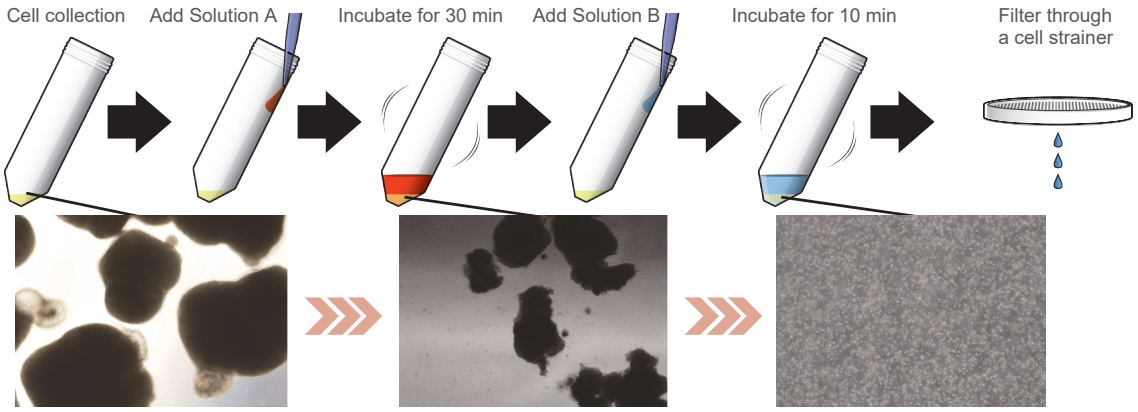
AscleStem® is a registered trademark of Nacalai Tesque, Inc.

AscleStem® Cardiomyocyte Dissociation Solution

This product is a reagent designed to effectively and gently dissociate human iPSC-derived cardiomyocytes. It can be applied to cardiomyocytes differentiated by either the monolayer culture method or the embryoid body formation method. In addition, it efficiently dissociates cardiomyocyte aggregates that are otherwise difficult to disperse.

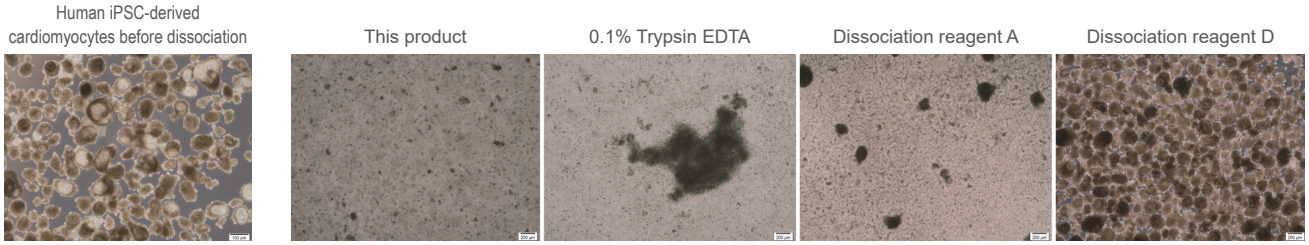
» **Effective and gentle dissociation**
» **Applicable to both monolayer culture and embryoid body formation methods**
» **Capable of dissociating long-term cultured cardiomyocyte aggregates**

Example of dissociation of cardiomyocytes differentiated by the embryoid body formation method



Comparison with other dissociation reagents

Various cell dissociation reagents, including this product, were applied for 40 minutes to human iPSC-derived cardiomyocytes differentiated by the embryoid body formation method (day 13 after the start of differentiation), and the degree of dissociation was compared.



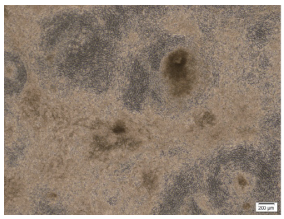
Using this product, cell aggregates that could not be sufficiently dissociated with other dissociation reagents were efficiently dissociated.

Application

Cell viability measurement after dissociation

Human iPSC-derived cardiomyocytes differentiated by the monolayer culture method (day 22 after the start of differentiation) were dissociated using this product, followed by trypan blue staining to determine cell viability. Use of this product enabled effective and gentle dissociation.

Before dissociation



After dissociation (before cell strainer filtration)

Cell viability after dissociation

Viable cells	178
Dead cells	9
Viability	95%

Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® Cardiomyocyte Dissociation Solution	F	17080-40	1 KIT

Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

AscleStem® is a registered trademark of Nacalai Tesque, Inc.

AscleStem® HSC Medium

» **Xeno-Free**
» **Enables expansion culture of hematopoietic stem cells (HSC)**
» **Experience in CiRA protocols for iPSC generation from human PBMCs**

Evaluation of hematopoietic stem cell expansion

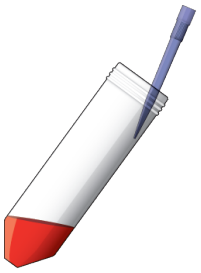
Human peripheral blood mononuclear cells (PBMCs) were cultured for five days in this medium supplemented with cytokines. After cell counting, expression of CD34 and CD45 was analyzed by flow cytometry. The results demonstrate that this medium efficiently supports the expansion of hematopoietic stem cells ($CD45^{dim}$ $CD34^{+}$).

Culture protocol overview

Cell culture was performed with reference to a protocol of CiRA Foundation.*

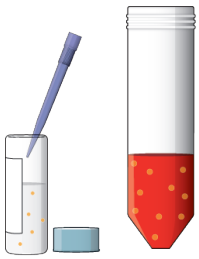
* https://www.cira-foundation.or.jp/j/assets/file/publications/EpisomalVector_iPS_protocol.pdf

Supplemented with 6 cytokines

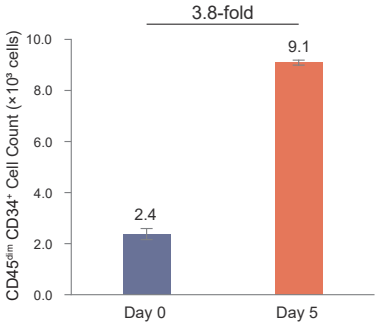


- FLT3L
- G-CSF
- IL-3
- IL-6
- SCF
- TPO

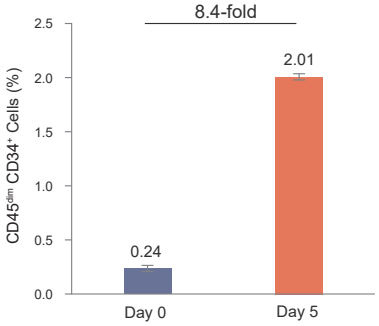
Adjust PBMCs to a cell density of 1.0 × 10^6 cells/mL and seed.



Number of HSC marker–positive cell



Percentage of HSC marker–positive cells



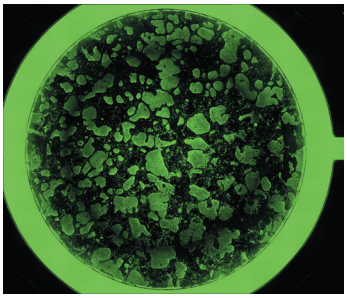
Application of this medium to iPS cell derivation

Human peripheral blood mononuclear cells (PBMCs) were cultured in this medium supplemented with six cytokines, followed by generation of iPS cells based on the RNA reprogramming method*.

* Nakagawa M, Nogi M, Doi H, et al. MDM4 enables efficient human iPS cell generation from PBMCs using synthetic RNAs. Sci Rep. 2025; 15: 30620. DOI: https://doi.org/10.1038/s41598-025-16446-y

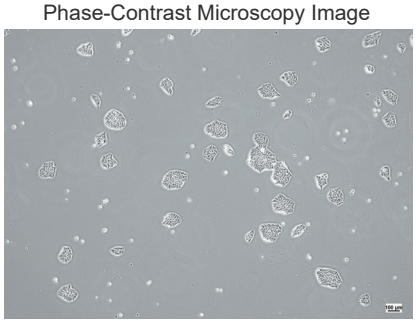
Confirmation of undifferentiated marker expression (Day 14 after introduction of reprogramming factors)

TRA-1-60–Positive Cells (Green)



Morphology of established iPS cells (Cells at passage 5)

Phase-Contrast Microscopy Image



These data were provided by Dr. Masato Nakagawa, Specially Appointed Associate Professor (Full Time), Human Metaverse Medicine, The University of Osaka.

Reference

Human IPS Cells—Reprogramming Human Peripheral Blood-Derived Mononuclear Cells (PBMCs) Using Synthetic RNA
https://www.cira.kyoto-u.ac.jp/e/research/protocol.html

Ordering information

Product Name	Storage	Product No.	PKG Size
AscleStem® HSC Medium	F	22562-25	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

AscleStem® is a registered trademark of Nacalai Tesque, Inc.

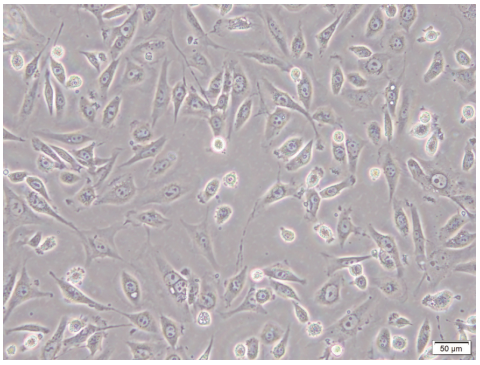
AscleStem® 0.1%-Gelatin Solution

» **Ready-to-use**

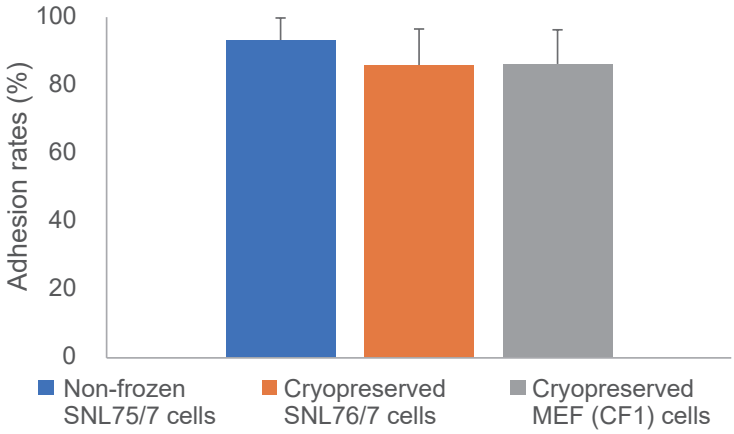
» **Good for cultivation of feeder cells**

Application 1: Adhesion of feeder cells

After coating a cell culture plate with AscleStem® 0.1%-Gelatin Solution, non-freezed SNL76/7 cells, cryopreserved SNL76/7 cells or MEF (CF1) cells were cultivated, then adhesion rates were measured.



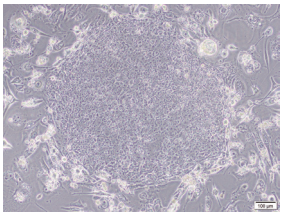
Mitomycin C treated SNL76/7 cells



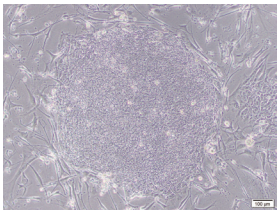
Application 2: Maintenance culture of hiPSCs

SNL76/7 cells and MEF (CF1) cells were seeded onto plates coated with this product, on which the 253G1 hiPSCs was maintained cuture and confirmed colony morphology and undifferentiated characteristics (alkaline phosphatase staining).

Morphological observation

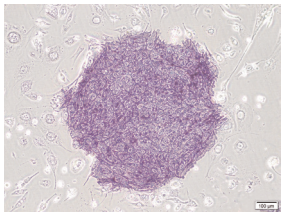


253G1 on SNL76/7 feeder cells

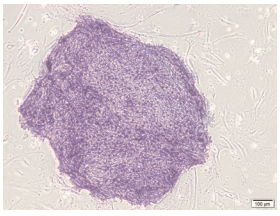


253G1 on MEF (CF1) feeder cells

Alkaline phosphatase staining



253G1 on SNL76/7 feeder cells



253G1 on MEF (CF1) feeder cells

Ordering Information

Product Name	Storage	Product No.	PKG Size
AscleStem® 0.1%-Gelatin Solution	RT	19895-75	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

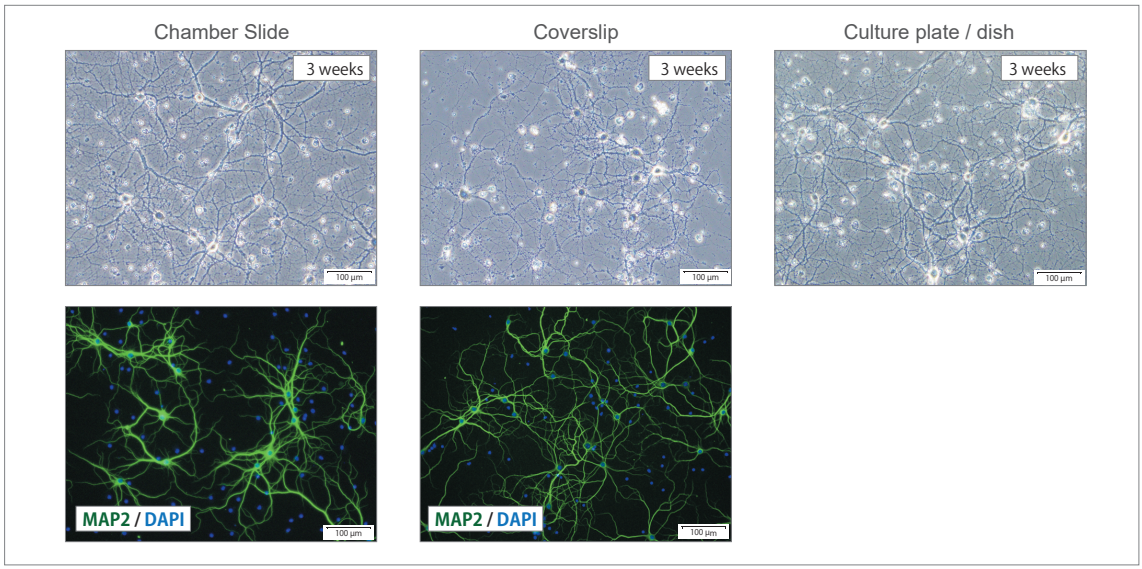
AscleStem® is a registered trademark of Nacalai Tesque, Inc.

0.01%-Polyethyleneimine Coating Solution

» **Applicable for primary neuronal cell culture**
» **Suitable for various types of cell culture labware**
» **Store at room temperature**

Application

Primary cultured neurons from the mouse fetal brain hippocampus were successfully maintained and cultured on various cell culture labware coated with this product.



Ordering Information

Product Name	Storage	Product No.	PKG Size
0.01%-Polyethyleneimine Coating Solution	RT	21958-14	100 mL

Mitomycin C Solution (1 mg/mL) for preparation of feeder cells

» **Ready-to-use: Sterility-tested for cell culture, does not solidify in freezer**
» **High stability: 2 years in freezer, protected from light**

Ordering Information

Product Name	Storage	Product No.	PKG Size
Mitomycin C Solution (1mg/mL)	F	20898-21	1 mL

Related Products

Product Name	Storage	Product No.	PKG Size
Y-27632	F	08945-71	1 mg
		08945-84	5 mg

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

N-2 Supplement (100X)

N-2 Supplement (100X) is a serum substitute used for culturing neural cells. It enables the maintenance of undifferentiated neural stem cells, which would otherwise undergo differentiation when exposed to serum. In addition, it can be used as a supplement for a variety of neural cell cultures in combination with growth factors and other serum substitutes.

» **Enhances neural stem cell growth and expansion**
» **Xeno-free**

Ingredients

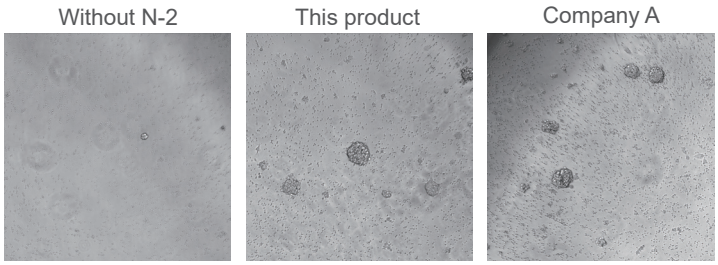
- Recombinant human insulin
- Human holo-transferrin (iron-saturated)
- Sodium selenite
- Putrescine
- Progesterone

Culturing neural cells using the neurosphere method

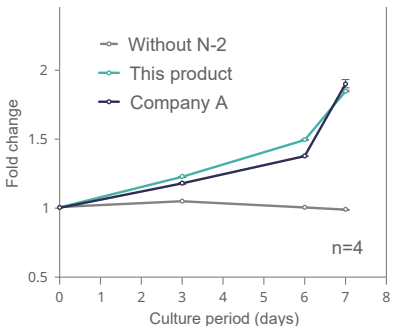
A single-cell suspension was prepared from the brains of E14 mouse embryos and cultured using the neurosphere method in medium* supplemented with either this product or a competitor's product (Company A). Evaluation of cell morphology and proliferation rate showed that this product achieved results comparable to those of Company A's product.

* Medium: DMEM/Ham's F-12 (#08460-95), 20 ng/mL bFGF, 20 ng/mL EGF

Cell morphology (Day 6 of culture)



Cell proliferation assay (WST-8 method)



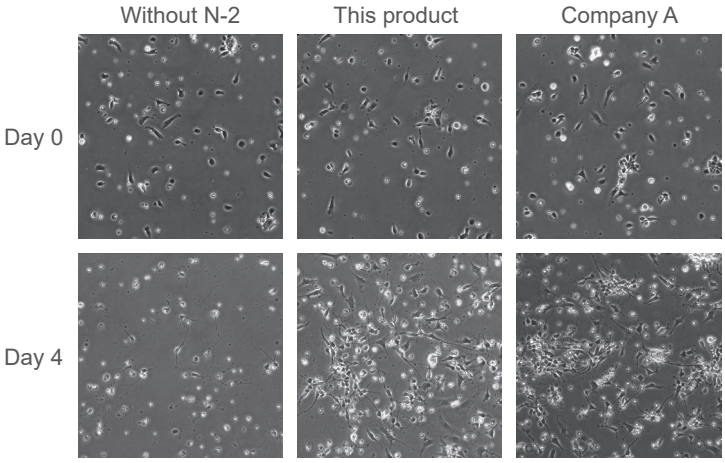
Culturing neural cells using the adherent culture method

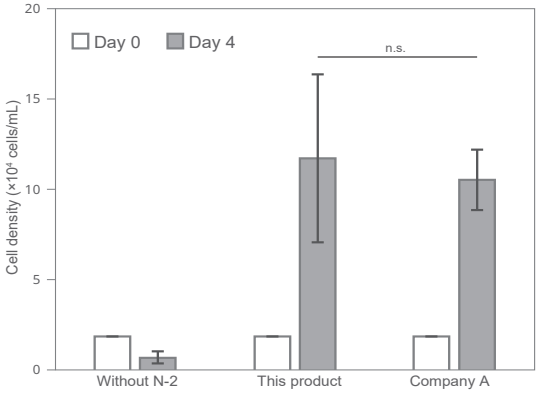
A single-cell suspension was prepared from the brains of E10 mouse embryos and cultured on, Poly-L-lysine-coated plates using medium* supplemented with either this product or a competitor's product (Company A). Results demonstrated that this product, like Company A's product, supports the proliferation of cells while maintaining them in an undifferentiated state.

* Medium: DMEM/Ham's F-12(#08460-95), 20 ng/mL bFGF, 20 ng/mL EGF

Comparison of cell morphology and growth

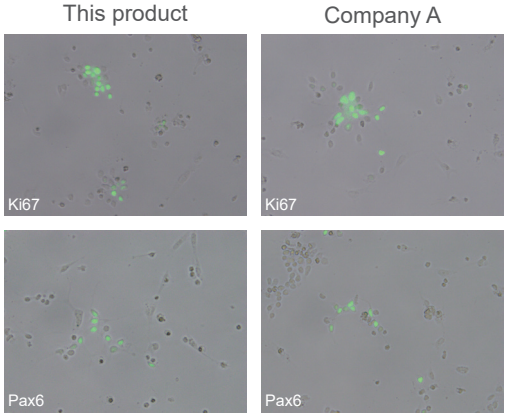
After 4 days of culture, evaluation of cell morphology and cell number showed that this product produced results comparable to those of Company A's product.

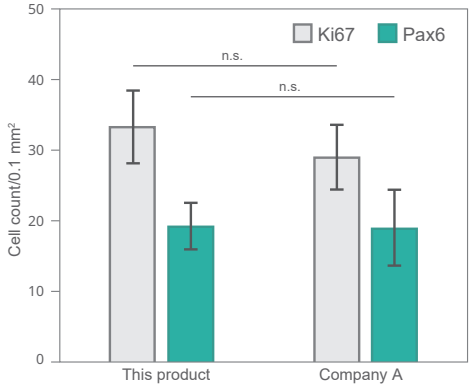




Confirmation of the undifferentiated state

Immunostaining was performed on cells after 3 days of culture to examine the expression of Ki67 (a proliferation marker) and Pax6 (a neural stem/progenitor cell marker). No significant differences were observed in the number of marker-positive cells between cultures supplemented with this product and those with Company A's product, indicating that the cells maintained an undifferentiated state.





Ordering Information

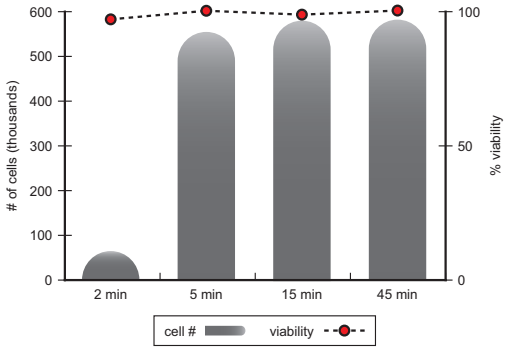
Product Name	Storage	Product No.	PKG Size
N-2 Supplement (100X)	F	22666-04	5 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Cell Dissociation Reagent: Accutase™

» **Gentle and efficient dissociation of any adherent cell line**
» **No mammalian or bacterial components are contained**
» **No neutralization steps by serum or trypsin inhibitors are required**
» **Works extremely well on embryonic and neuronal stem cells**

Application



Human MG63 Fibrosarcoma cells cultured in DMEM + 10% FBS were treated with Accutase™.

Treatment resulted in rapid cell detachment, a single cell suspension, and high viability.

Accutase™ is gentle on cells; viability was 97 ± 3% even after 45 minutes in Accutase™.

Cell Lines dissociated with Accutase™

- hESCs
- vascular endothelial cells
- hepatocyte progenitors
- adherent CHO cells
- 293 cells
- 3T3
- HeLa
- M24 metastatic melanoma
- gliomas D54
- fibroblasts
- hepatocytes
- primary chick embryo neuronal cells
- adherent BHK cells
- L929 cells
- Vero
- NT2
- A375 metastatic melanoma
- HT1080 fibrosarcoma cells
- keratinocytes
- vascular smooth muscle cells
- bone marrow stem cells
- macrophages
- immortalized mouse testicular germ cells
- COS
- MG63
- gliomas U251
- Sf9 insect cells

Ordering Information

Product Name	Storage	Product No.	PKG Size
Accutase™	F	12679-54	100 mL

Related Products

Product Name	Storage	Product No.	PKG Size
Accumax	F	13766-74	100 mL
2.5g/l-Trypsin Solution	F	35555-54	100 mL
5.0g/l-Trypsin/5.3mmol/l-EDTA Solution	F	35556-44	100 mL
2.5g/l-Trypsin/1mmol/l-EDTA Solution	F	35554-64	100 mL
2.5g/l-Trypsin/1mmol/l-EDTA Solution, with Phenol Red	F	32777-44	100 mL
0.5g/l-Trypsin/0.53mmol/l-EDTA Solution	F	35553-74	100 mL
0.5g/l-Trypsin/0.53mmol/l-EDTA Solution, with Phenol Red	F	32778-34	100 mL
0.2g/l-EDTA Solution	R	14367-74	100 mL

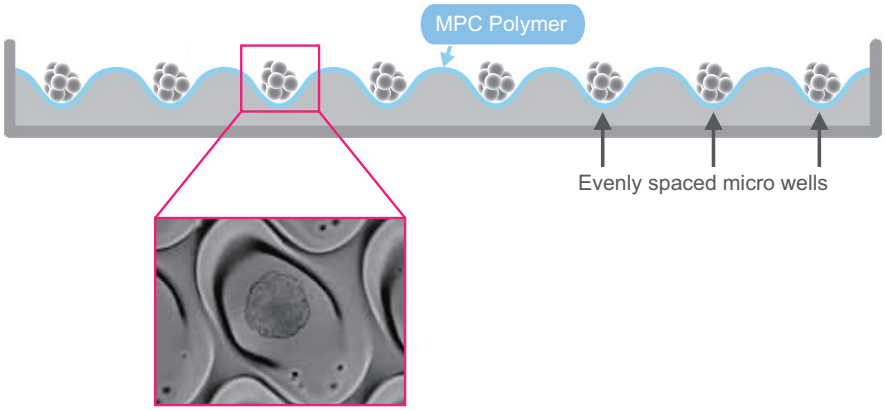
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

EZSPHERE for 3D Cell Culture with Microwells

Three dimensional (3D) cell culture systems have gained in popularity as invaluable tools in broad applications of cell biology. 3D multi-cellular cell aggregates (Spheroid) can be formed by using a low attachment culture surface. However, variability in forming spheroids has been a persistent problem. EZSPHERE is specifically designed to form a large number of uniformly sized spheroids and embryoid bodies (EBs).

» **Coated with very low binding 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer**
» **Form uniformly sized spheroids efficiently in the round shape wells**

Well cross-section



All product names, trademarks, and registered trademarks are the property of their respective owners. Use of these names does not imply any affiliation or endorsement.

Ordering Information

Product Name	Storage	Product No.	PKG Size
EZSPHERE SP, 35 mm Dish	RT	4000-900SP	10 ea
EZSPHERE SP, 60 mm Dish	RT	4010-900SP	10 ea
EZSPHERE SP, 100 mm Dish	RT	4020-900SP	10 ea
EZSPHERE SP, 24-well Plate	RT	4820-900SP	5 ea
EZSPHERE SP, 96-well Plate	RT	4860-900SP	5 ea
EZSPHERE SP, 35 mm Dish Type 902	RT	4000-902SP	10 ea
EZSPHERE SP, 35 mm Dish Type 903	RT	4000-903SP	10 ea
EZSPHERE SP, 35 mm Dish Type 904	RT	4000-904SP	10 ea
EZSPHERE SP, 35 mm Dish Type 905	RT	4000-905SP	10 ea
EZSPHERE SP Microplate 6-well EZ-TRY	RT	TCI-4810-EZ-TRY-SP-N	5 ea
EZSPHERE SP Microplate 24-well EZ-TRY	RT	TCI-4820-EZ-TRY-SP	5 ea

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

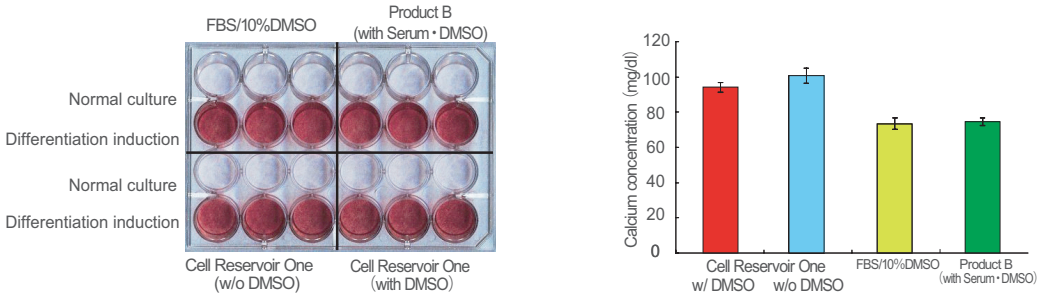
Serum-free Cell Freezing Media: Cell Reservoir One

Cell Reservoir One is a serum-free cell culture freezing medium, which contains a water-soluble glycoprotein sericin isolated from the silkworm cocoon as a major constituent. Sericin shows the same high efficacy of cryopreservation as with FBS, and reduces the cell toxicity of DMSO. As DMSO is known to have adverse effects on cellular functions, especially embryonic stem cells, Cell Reservoir One is available both with and without DMSO.

- **No programmed freezer or special vessel necessary**
- **Ready-to-use solution**
- **Serum-free with no animal-derived components**
- **High cell recovery and viability**

Application 1

Human mesenchymal stem cell (hMSC): Bone differentiation

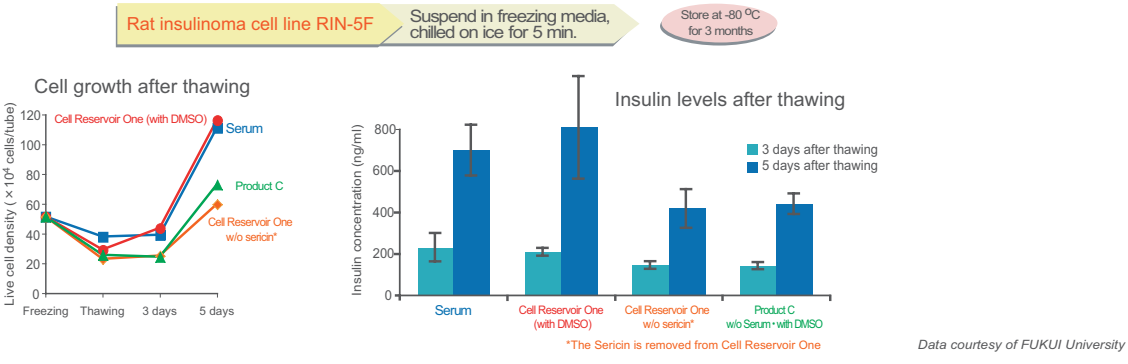


Cell Reservoir One maintains bone differentiation ability of hMSC.
Freezing condition: -80ºC, 1 month

Condition:
(Left) Alizarin Red staining after 4 weeks differentiation induction of hMSC
(Right) Quantification of calcium deposition after differentiation induction

Application 2

Pancreatic islet transplantation model



Data courtesy of FUKUI University

Procedure for cell freezing

1. Collect cells in logarithmic growth phase.
2. Suspend the cells in Cell Reservoir One (5 x 10^5 - 1 x 10^7 cells in 1 mL of Cell Reservoir One).
3. Dispense the suspension to a cryo-cell tube.
4. Store it at -80ºC without pre-freezing.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Cell Reservoir One (with DMSO)	R	07485-44	100 mL
Cell Reservoir One (without DMSO)	R	07579-24	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

ES/iPS cell Freezing Media: Cell Reservoir One, Vitrify

Vitrification has become an important alternative to standard slow programmable freezing methods for cryopreservation of primate ES cell lines including Human iPS cells because of the higher survival rates of cells after thawing. However, the vitrification requires an ultra-rapid freezing protocol, usually less than 15 seconds between making cell suspensions and freezing in liquid nitrogen. Cell Reservoir One (Vitrify) is a novel serum-free cell culture freezing medium for vitrification method, which contains a water-soluble glycoprotein sericin isolated from the silkworm cocoon as a major constituent. It provides high survival rates of primate cells, such as Monkey ES cells and Human iPS cells even with a longer freezing protocol; up to 60 second from the cell collection to freezing in liquid nitrogen.

**Cell Reservoir One (Vitrify) is produced in corporation with SEIREN. (Patented)*

» **High viability with a longer freezing protocol (up to 60 seconds)**

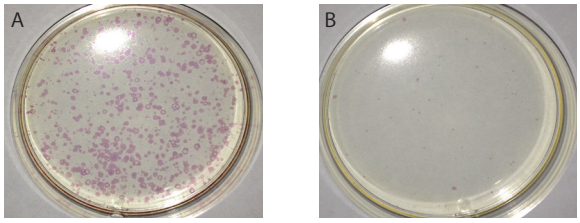
» **Low toxicity to cells (DMSO and acetamide free)**

Application

Comparison of survival rate of Human iPS cells (201B7 cell line*) *Takahashi, K. et al. *Cell*, Nov 30;**131**(5):861-872 (2007)

Freezing protocol: 60 seconds

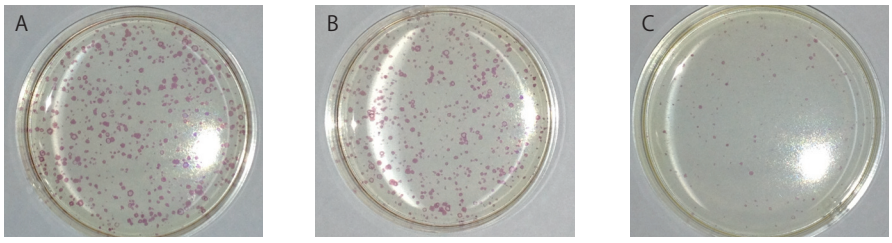
A: Cell Reservoir One (Vitrify) B: DAP213



Human iPS cells were cryopreserved for more than 2 weeks in Cell Reservoir One (Vitrify) or DAP213. Viability was detected using Alkaline Phosphatase 4 days after thawing. Cell Reservoir One (Vitrify) showed high survival rate, while most of cells in DAP213 were dead.

Freezing protocol: 15 seconds

A: Cell Reservoir One (Vitrify) B: DAP213 C: Company A



Human iPS cells were cryopreserved for more than 2 weeks in Cell Reservoir One (Vitrify), DAP213 or Company A's product. Viability was detected using Alkaline Phosphatase 4 days after thawing. Cell Reservoir One (Vitrify) showed the highest viability.

Data courtesy of a customer

Conclusion

Cell Reservoir One (Vitrify) showed high viability with both 15 and 60 seconds of freezing protocol. With 60 seconds protocol, the survival rate of cells in Cell Reservoir One (Vitrify) was significantly higher than other freezing media.

Freezing medium		The number of colony		
		Vitrification method		Slow freezing method
		60 Seconds	15 Seconds	
A	Cell Reservoir One (Vitrify)	672	563	-
B	DAP213	37	479	-
C	Company A	-	-	172

Ordering Information

Product Name	Storage	Product No.	PKG Size
Cell Reservoir One, Vitrify	R	11325-62	25 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Related Product

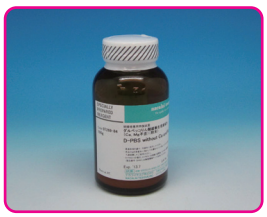
Product Name	Grade	Storage	Product No.	PKG size
Dimethyl Sulfoxide	SP (For TC)	RT	13408-64	5 x 5 mL
Glycerol	SP (Biotechnology grade)	RT	09886-05	500 mL
Propylene Glycol	SP (Biotechnology grade)	RT	11740-25	500 g

Balanced Salines

» **Quality control items: pH, Osmotic pressure, Sterility test, Endotoxin test, Mycoplasma test**

D-PBS(-) w/o Ca and Mg, liquid
14249-95

D-PBS(-) w/o Ca and Mg, powder
07269-84



D-PBS(+) Preparation Reagent
(Ca,Mg Solution) (100x)
02492-94

Product List and Ordering Information

Product Name	KCl	Ca • Mg	Phenol Red	Storage	Product No.	PKG size	GIBCO	Sigma
D-PBS(-) without Ca and Mg, liquid	○	-	-	RT	14249-95 14249-24	500 mL 10 x 500 mL	14190-144 14190-250	D8537
D-PBS(-) without Ca and Mg, liquid(10x)	○	-	-	RT	11482-15	500 mL	14200-075	D1408
D-PBS without Ca and Mg, Powder	○	-	-	RT	07269-84	100 g	21600-069	D5652
Phosphate Buffered Saline without KCl(pH 7.2)	-	-	-	RT	11480-35	500 mL	20012-027	
Phosphate Buffered Saline without KCl(10x)(pH 7.2)	-	-	-	RT	11481-25	500 mL	70013-032	
Phosphate Buffered Saline without KCl(pH 7.4)	-	-	-	RT	13397-85	500 mL	10010-023	
D-PBS(+)Preparation Reagent(Ca, Mg Solution)(100x)	-	○	-	RT	02492-94	30 mL		
HBSS(+) with Ca, Mg and Phenol Red, liquid	○	○	○	RT	17459-55	500 mL	24020-117	H9269
HBSS(+) with Ca, Mg, without Phenol Red, liquid	○	○	-	RT	09735-75	500 mL	14025-092	H8264
HBSS(-) without Ca and Mg, with Phenol Red, liquid	○	-	○	RT	17460-15	500 mL	14170-112	H9394
HBSS(-) without Ca, Mg and Phenol Red, liquid	○	-	-	RT	17461-05	500 mL	14175-095	H6648

Sterile Water

» **Quality control items: Sterility test, Endotoxin test, Nuclease test, Protease test**

Product List and Ordering Information

Product Name	Grade	Product No.	Storage	PKG size	GIBCO	Sigma
Water deionized & sterilized	SP (For molecular biology)	06442-95	RT	500 mL	15230-162	W3500
Water deionized & sterilized	SP (For molecular biology)	20620-31	F	10 x 1 mL	15230-162	-

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Media Additives

Prepared media additive solution

» **Quality control items: Sterility test, Endotoxin test, Mycoplasma test**

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size	GIBCO	Sigma
200mmol/l L-Alanyl-L-glutamine Solution(100x)	SP (For TC)	R	04260-64	100 mL	35050-061	G8541
200mM-L-Glutamine Stock Solution	SP (For TC)	F	16948-04	100 mL	25030-081	G7513
0.1mol/l-Calcium Chloride Solution	SP (For TC)	RT	16973-64	20 mL		
45(w/v)%-D-(+)-Glucose Solution	SP (For TC)	RT	16974-54	100 mL		G8769
1mol/l-HEPES Buffer Solution	SP (For TC)	R	17557-94	100 mL	15630-080	H0887
MEM Non-Essential Amino Acids Solution(100x)	SP (For TC)	R	06344-14 06344-56	20 mL 100 mL	11140-076 11140-050	M7145
100mM-Sodium Pyruvate Solution(100x)	SP (For TC)	R	06977-34	100 mL	11360-070	S8636

Amino acids, vitamins and others

» **Quality control items: Endotoxin test, cell culture test**

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
Amino Acids				
L-α-Alanine, Animal-Free	SP (For TC)	RT	12998-32	25 g
L-Alanyl-L-glutamine, Animal-Free	SP (For TC)	R	18189-52	25 g
L-Arginine, Animal-Free	SP (For TC)	RT	11984-32 11984-45	25 g 500 g
L-(+)-Arginine Monohydrochloride, Animal-Free	SP (For TC)	RT	12999-22	25 g
L-Asparagine Monohydrate, Animal-Free	SP (For TC)	RT	13000-42	25 g
L-Aspartic Acid, Animal-Free	SP (For TC)	RT	13002-22	25 g
L-Cysteine Hydrochloride Monohydrate, Animal-Free	SP (For TC)	R	11983-42 11983-55	25 g 500 g
L-Cystine Dihydrochloride, Animal-Free	SP (For TC)	RT	13003-12	25 g
L-Glutamic Acid, Animal-Free	SP (For TC)	RT	13012-92	25 g
L-Glutamine, Animal-Free	SP (For TC)	RT	13004-02 13004-15	25 g 500 g
Glycine, Animal-Free	SP (For TC)	RT	12997-42	25 g
L-Histidine(free base), Animal-Free	SP (For TC)	RT	13014-72	25 g
L-Histidine Monohydrochloride Monohydrate, Animal-Free	SP (For TC)	RT	13017-42	25 g
L-Hydroxyproline, Animal-Free	SP (For TC)	RT	13018-32	25 g
L-(+)-Isoleucine, Animal-Free	SP (For TC)	RT	13035-02	25 g
L-Leucine, Animal-Free	SP (For TC)	RT	13036-92	25 g
L-Lysine Monohydrochloride, Animal-Free	SP (For TC)	RT	13037-82	25 g
L-Methionine, Animal-Free	SP (For TC)	RT	13038-72	25 g
L-(-)-Phenylalanine, Animal-Free	SP (For TC)	RT	13039-62	25 g
L(-)-Proline, Animal-Free	SP (For TC)	RT	13040-22	25 g
L-Serine, Animal-Free	SP (For TC)	RT	13041-12	25 g
L-Threonine, Animal-Free	SP (For TC)	RT	13042-02	25 g
L-Tryptophan, Animal-Free	SP (For TC)	RT	13043-92	25 g
L-Tyrosine Disodium Salt Dihydrate, Animal-Free	SP (For TC)	R	11985-22 11985-35	25 g 500 g

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

L-Valine, Animal-Free	SP (For TC)	RT	13046-62	25 g
Vitamins				
p-Aminobenzoic Acid, Animal-Free	SP (For TC)	RT	13047-52	25 g
L(+)-Ascorbic Acid, Animal-Free	SP (For TC)	RT	13048-42	25 g
D-Biotin, Animal-Free	SP (For TC)	RT	13049-61	1 g
Choline Chloride, Animal-Free	SP (For TC)	R	13050-34	5 g
myo-Inositol, Animal-Free	SP (For TC)	RT	13051-82	25 g
Pyridoxine Hydrochloride, Animal-Free	SP (For TC)	RT	13053-04	5 g
Vitamin B1 Hydrochloride, Animal-Free	SP (For TC)	RT	13052-14	5 g
Vitamin B12, Animal-Free	SP (For TC)	R	13054-94	100 mg
Others				
D-(+)-Glucose, Animal-Free	SP (For TC)	RT	13057-35	500 g
Glutathione(Reduced Form), free acid, Animal-Free	SP (For TC)	R	13056-61	1 g
Hypoxanthine, Animal-Free	SP (For TC)	RT	13055-71	1 g
Sodium Pyruvate, Animal-Free	SP (For TC)	R	13058-12	25 g

Low molecular compounds

(Solution)

» **Quality control items: Sterility test, Mycoplasma test, Endotoxin test**

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
AscleStem® CHIR99021 Solution(10mM), Animal-Free	SP (For TC)	F	21068-74	5 x 100 µL
AscleStem® SB 431542 Solution(10mM), Animal-Free	SP (For TC)	F	21235-74	5 x 100 µL
AscleStem® IWP-2 Solution(5mM), Animal-Free	SP (For TC)	F	21236-64	5 x 100 µL
AscleStem® Estradiol Solution(1mM), Animal-Free	SP (For TC)	F	21237-25	500 µL
10mmol/L-Y-27632 Solution	SP (For TC)	F	18190-96 18190-54	100 µL 1.5 mL

(Powder)

» **Quality control items:** refer to the specification sheets for each product.

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
17β-Estradiol, Animal-Free	SP (For biochemical research)	R	18192-92	25 mg
IWP-2, Animal-Free	SP (For biochemical research)	R	18175-64	5 mg
SB 431542, Animal-Free	SP (For biochemical research)	RT	18176-54	5 mg
Y-27632, Animal-Free	SP (For biochemical research)	F	18188-04	10 mg

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Recombinant proteins

(Solution)

» **Quality control items: Sterility test, Endotoxin test**

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
Activin A Solution, Human, Recombinant	SP (For TC)	F	18585-36	10 µg
			18585-94	50 µg
			18585-81	1 mg
bFGF (basic FGF) Solution, Human, Recombinant (154 a.a.), Animal-Free	SP (For TC)	F	19155-07	10 µg
			19155-36	5 x 10 µg
			19155-81	1 mg
SCF Solution, Human, Recombinant	SP (For TC)	F	21065-04	10 µg
			21065-46	50 µg
			21065-91	1 mg
KGF (FGF-7) Solution, Human, Recombinant	SP (For TC)	F	21185-34	10 µg
			21185-76	50 µg
			21185-21	1 mg
VEGF165 Solution, Human, Recombinant	SP (For TC)	F	21957-24	10 µg
			21957-66	50 µg
			21957-11	1 mg
BMP-4 Solution, Human, Recombinant	SP (For TC)	F	22247-94	10 µg
			22247-36	50 µg
			22247-81	1 mg

(Powder)

» **Quality control items:** refer to the specification sheets for each product.

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
Albumin, Human, Recombinant expressed in Rice, Animal-Free	SP (For TC)	F	19597-01	1 g
			19597-14	5 g
Insulin, Human, Recombinant expressed in Yeast, Animal-Free	SP (For TC)	F	12878-86	50 mg
			12878-44	250 mg
Transferrin, Human, Recombinant expressed in Rice, Animal-Free	SP (For TC)	R	12879-34	100 mg
			12879-21	1 g
			12879-76	5 g

Proteins

(Powder)

» **Quality control items:** refer to the specification sheets for each product.

Product List and Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
apo-Transferrin from Human	SP (For TC)	R	34401-84	100 mg
			34401-55	500 mg
Albumin, Bovine Serum, Low Endotoxin, pH5.2	SP (For biochemical research)	R	01861-84	10 g
			01861-97	100 mg
Albumin, Bovine Serum, Fatty Acid Free, pH7.0	SP (For biochemical research)	R	08587-26	10 g
			08587-42	25 g
			08587-84	50 g

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Reagents for Cell Dissociation

» **Quality control items: pH, Sterility test, Mycoplasma test, Enzyme actinity (USP unit)**

Product List and Ordering Information

Product Name	Phenol Red	Buffer	Grade	Storage	Product No.	PKG size
0.5g/l-Trypsin/0.53mmol/l-EDTA Solution	-	HBSS(-)	SP (For TC)	F	35553-74	100 mL
0.5g/l-Trypsin/0.53mmol/l-EDTA Solution, with Phenol Red	○	HBSS(-)	SP (For TC)	F	32778-34	100 mL
					32778-05	500 mL
2.5g/l-Trypsin/1mmol/l-EDTA Solution	-	HBSS(-)	SP (For TC)	F	35554-64	100 mL
2.5g/l-Trypsin/1mmol/l-EDTA Solution, with Phenol Red	○	HBSS(-)	SP (For TC)	F	32777-44	100 mL
					32777-15	500 mL
5.0g/l-Trypsin/5.3mmol/l-EDTA Solution	-	HBSS(-)	SP (For TC)	F	35556-44	100 mL
2.5g/l-Trypsin Solution	-	HBSS(-)	SP (For TC)	F	35555-54	100 mL
25g/l-Trypsin Solution	-	Saline	SP (For TC)	F	18172-94	100 mL
0.2g/l-EDTA Solution	-	PBS(-)	SP (For TC)	R	14367-74	100 mL
0.5mmol/l-EDTA/PBS Solution	-	PBS(-)	SP (For TC)	RT	13567-84	100 mL
Accutase	○	PBS(-)	SP (For TC)	F	12679-54	100 mL
Accumax	-	PBS(-)	SP (For TC)	F	17087-54	100 mL

Antibiotics

Product List and Ordering Information

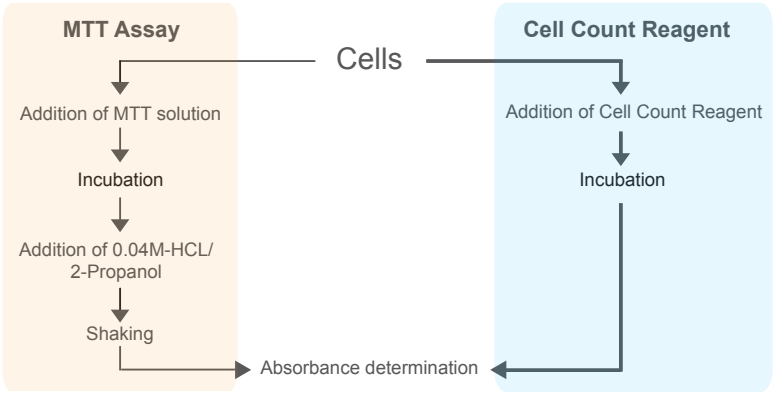
Product Name	Composition	Application	Grade	Storage	Product No.	PKG size
Actinomycin D Solution(1mg/mL)	1 mg/mL	Other	SP (For TC)	F	00393-41	1 mL
Antibiotic-Antimycotic Mixed Stock Solution(100x)	Penicillin 10,000 u/mL Streptomycin Potency: 10,000 µg/mL Amphotericin B Potency: 25 µg/mL	Bacteria, Fungi, Yeast	SP (For TC)	F	02892-54	100 mL
Antibiotic-Antimycotic Mixed Stock Solution(100x)(Stabilized)			SP (For TC)	F	09366-44	100 mL
G 418 Disulfate	-	Selection Antibiotics	SP (For TC)	RT	08973-01	1 g
					08973-14	5 g
					08973-72	25 g
G 418 Disulfate Aqueous Solution	Potency: 50 mg/mL		SP (For TC)	R	09380-86	20 mL
					09380-44	100 mL
G 418 Disulfate	-		SP (For TC)	RT	08973-01	1 g
					08973-14	5 g
G 418 Disulfate Aqueous Solution	Potency: 50 mg/mL		SP (For TC)	R	09380-86	20 mL
					09380-44	100 mL
Gentamicin Sulfate	-	Bacteria, Mycoplasma	SP (For TC)	R	08975-81	1 g
					08975-94	5 g
Gentamicin Sulfate Solution(10mg/mL)	Potency: 10 mg/mL		SP (For TC)	R	16672-04	10 mL
Gentamicin Sulfate Solution(50mg/mL)	Potency: 50 mg/mL		SP (For TC)	R	11980-14	10 mL
Hygromycin B	-	Selection Antibiotics	SP (For biochemical research)	R	07296-66	100 mg
					07296-11	1 g
					07296-24	5 g
Hygromycin B Solution	Potency: 50 mg/mL		SP (For TC)	R	09287-84	20 mL
Kanamycin Monosulfate	-	Bacteria, Mycoplasma	SP (For TC)	RT	08976-71	1 g
					08976-84	5 g
Kanamycin Sulfate Solution(50mg/mL)	Potency: 50 mg/mL		SP (For TC)	R	11981-04	20 mL
Mitomycin C Solution(1mg/mL)	1 mg/mL	Other	SP (For TC)	F	20898-21	1 mL
Penicillin-Streptomycin Mixed Solution	Penicillin 10,000 u/mL Streptomycin Potency: 10,000 µg/mL	Bacteria	SP (For TC)	F	26253-84	100 mL
Penicillin-Streptomycin Mixed Solution (Stabilized)			SP (For TC)	F	09367-34	100 mL
Penicillin-Streptomycin Mixed Solution	Penicillin 5,000 u/mL Streptomycin Potency: 5,000 µg/mL		SP (For TC)	F	26252-94	100 mL
Penicillin-Streptomycin-Glutamine Mixed Solution	Penicillin 10,000 u/mL Streptomycin Potency: 10,000 µg/mL L-Glutamine 29.2 mg/mL		SP (For TC)	F	06168-34	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

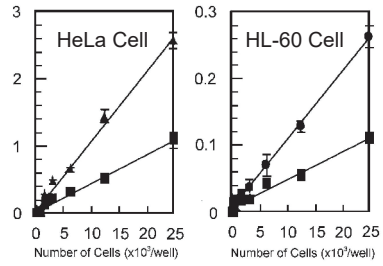
Cell Count Reagent SF, Based on WST-8

» **More sensitivity than other water-soluble tetrazolium salts, such as XTT and MTS**
» **No radioisotope**
» **Ready-to-use**

Comparison of assay procedure with MTT and Cell Count Reagent SF



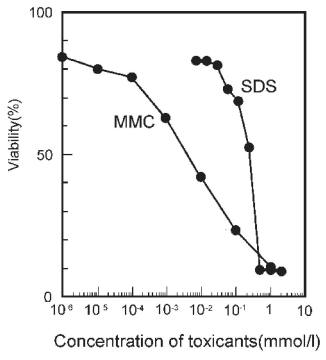
Application for cell proliferation assay



1. Prepare a cell suspension using an appropriate culture media, and dispense 100 µl of cell suspension into each well of a 96-well plate after counting cells.
2. Pre-incubate the plate in CO_2 incubator.
3. Add 10 µl of Cell Count Reagent SF to each well.
4. Incubate the plate for 1-4 hours in the CO_2 incubator.
5. Measure the absorbance at 450 nm (calibration wavelength: 600 nm or more) by micro plate reader.

Incubation
Hela cells : 5% CO_2, 37ºC, 1 hour (■), 2 hours (▲)
HL-60 : 5% CO_2, 37ºC, 1 hour (■), 3 hours (●)

Application for cytotoxicity assay



1. Prepare a cell suspension with 5,000 cells/well using an appropriate culture media, and dispense 100 µl of cell suspension into each well of a 96-well plate after counting cells.
2. Pre-incubate the plate in CO_2 incubator for 24 hours.
3. Add 10 µl of a compoud prepared to appropriate concentration into each well.
4. Incubate the plate for 48 hours in the CO_2 incubator.
5. Add 10 µl of Cell Count Reagent SF to each well.
6. Incubate the plate for 1-4 hours in the incubator.
7. Measure the absorbance at 450 nm (calibration wavelength: 600 nm or more) by micro plate reader.

Cell : Hela cells in DMEM (10% FCS)
Compounds applied : MMC (Mitomycin C)
SDS (Sodium Dodecylsulfate)
Treatment / incubation period : 5% CO_2, 37ºC, 48 hours / 5% CO_2, 37ºC, 2 hours
Wavelength : 450 nm (reference: 650 nm)

References

M. Ishiyama, Y. Miyazono, K. Sasamoto, Y. Ohkura, K. Ueno, *Talanta*, 44, 1299 (1997)
H. Tominaga, M. Ishiyama, F. Ohseto, K. Sasamoto, T. Hamamoto, K. Suzuki and M. Watanabe, *Anal. Commun*, 36 (2), 47 (1999)

Ordering Information

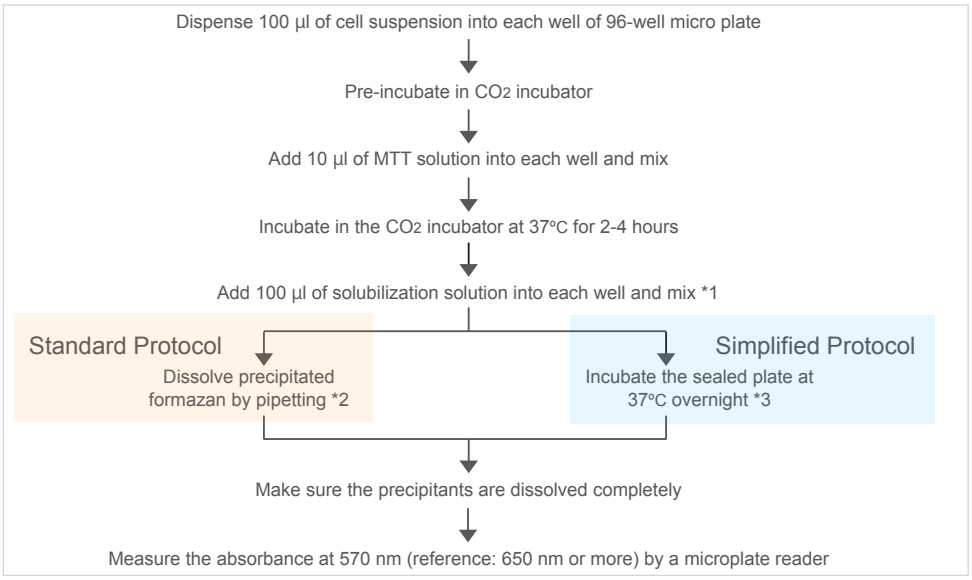
Product Name	Storage	Product No.	PKG Size
Cell Count Reagent SF	R	07553-15	500 tests
		07553-44	2500 tests

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

MTT Cell Count Kit, Based on Reduction of MTT

» **No radioisotope**
» **Ready-to-use**

Cell proliferation assay procedure



*1 Mix well Solubilization Solution and media as serum proteins might appear as precipitants.
*2 Aviod hard pipetting and shaking for a long time as that might help Solubilization Solution volatilize and affect the assay result.
*3 Make sure the plate is sealed completely. Alternatively, use a CO_2 incubator at 37ºC.

Ordering Information

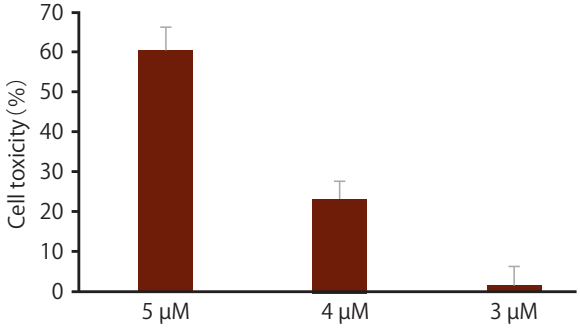
Product Name	Storage	Product No.	PKG Size
MTT Cell Count Kit	F	23506-80	1 KIT

LDH Cytotoxicity Assay Kit

» **Economical packaging size (100 and 500-test sizes available)**
» **Ready-to-use**
» **Storable at 4ºC for 3 months and at -20ºC for 2 years without loss performance**
» **By using cell suspension, enables unique assays with variety of other assays**

Application

Cell damage assay with melittin



Add represented concentration of melittin to 24-hour-cultured B16/BL6 cell (1 x 104 cells/well), then assay after 30-minute incubation. Substrate solution was directly added to each micro well. The higher concentration of melittin added, the stronger cell toxicity observed.

Date courtesy of Dr. Takuma Yamashita, Department of Biopharmaceutics and Drug Metabolism, Kyoto University.

Ordering Information

Product Name	Storage	Product No.	PKG Size
LDH Cytotoxicity Assay Kit	F	18250-64	100 tests
		18250-35	500 tests

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Trypan Blue Solution

» **Ready-to-use**

Ordering Information

Product Name	Storage	Product No.	PKG Size
Trypan Blue Solution	RT	20577-34	100 mL

Reagents for Apoptosis Research

Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
Annexin V-633 Apoptosis Detection Kit	SP (For fluorescence analysis)	R	18146-44	100 tests
Annexin V-FITC Apoptosis Detection Kit	SP (For fluorescence analysis)	R	15342-54	100 tests
Actinomycin D Solution (1mg/mL)	SP (For TC)	F	00393-41	1 mL
Colcemid Solution (10μg/mL)	SP (For TC)	R	09356-74	10 mL
Mitomycin C Solution (1mg/mL)	SP (For TC)	F	20898-21	1 mL

Reagents for Alkaline Phosphatase Staining

Ordering Information

Product Name	Grade	Storage	Product No.	PKG size
BCIP-NBT Solution (Ready To Use)	SP (Reagents for alkaline phosphatase staining)	R	19880-84	100 mL
4%-Paraformaldehyde Phosphate Buffer Solution	SP (For histochemistry research)	R	09154-14 09154-56 09154-85	5 x 10 mL 100 mL 500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Medium for Bacteria, Plusgrow II

Plusgrow II is high performance medium for bacteria that offers easy procedures for weighing, dissolving and autoclave treatments.

» **Higher fungus density than conventional products**
» **High plasmid collection**

Comparison with conventional products

Bacteria growth test

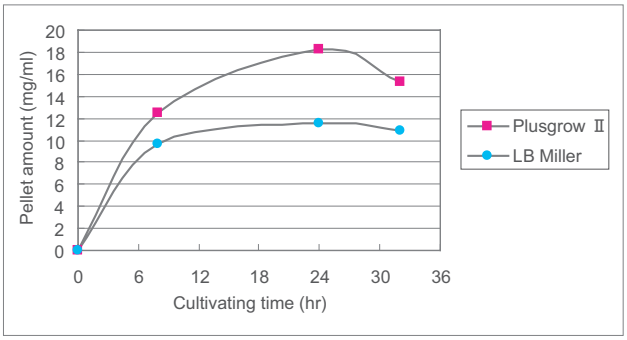


Figure 1. Bacteria growth curve
E. coli is first cultivated in ampicillin (50 µg/mL) then added to the medium at 37ºC and shaken. Then culture fluid is then centrifugally processed. Bacteria levels can then be evaluated by pellet amounts.
E. coli cell line : JM109
Plasmid : pGEM-3zf(+)

Plasmid collection test

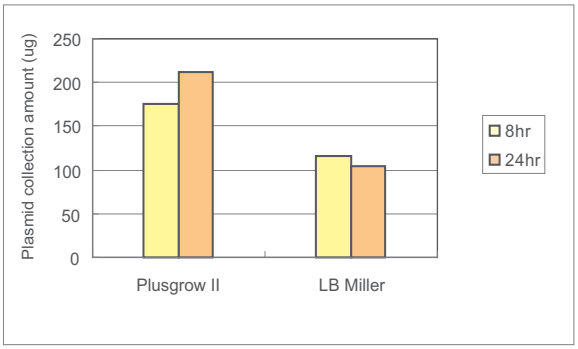


Figure 2. Plasmid collection amount (per 1mL of culture fluid)
Plasmid is collected and purified via the alkali-SDS method (containing phenol extract) following 8 and 24 hours of *E. coli* cultivation.

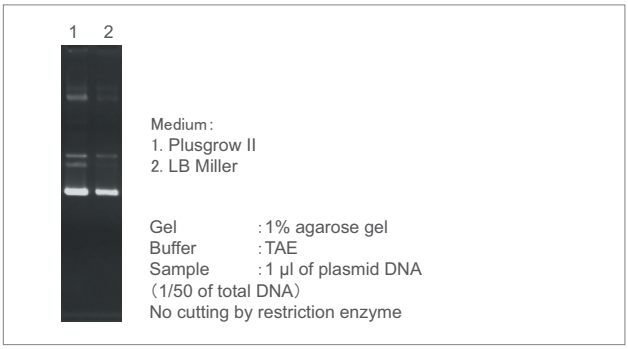


Figure 3. Electrophosis image of collection of plasmid (following 24 hours of cultivation)

Ordering Information

Product Name	Storage	Product No.	PKG Size
Plusgrow II (One package for 1L) Dissolve → Autoclave at 121°C for 15 minutes	RT	08246-86 08246-44	40 g 10 x 40 g
Plusgrow II Weigh out 40 g → Dissolve in 1L → Autoclave at 121°C for 15 minutes	RT	08202-04 08202-75	100 g 500 g

Related Products

Product Name	Storage	Product No.	PKG Size
LB Agar, Lennox	RT	20067-85	500 g
LB Agar, Miller	RT	20069-65	500 g
LB Broth, Lennox	RT	20066-95 20066-24	500 g 2 kg
LB Broth, Miller	RT	20068-75	500 g
Agar, powder	RT	01028-85	500 g
Agar Purified, powder	RT	01162-15	500 g
Extract Yeast Dried	RT	15838-45	500 g
Tryptone	RT	35640-95	500 g

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

IPTG and X-gal Solutions

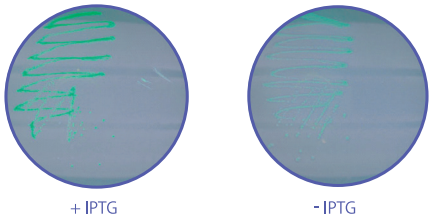
100mmol/l-Isopropyl-β-D-thiogalactopyranoside [IPTG] Solution

» **0.22 µm filtrated ready-to-use solution**
» **Sterilized**
» **No need to adjust concentration**
» **1 mL package size allows for easy application**

Application

Recombinant protein expressions are evaluated by the green fluorescent protein (GFP) expressing vector in *E. coli*.

Induced expression by IPTG



Numerous fluorescent proteins can be expressed by adding IPTG. In situations where IPTG is not applied, expressions occur at the basal level.

E. Coli : BL21 (DE3) pLysS (Novagen)
Vector : pQBI-T7-GFP (Q-Bio gene)
IPTG concentration : 1mM
Detection : Transilluminator

5-Bromo-4-chloro-3-indolyl-β-D-galactoside [X-gal] Solution (20 mg/mL)

5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) is widely used for Blue-white selection.

» **Ready-to-use DMF solution**
» **1 mL package size allows for easy application**

Ordering Information

Product Name	Storage	Product No.	PKG Size
100mmol/l-Isopropyl-β-D-thiogalactopyranoside [IPTG] Solution	F	07496-91	10 x 1 mL
5-Bromo-4-chloro-3-indolyl-β-D-galactoside Solution(20 mg/mL)	F	03971-71	10 x 1 mL

Related product

Product Name	Storage	Product No.	PKG Size
Isopropyl-β-D-thiogalactopyranoside [IPTG], Dioxane free	R	19742-36	100 mg
		19742-81	1 g
		19742-94	10 g
5-Bromo-4-chloro-3-indolyl-α-D-galactoside [X-α-Gal]	R	02897-04	100 mg
5-Bromo-4-chloro-3-indolyl-β-D-galactoside [X-Gal]	R	05627-86	10 mg
		05627-57	100 mg
		05627-31	1 g
		05627-44	5 g
		05644-14	5 x 20 mg
5-Bromo-4-chloro-3-indolyl-β-D-glucuronide Cyclohexylammonium Salt	F	05646-94	10 mg
		05646-36	100 mg

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Gelling Agent for Plant Study: Gellan Gum

» **High transparency**

Comparison data with agar gel: Root growth observation

0.8% Agar Gel

0.2% Gellan Gum

These photos were taken on the 6th day.
Temperature : 27 °C
Light Period : 13 hours
Dark Period : 11 hours

Seeds of Komatsuna (Brassica rapa var. perviridis) were incubated in petri dishes containing a MS medium with either 0.8% agar or 0.2% Gellan Gum under sterile conditions. The seedlings were transferred to plant boxes containing the same medium 5 days later.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Gellan Gum	RT	12389-96	50 g
		12389-54	250 g

[Storage] RT = Room Temperature,_ R = Refrigerator, F = Freezer

Zymolyase™ (from Arthrobacter Luteus)

Zymolyase™, produced by a submerged culture of Arthrobacter luteus[1], has strong lytic activity against living yeast cell walls[2],[3] to produce protoplast or spheroplast of various strains of yeast cells. An essential enzyme for the lytic activity of Zymolyase™ is β-1,3-glucan laminaripentaohydrolase. It hydrolyzes linear glucose polymers with β-1,3-linkages and releases specifically laminaripentaose as the main and minimum product unit[4], [5], [10], [11]. There are two preparations of Zymolyase™, Zymolyase™-20T and Zymolyase™-100T, having lytic activity of 20,000 units/g and 100,000 units/g respectively. Zymolyase™-20T is ammonium sulfate precipitate while Zymolyase™-100T is a further purified preparation by affinity chromatography[9]. Lytic activity varies depending on yeast strain, growth stage of yeast, or cultural conditions[6-8]. Further information related to Zymolyase™ can be obtained in the reference section below[12-16].

Specifications

Product Name		Zymolyase™-20T	Zymolyase™-100T
Form		Lyophilized Powder	
Purification		Ammonium Sulfate Precipitation	Affinity Chromatography
Activity		20,000 units/g	100,000 units/g
Essential enzyme		β-1,3-glucan laminaripentaohydrolase	
Other activities contained(*1)	β-1,3-glucanase	approx. 1.5 x 10^6 units/g	approx. 1.0 x 10^7 units/g
	protease	approx. 1.0 x 10^4 units/g	approx. 1.7 x 10^4 units/g
	mannanase	approx. 1.0 x 10^6 units/g	approx. 6.0 x 10^4 units/g
Contaminants	Amylase, Xylanase, Phosphatase	Trace amount	Not detectable
Optimum pH and Temp.		pH7.5, 35°C (for lysis of viable yeast cells) pH6.5, 45°C (for hydrolysis of yeast glucan)	
Stability	2°C	No loss of activity was found after storage for 1 year	
Heat stability	30°C	70% of the lytic activity is lost after storage for 3 months	90% of the lytic activity is lost after storage for 3 months
	60°C	Lytic activity is lost on incubation for 5 minutes	
Specificity (Lytic Spectrum)		*Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Kloeckera, kluyveromyces, Lipomyces, Metschnikowia, Pichia, Pullularia, Torulopsis, Saccharomyces, Saccharomycopsis, Saccharomycodes, Schwanniomyces, etc.*	

(*1) See reference, Kitamura, K., Kaneko, T., Yamamoto, Y., J. Gen. *Appl. Microbiol.*, **18**, 57 (1972) as to the definition of each enzyme units.

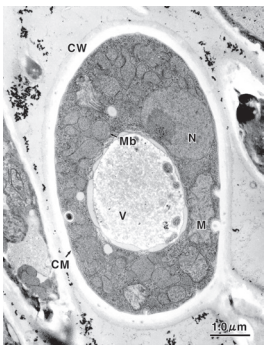
Unit Definition

One unit of lytic activity is defined as that amount which indicates 30% of decrease in absorbance at 800 nm (A_{800}) of the reaction mixture under the following condition.

[Reaction Mixture]

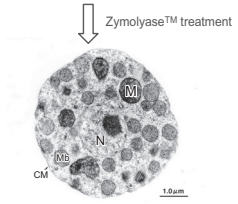
Enzyme solution	: 1 mL (0.05-0.1 mg/mL for Zymolyase™-20T) (0.012-0.024 mg/mL for Zymolyase™-100T)
Brewer's yeast cell suspension	: 3 mL (2 mg/mL)
1/15M Phosphate buffer	: 5 mL (pH7.5)
Distilled water	: 1 mL

After incubation for 2 hours at 25°C with gentle shaking, A_{800} of the mixture is determined. When 60% of A_{800} decrease, equivalent to 2 units, is observed in the reaction system, the brewer's yeast cells are completely lysed, namely 1 unit of Zymolyase™ lyses 3 mg dry weight of brewer's yeast.



Electron microscopical photo of yeast cell (Candida tropicalis)

CW : Cell Wall Mb : Microbody
CM : Cell Membrane N : Nucleus
M : Mitochondria V : Vacuole



Data courtesy of Masako Osumi, Emeritus Professor at Nippon Women's University

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13. Iizuka Masaru, Torii Yasuhiko, Yamamoto Takehiko: *Agric. Biol. Chem.*, **47** (12), 2267 (1983)
14. Shibata Nobuyuki, Kobayashi Hidemitsu, tojo Menehiro, Suzuki Shigeo: *Arch. Biochem. Biophys.*, **251** (2), 697 (1986)
15. Iijima Y., Yanagi S. O.: *Agric. Biol. Chem.*, **50** (7), 1855 (1986)
16. Herrero Enrique, Sanz Pascual. Sentandreu Rafael: *J. Gen. Microbiol.*, **133** (10), 2895 (1987)

Ordering Information

Product Name	Storage	Product No.	PKG Size
Zymolyase™ 20T	R	07663-91	1 g
Zymolyase™ 100T	R	07665-55	500 mg

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Cell Lysis Solution: Cell Lysis Buffer

Cell Lysis Buffer is designed for protein extraction, similar to RIPA Buffer, and is available in two forms: a ready-to-use type and a 10x concentrated type. The 10x concentrated type is packaged separately with SDS solution, which may adversely affect immunoprecipitation.

» **Available for protein extraction in the same way as RIPA Buffer**
» **Does not include OPE/NPE (e.g., NP-40), substances regulated under REACH**
» **Available in two forms**

Lineup

Available in two forms as below.

Product name	Cell Lysis Buffer	Cell Lysis Buffer (10x)
Product type	1x (ready to use)	10x
Protease inhibitor cocktail	Not included	Included
SDS	Included	Not included (10x SDS solution is packed separately)
Storage	Refrigerator	Freezer

Comparison

Protein extraction by using Cell Lysis Buffer or Company A's product

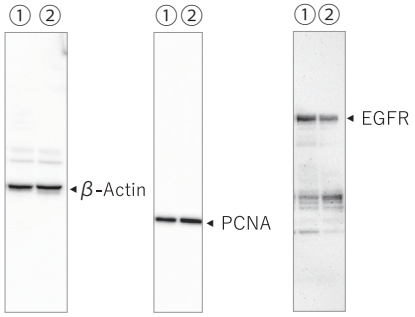
CBB staining

Protein was extracted from rat kidney by using this product or Company A's, then subjected to electrophoresis, and CBB staining.The result shows protein extraction efficiency of this product is equal to that of Thermo's RIPA Buffer.

<Conditions>
Sample : ① Protein extracted by using this product (15 µg)
② Protein extracted by using Company A's product (15 µg)
③ Protein Ladder One Plus, Triple-color for SDS-PAGE (#19593-25)
SDS-PAGE : Bullet PAGE Plus Precast Gel, 5-15%, 13wells (#21791-84) 400 V
Staining : Bullet CBB Stain Lite (#21964)

Western blotting

Protein was extracted from mouse liver by using this product or Company A's, then subjected to western blotting. The results show that this product is applicable to western blotting, similar to Company A's.



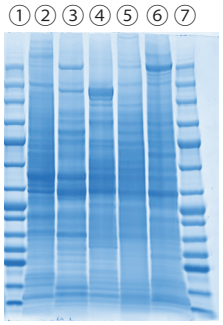
<Conditions>
Sample : ① Protein extracted by using this product
② Protein extracted by using Company A's product
SDS-PAGE : Bullet PAGE Plus Precast Gel, 5-15%, 13wells (#21791-84) 400 V
Transfer : Bullet Semi-dry Transfer One (#15353-01) 25 V
Blocking : Bullet Blocking One for Western Blotting (#13779)
Primary antibody : [β-Actin] β-Actin antibody (C4) (Santa Cruz #sc-47778) 1,000x diluted
[PCNA] PCNA antibody (Novus #NB100-456) 1,000x diluted
[EGFR] EGFR antibody (CST #4267) 2,000x diluted
Secondary antibody : [β-Actin] Anti-Mouse IgG HRP (#21860) 20,000x diluted
[PCNA] Anti-Rabbit IgG HRP (#21858) 20,000x diluted
[EGFR] Anti-Rabbit IgG HRP (#21858) 20,000x diluted
Detection : Chemi-Lumi One Super (#02230)

Applications

Protein extraction by using Cell Lysis Buffer (10x)

CBB staining

Protein was extracted from various mouse organs by using this product with SDS, subject to electrophoresis and CBB staining.

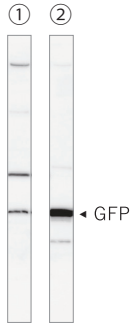


<Conditions>

Sample	: ①, ⑦ Protein Ladder One Plus, Triple-color for SDS-PAGE (#19593-25)
	② Protein extracted from brain (12 µg)
	③ Protein extracted from heart (12 µg)
	④ Protein extracted from liver (12 µg)
	⑤ Protein extracted from kidney (12 µg)
	⑥ Protein extracted from stomach (12 µg)
SDS-PAGE	: Bullet PAGE Plus Precast Gel, 5-15%, 13wells (#21791-84) 400 V
Stain	: Bullet CBB Stain Lite (#21964)

Immunoprecipitation

Protein was extracted from GFP-expressing HEK293 cells using this product without SDS and immunoprecipitated using rat anti-GFP antibody-conjugated agarose beads. GFP was detected by western blotting of the samples before and after immunoprecipitation. GFP in the samples before and after immunoprecipitation was detected by western blotting.



<Conditions>

Sample	: ① Sample before immunoprecipitation (3 µL)
	② Sample after immunoprecipitation (3 µL)
SDS-PAGE	: Bullet PAGE Plus Precast Gel, 5-15%, 13wells (#21791-84) 400 V
Transfer	: Bullet Semi-dry Transfer One (#15353-01) 25 V
Blocking	: Bullet Blocking One for Western Blotting (#13779)
Primary antibody	: Anti-GFP (Mouse IgG1-κ) (#04363) 5,000x diluted
Secondary antibody	: Anti-Mouse IgG HRP (#21860) 20,000x diluted
Detection	: Chemi-Lumi One Super (#02230)

Ordering Information

Product Name	Storage	Product No.	PKG Size
Cell Lysis Buffer	R	22352-04	100 mL
Cell Lysis Buffer (10x)	F	22353-81	1 set

Related Products

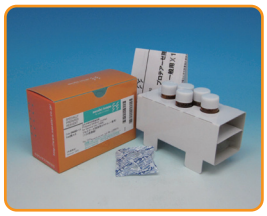
Product Name	Storage	Product No.	PKG Size
Protease Inhibitor Cocktail for General Use (100x)	F	04080-11	5 x 1 mL
Protease Inhibitor Cocktail (EDTA free) (100x)	F	03969-21	5 x 1 mL
Protease Inhibitor Cocktail for Use with Mammalian Cell and Tissue Extracts	F	25955-11	5 x 1 mL
Phosphatase Inhibitor Cocktail (EDTA free)	R	07575-51	1 mL
Phosphatase Inhibitor Cocktail	R	07574-61	1 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Protease Inhibitor Cocktail

Inhibition of intra and extra cellular proteases is vital to purify and collect the expressed proteins. Saving trouble of finding adequate inhibitors, a wide range of protease is inhibited by the Protease Inhibitor Cocktail.

» **Contains inhibitors for a variety of protease**
» **Available in 3 types: General use, Mammalian cell and tissue, and EDTA free**



Composition of each Protease Inhibitor Cocktail

Inhibitors	Target Protease	#04080-11	#03969-21	#25955-11
4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF)	Serin protease	✔	✔	✔
Aprotinin	Serin protease and Esterase	✔	✔	✔
E-64	Cysteine protease	✔	✔	✔
Leupeptin hemisulfate monohydrate	Cysteine protease and Trypsin-like protease	✔	✔	✔
Disodium dihydrogen ethylenediaminetetraacetate dihydrate	Metalloprotease	✔		
Bestatin	Aminopeptidase and Leucine aminopeptidase			✔
Pepstatin A	Aspartic protease			✔

Reference

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3. Iyama, T. *et al. Nucl. Acids Res.* **38**(14), 4834-4843 (2010)
4. Kimura, Y. *et al. Cancer Research* **70**(2), 501-511 (2010)
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6. Hagiwara B *et al. J. Biochem*., **45**, 185-194 (1958)

Ordering Information

Product Name	Storage	Product No.	PKG Size
Protease Inhibitor Cocktail for General Use (100x)	F	04080-11	5 x 1 mL
Protease Inhibitor Cocktail (EDTA free) (100x)	F	03969-21	5 x 1 mL
Protease Inhibitor Cocktail for Use with Mammalian Cell and Tissue Extracts	F	25955-11	5 x 1 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Phosphatase Inhibitor Cocktail

Phosphatase Inhibitor Cocktail is a mixture of several inhibitors to protect valuable proteins from dephosphorylation. The product preserves phosphorylated proteins existing in small quantity in cells and tissues.

» **Contains 6 kinds of phosphatase inhibitors for different targets**
» **100 times concentrated stock solution**
» **Compatible with protein assay**
» **Ready-to-use**

Composition of each Phosphatase Inhibitor Cocktail

Inhibitors	Target Phosphatase	#07575-51 EDTA free	#07574-61
Sodium orthovanadate (V)	Tyrosine phosphatase and Alkaline Phosphatase	✔	✔
Disodium molybdate (VI) dihydrate	Acid phosphatase	✔	✔
Sodium (+) -tartrate dihydrate	Acid phosphatase	✔	✔
Imidazole	Alkaline Phosphatase	✔	✔
Sodium fluoride	Acid phosphatase	✔	✔
b-Glycerophosphoric acid disodium salt	Serine-threonine phosphatase	✔	✔
tetra-Sodium ethylenediaminetetraacetate	Alkaline Phosphatase		✔

* 100 times concentrated aqueous solution

Comparison data

Figure 1.
The detection of phosphorylated proteins in Hela cells with Anti-p-Thr antibody

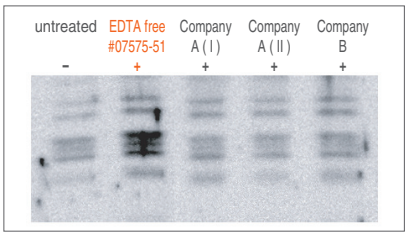


Figure 2.
The phosphatase inhibition efficiency assayed by fluorescence labeled p-Tyr peptide substrate

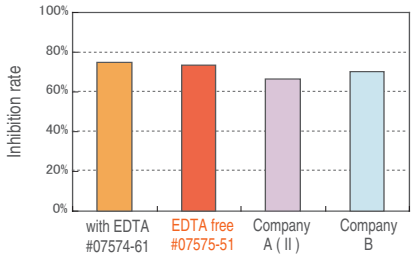
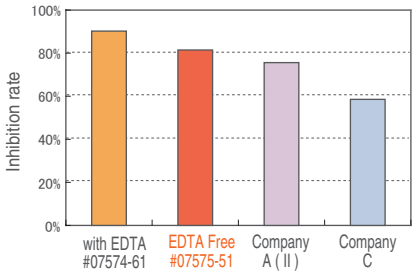


Figure 3.
The phosphatase inhibition efficiency assayed using p-nitrophenylphosphoric acid



Reference

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2. Selamat, W. *et al. Neuroscience Letters* **450**(2), 163-166 (2009)
3. Saito, T. *et al. Biochemical and Biophysical Research Communications* **357**(2), 371-376 (2007)
4. Murakami, Y. *et al. J. Biochem.*, **141**, 401-410 (2007)
5. Takenaga, M. *et al. J. Cell Sci.*, **120**, 2078-2090 (2007)

Ordering Information

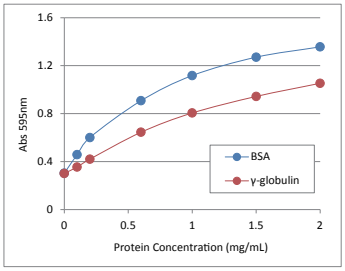
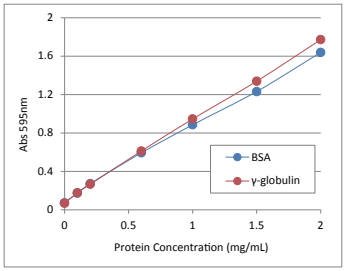
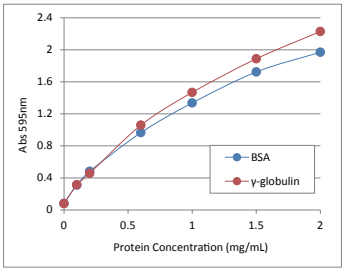
Product Name	Storage	Product No.	PKG Size
Phosphatase Inhibitor Cocktail(EDTA free)	R	07575-51	1 mL
Phosphatase Inhibitor Cocktail	R	07574-61	1 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Determination of Protein Concentration: Protein Assay

The protein assay is one of the most important key techniques in proteomics. To determine protein concentration, three products with spectrophotometric method are available as follows.

Comparison of each method

Assay Method	Bradford	BCA	Lowry
Product Name	Protein Assay CBB Solution	Protein Assay BCA Kit	Protein Assay Lowry Kit
			
Linearity			
	+	+++	++
Convenience	+++	++	+
Absorbance	595 nm	562 nm	750 nm
Incompatible with	Detergents	Reducing agents	Reducing agents
	Condition: Mixing BSA (left: 0 mg/mL, right: 1 mg/mL) and each substance described in the column left below		
Incubate with Water			
Incubate with 0.1% SDS			
Incubate with 1 mM DTT			
Incubate with 0.1% SDS and 1 mM DTT			
Remarks	For protein samples containing detergents, BCA assay method or removal of detergents by CBB Clean Up Kit (Prod No. 11611) is helpful.	For protein samples containing reducing agents, BCA Kit with BCA Reductant Adaptable Reagent (Prod No. 21014) or the Bradford method is useful.	For protein samples containing reducing agents, the Bradford method is useful.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Protein Assay CBB Solution (Ready To Use)	R	11617-71	1 L
Protein Assay BCA Kit	RT	06385-00	1 KIT
Protein Assay Lowry Kit	RT	29470-60	1 KIT

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

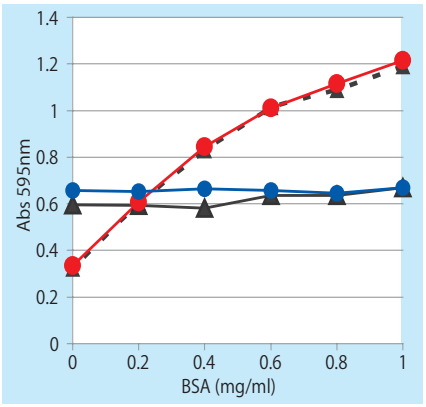
Protein Assay CBB Clean Up Kit

» **Removal interfering substances within 5 minutes**

» **Designed for the Bradford protein assay**

Comparison data of determination of BSA concentration with competitor's clean up kit

Protein Assay CBB Clean Up Kit is helpful to get rid of reducing agents and detergents that cause interfering with the Bradford assay, and enables better quantitative assays compared to the competitor's clean up kit.



Internal Control : BSA in water
No clean up : BSA in the Sample Buffer
Clean up with Nacalai's kit : BSA in the Sample Buffer
Clean up with Competitor's kit : BSA in the Sample Buffer

Sample Buffer Component:
62.5mM Tris-HCl pH6.8, 2%SDS, 10% Glycine, 5% 2-Mercaptoethanol, 0.005% BPB

Components

Reagent Name	Volume	Quantity
Solution A	2.5 mL	1 bottle
Solution B	2.5 mL	1 bottle
Solution C *Add 185 mL of ethanol (99.5%) into Solution C bottle, and mix it thoroughly	80 mL	2 bottles

Ordering Information

Product Name	Storage	Product No.	PKG Size
Protein Assay CBB Clean Up Kit	RT	11611-60	1 KIT
Protein Assay CBB Solution (Ready To Use)	R	11617-71	1 L
Albumin, Bovine Serum, Solution (2mg/mL) for Protein Assay	F	00653-31	10 x 1 mL

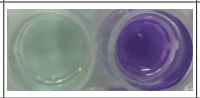
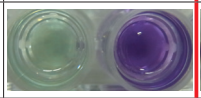
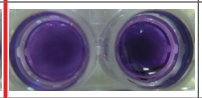
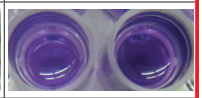
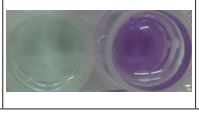
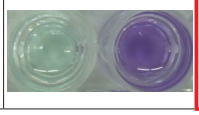
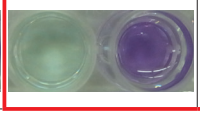
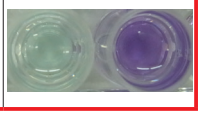
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Reductant Adaptable Reagent for Protein Assay BCA Kit

» **This product enables measurement of samples containing reducing agents**
» **Quick and easy: Pretreatment takes 15 mins. and measurements are completed in the same well**
» **Easy dispensing and homogenizing protocols**

Comparison

Measurement of samples containing reducing agents and surfactants

	PBS	0.1% SDS	1 mM DTT	0.1% SDS 1 mM DTT
BCA Kit				
Product pre-treatment operation + BCA Kit				

Effective for samples with both surfactants and reductants as well!

*Refer to the table of possible coexisting substances (instruction manual) for interfering substances that can be used in combination with reducing agents.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Protein Assay BCA Reductant Adaptable Reagent	R	21014-80	1 KIT
Protein Assay BCA Kit	RT	06385-00	1 KIT
Albumin, Bovine Serum, Solution(2mg/mL) for Protein Assay	F	00653-31	10 x 1 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

COSMOGEL® Ig-Accept Protein G

This product utilizes the binding of the Fc region of immunoglobulin (Ig) with Protein G, and is mainly used for the purification of IgG.

Specification

Carrier Name	COSMOGEL® Ig-Accept Protein G
Ligand	Protein G
Particle size	50 - 150 µm
Matrix	4% Cross-linked agarose
Recommended Line Velocity	26 cm/h
Limit pressure	3.6 psi (0.25 bar)
Supply condition	50 vol% suspension (20 vol% ethanol solution)

Application

COSMOGEL® Ig-Accept Protein G

Sample : Mouse serum (ammonium sulfate precipitation fraction)
Elution : Glycine - Hydrochloric acid buffer (pH 2.8)
Lane : 1. Protein Markers (10x) (#29458-24) in 1x
2. Sample purified by COSMOGEL® Ig-Accept Protein G

Ordering Information

Product Name	Storage	Product No.	PKG Size
COSMOGEL® Ig-Accept Protein G	R	02198-64	5 mL
		02198-22	25 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

COSMOGEL® GST-Accept

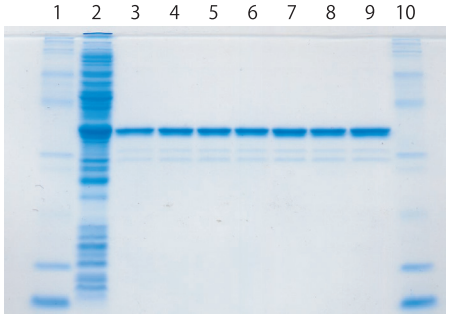
» **Superior durability**

» **Superior cost performance**

Application

10 consecutive purifications of GST-tag fusion proteins

We performed 10 consecutive purifications of GST-His fusion proteins by using the same resin from *E. coli* lysate. The 10th purification was also successful.



Lane:

1. Use Protein Markers(10x)(#29458-24)1x
2. E. coli lysate
3. 1st elution fraction
4. 2nd elution fraction
5. 3rd elution fraction
6. 4th elution fraction
7. 6th elution fraction
8. 8th elution fraction
9. 10th elution fraction
10. Use Protein Markers(10x)(#29458-24)1x

Sample : *E. coli* (BL21(DE3)pLysS) lysate transformed with GST-His(pET-41b(+))
Binding buffer : Use Phosphate Buffered Saline(10x)(pH 7.4)(#27575-31)1x
Elution buffer : 10 mM Glutathione, 50 mM Tris-HCl, pH 8.0

Ordering Information

Product Name	Storage	Product No.	PKG Size
COSMOGEL® GST-Accept	R	09277-56	5 mL
		09277-72	25 mL
		09277-14	100 mL

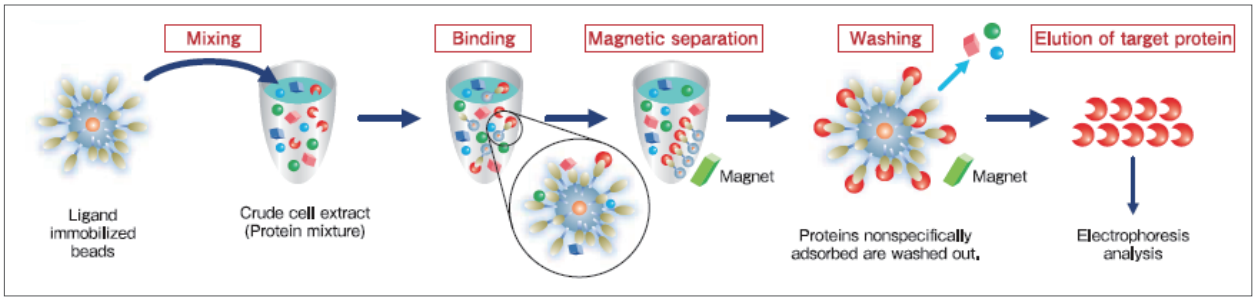
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

High Performance Magnetic Nanoparticles: FG beads

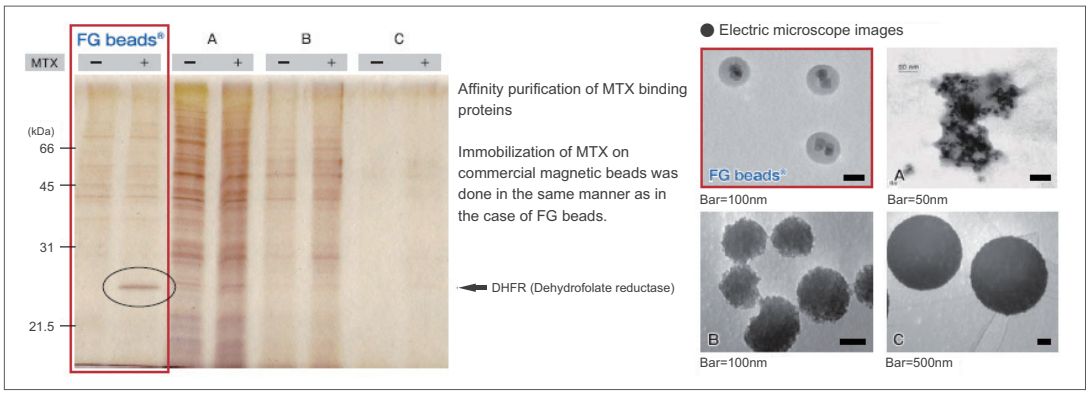
» **Excellent recovery of target proteins**
» **Extremely low non-specific binding**
» **High stability in organic solvents**

The FG beads developed by Tokyo Institute of Technology consists of ferrite nanoparticles coated firmly with a polymer layer and its diameter is approx. 200 nm. The FG beads are used as carriers for affinity purification of target proteins.[1)]

Purification process



Comparison with other magnetic beads [2)]



1. *S. Sakamoto et al., Chem. Rec. 9 (2009) 66*
2. *K. Nishio et al., Colloids Surfaces. B. 64 (2008) 162*

All product names, trademarks, and registered trademarks are the property of their respective owners. Use of these names does not imply any affiliation or endorsement.

Ordering Information

Product Name	Density	Storage	Product No.	PKG Size
Plain beads	20mg/mL	R	TAS8848N1010	0.5mL
Linker beads (Epoxy beads)	20mg/mL	R	TAS8848N1110	0.25mL
OH beads	20mg/mL	R	TAS8848N1120	0.25mL
NH2 beads	20mg/mL	R	TAS8848N1130	0.25mL
COOH beads	20mg/mL	R	TAS8848N1140	0.25mL
NHS beads	20mg/mL	F	TAS8848N1141	0.25mL
Ts beads	20mg/mL	R	TAS8848N1150	0.25mL
Azide beads	20mg/mL	R	TAS8848N1160	0.25mL
Alkyne beads	20mg/mL	R	TAS8848N1161	0.25mL
Streptavidin beads	20mg/mL	R	TAS8848N1170	0.25mL
NeutrAvidin beads	20mg/mL	R	TAS8848N1171	0.25mL
Protein A beads	20mg/mL	R	TAS8848N1172	0.25mL
Protein G beads	20mg/mL	R	TAS8848N1173	0.25mL
HM-Streptavidin beads	20mg/mL	R	TAB8848N3170	0.25mL
HM-NeutrAvidin beads	20mg/mL	R	TAB8848N3171	0.25mL
HM-Protein A beads	20mg/mL	R	TAB8848N3172	0.25mL
HM-Protein G beads	20mg/mL	R	TAB8848N3173	0.25mL
Magnetic Stand (for 1.5 mL tube)		RT	TAB4899N12	1ea
Magnetic Stand (for 15 mL tube)		RT	TAB4899N20	1ea
Magnetic Stand (for 50 mL tube)		RT	TAB4899N30	1ea
Magnetic Stand (for PCR tube)		RT	TAB4899N41	1ea

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

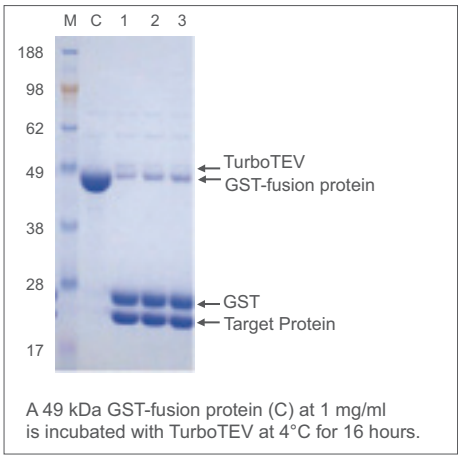
TurboTEV Protease & Turbo3C Protease

» **Both GST and His tags to facilitate its removal from the digested protein sample**

» **Activity over a broad temperature (4°C to 37°C) and pH (6.5 to 8.5) range**

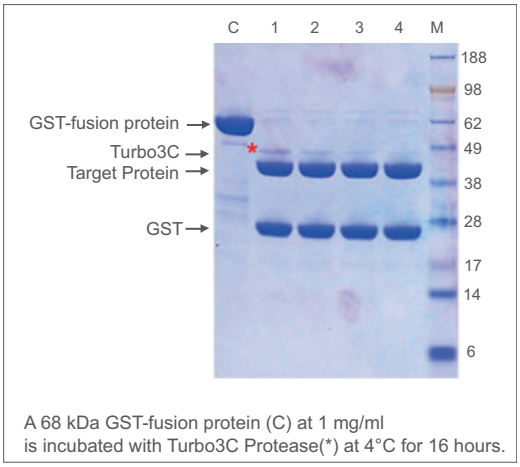
Application

TurboTEV Protease



A 49 kDa GST-fusion protein (C) at 1 mg/ml
is incubated with TurboTEV at 4°C for 16 hours.

Turbo3C Protease



A 68 kDa GST-fusion protein (C) at 1 mg/ml
is incubated with Turbo3C Protease(*) at 4°C for 16 hours.

GST-fusion protein (C) at 1 mg/mL is incubated with TurboTEV or Turbo3C Protease at a ratio of (1) 1:50, (2) 1:100, (3) 1:200 (w/w) in a buffer of 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM 2-mercaptoethanol at 4°C for 16 hours.

Specification

	TurboTEV Protease	Turbo3C Protease
Molecular weight	52 kDa	47 kDa
Cleavage site	Glu-Asn-Leu-Tyr-Phe-Gln↓Gly	Leu-Glu-Val-Leu-Phe-Gln↓Gly-Pro
Enzymatic activity	One unit cleaves >85% of 3 µg control substrate at 30°C for 1 h	One unit cleaves >95% of 100 µg control substrate at 4°C for 16 h
Concentration	20,000 units/mL	2,000 units/mL
Cleavage condition	A broad temperature (4°C to 37°C) and pH (6.5 to 8.5) range	
Formulation	25 mM Tris-HCl(pH8.0), 50 mM NaCl, 1 mM TCEP, 50% Glycerol	

Ordering Information

Product Name	Storage	Product No.	PKG Size
TurboTEV (TEV Protease) 2 mg/mL	F	NU0102S	1,000 units (0.1 mg)
		NU0102M	10,000 units (1 mg)
		NU0102L	100,000 units (10 mg)
Turbo3C (HRV3C Protease) 2 mg/mL	F	NU0101S	1,000 units (1 mg)
		NU0101M	10,000 units (10 mg)

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

TurboTEV Protease & Turbo3C Protease are produced by Nacalai USA.

[Ultra-fast Precast Gel]

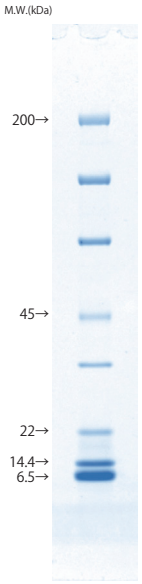
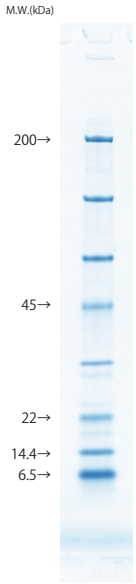
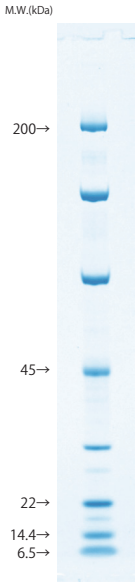
Bullet PAGE Plus Precast Gel

» **Only 10 minutes with 400 V**
» **Wide range of gel concentrations available**
» **Compatible with conventional Laemmli running buffer and sample buffer**
» **Surfactant-free, so can be used for nucleic acid analysis (refer to p11)**

*Please use 25 mM Tris and 192 mM glycine buffer for nucleic acid electrophoresis

Performance comparison

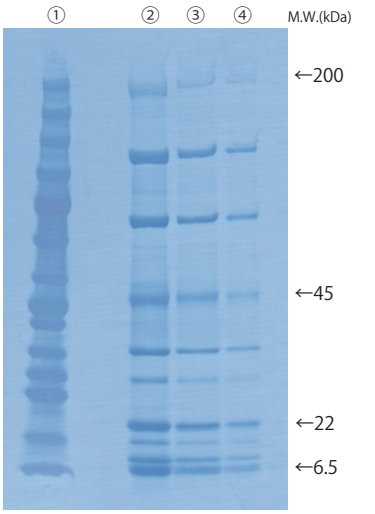
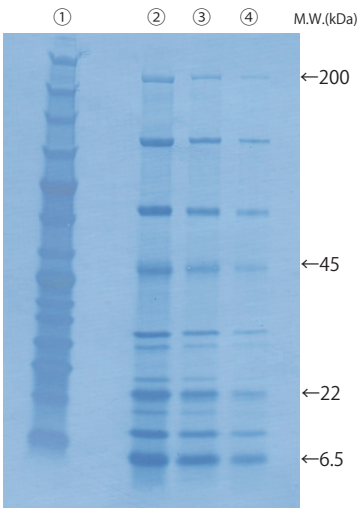
This product demonstrates separation ability and band sharpness comparable to competitors' gels, despite a short running time of about 10 minutes.

Product name	Bullet PAGE Plus	Company A	Company B
Gel %	5 - 20%	4 - 20 %	4 - 12%
Prod no.	21794-54	-	-
Running time	10 m 07 s	29 m 35 s	48 m 32 s
Separation image			

<Condition>
Sample : Protein Markers(10x)(# 29458-24), 3µl
Gel staining : CBB Stain One (#04543)
Voltage constant : Bullet PAGE Plus: 400V
Company A and Company B: 200V

Blotting performance: Comparison of protein transfer efficiency

This product shows a transfer efficiency equivalent to Company A's product.

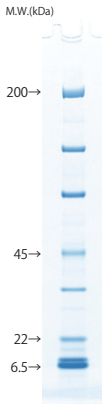
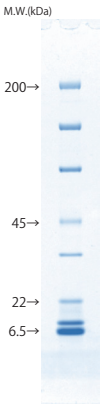
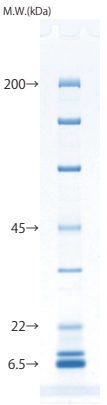
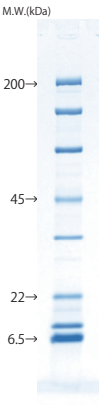
Transfer solution	Bullet Semi-dry Transfer One (#15353-01)	
Transfer condition	25 V, 10 min	
Gel	Bullet PAGE Plus 5 - 20 %	Company A 4 - 20 %
Image of membrane		

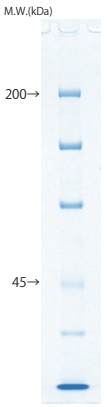
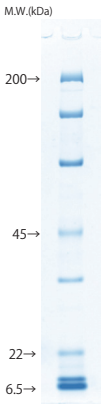
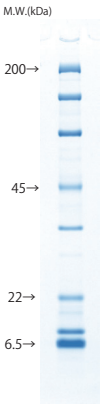
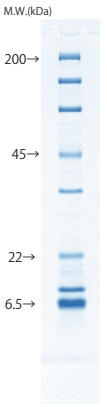
<Condition>

Sample : ① Protein Ladder One Plus, Triple-color for SDS-PAGE (#19593-25)
Dilute Protein Marker (10x) (#29458-24) to achieve ② 3x, ③ 1x, and ④ 1/3x, 2 µL of each.

Staining : Stain the PVDF membrane with CBB Stain One (Ready To Use) (#04543)

Gel types

Gel type	Gradient gel				
Gel %	5 - 10%	5 - 15%	5 - 20 %	7.5 - 15 %	10 - 20 %
Prod no. for 13-well for 17-well	21789-34 21790-94	21791-84 21792-74	21793-64 21794-54	21795-44 21796-34	21797-24 21798-14
Separation Image					

Gel type	Single-percentage gel			
Gel %	7.5%	10%	12.5%	15%
Prod no. for 13-well for 17-well	21799-04 21800-54	21801-44 21806-94	21807-84 21811-14	21853-74 21854-64
Separation image				

Specification

Glass plate size	: W100 mm x H80 mm x T3.2 mm (made of glass)
Gel size	: W80 mm x H60 mm x T1.0 mm
Sample well configuration / maximum load volume	: 13-well / 40 µl, 17-well / 28 µl
Shelf life	: 9 months

Ordering Information

Product Name	Storage	Product Number	PKG Size
Bullet PAGE Plus Precast Gel, 5-10%, 13wells	R	21789-34	10 Sheets
Bullet PAGE Plus Precast Gel, 5-10%, 17wells	R	21790-94	10 Sheets
Bullet PAGE Plus Precast Gel, 5-15%, 13wells	R	21791-84	10 Sheets
Bullet PAGE Plus Precast Gel, 5-15%, 17wells	R	21792-74	10 Sheets
Bullet PAGE Plus Precast Gel, 5-20%, 13wells	R	21793-64	10 Sheets
Bullet PAGE Plus Precast Gel, 5-20%, 17wells	R	21794-54	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5-15%, 13wells	R	21795-44	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5-15%, 17wells	R	21796-34	10 Sheets
Bullet PAGE Plus Precast Gel, 10-20%, 13wells	R	21797-24	10 Sheets
Bullet PAGE Plus Precast Gel, 10-20%, 17wells	R	21798-14	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5%, 13wells	R	21799-04	10 Sheets
Bullet PAGE Plus Precast Gel, 7.5%, 17wells	R	21800-54	10 Sheets
Bullet PAGE Plus Precast Gel, 10%, 13wells	R	21801-44	10 Sheets
Bullet PAGE Plus Precast Gel, 10%, 17wells	R	21806-94	10 Sheets
Bullet PAGE Plus Precast Gel, 12.5%, 13wells	R	21807-84	10 Sheets
Bullet PAGE Plus Precast Gel, 12.5%, 17wells	R	21811-14	10 Sheets
Bullet PAGE Plus Precast Gel, 15%, 13wells	R	21853-74	10 Sheets
Bullet PAGE Plus Precast Gel, 15%, 17wells	R	21854-64	10 Sheets

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

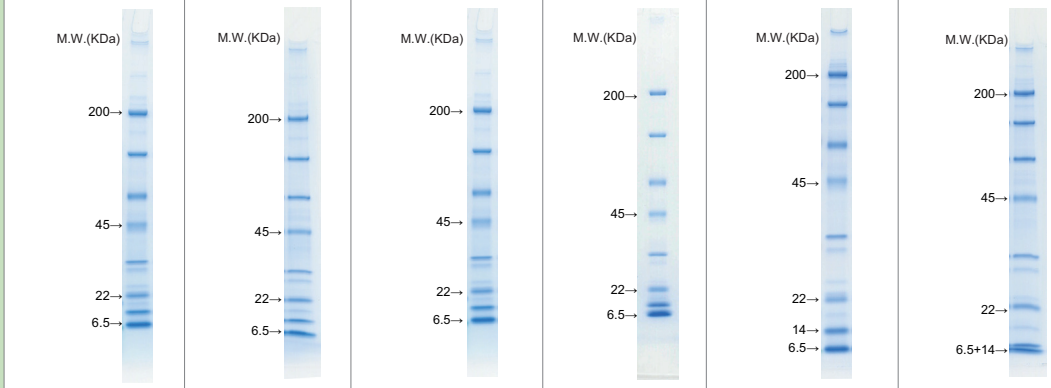
[General-use Precast Gel]

Extra PAGE One Precast Gel

» **Superior resolution**
» **Sharp bands**
» **High transfer efficiency**
» **Compatible with conventional Laemmli sample and running buffers**

Performance Comparison

Extra PAGE One Precast Gel offers higher resolution than other gels. Especially 5-20% gradient gel and 12.5% gel provide improved resolution for low molecular weight.

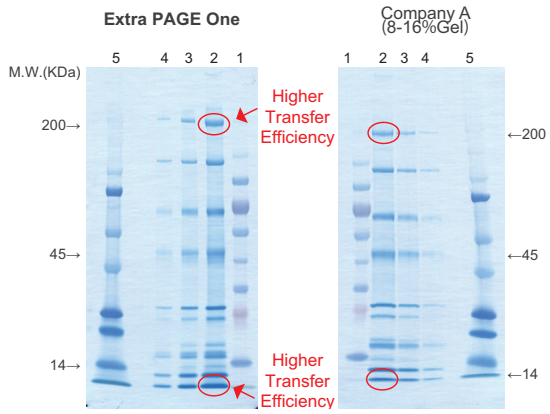
Gel	Gradient gel				Single-percentage gel	
Product name	Extra PAGE One	Company A	Extra PAGE One	Company B	Extra PAGE One	Company A
Gel%	5 - 20%	4 - 20%	5 - 20%	5 - 20%	12.5%	12.5%
Product no.	11955-54	-	11955-54	-	11965-24	-
Migration patterns						

<Condition>

Sample : Protein Markers (#29458-24) 5ul.
Staining : CBB Stain One (#04543)
Electrophoresis condition : Extra PAGE One Precast Gel: 300 V, approx. 50 min.
Company A: followed the manufacture's instructions

Comparison of transfer efficiency

Extra PAGE One Precast Gel offers higher transfer efficiency than Company A for both high and low molecular weight proteins.



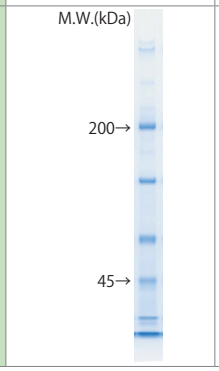
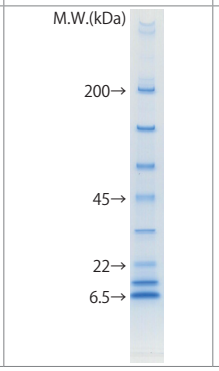
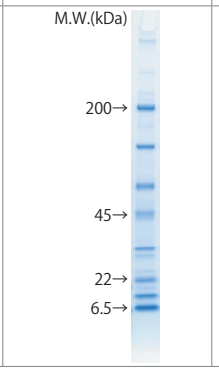
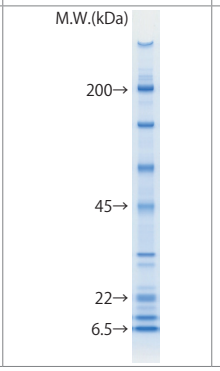
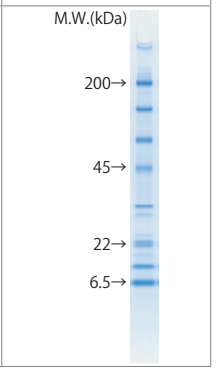
<Sample>
1. Protein ladder One (Triple-color) (Product No. 09547)
2. 2 ul of Protein Markers (3x)
3. 2 ul of Protein Markers (1x)
4. 2 ul of Protein Markers (1/3)
5. 10 ul of Bio-Rad Precision Plus Protein™, Kaleidoscope™

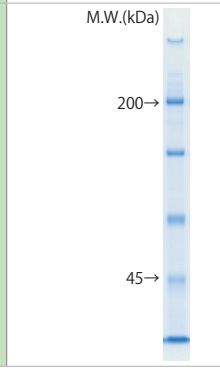
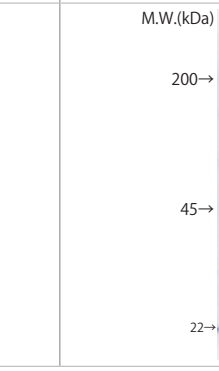
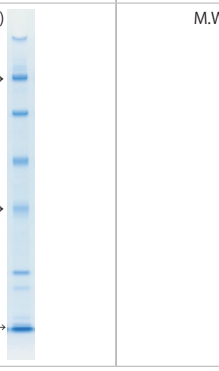
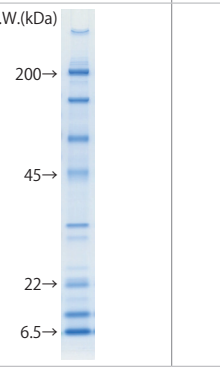
<Transfer>
Semi-dry with PVDF membrane and Blotting Buffer Solution for Western Blotting (Product No.: 30650-31) (Transfer time 20 min with 10V constant current)

<Staining>
CBB Stain One (Product No.: 04543)

Product lines

Extra PAGE One Precast Gel is available in gradient gels and single-percentage gels.

Gel	Gradient gel				
Gel %	5 - 10%	5 - 15%	5 - 20%	7.5 -15 %	10 - 20 %
Product no. 13well/17well	13059-44 / 13060-04	13061-94 / 13062-84	13063-74 / 13064-64	13065-54 / 13066-44	13067-34 / 13068-24
Migration patterns					

Gel	Single-percentage gel			
Gel %	7.5%	10%	12.5%	15%
Product no. 13well/17well	13069-14 / 13070-74	13071-64 / 13072-54	13073-44 / 13074-34	13075-24 / 13076-14
Migration patterns				

Note) Back ground may remain slightly when using Nacalai's Rapid Stain CBB Kit (Product No.: 30035-14) or other products. To reduce back ground, wash the gel with 10% acetic acid for 20 minutes.

Specifications

Cassette size : 100 mm(W) x 100 mm(H) x 3.2 mm(T) (made of glass)
Gel size : 80 mm(W) x 80 mm(H) x 1.0 mm(T)
Well : 13 wells, 17 wells
Shelf life expire date : 9 months

Note) Use electrophoresis tank for plate size 100mm(W) x 100mm(H) x 3.2mm(T).

Ordering Information

Product Name	Storage	Product Number	PKG Size
Extra PAGE One Precast Gel, 5-10%, 13wells	R	13059-44	10 Sheets
Extra PAGE One Precast Gel, 5-10%, 17wells	R	13060-04	10 Sheets
Extra PAGE One Precast Gel, 5-15%, 13wells	R	13061-94	10 Sheets
Extra PAGE One Precast Gel, 5-15%, 17wells	R	13062-84	10 Sheets
Extra PAGE One Precast Gel, 5-20%, 13wells	R	13063-74	10 Sheets
Extra PAGE One Precast Gel, 5-20%, 17wells	R	13064-64	10 Sheets
Extra PAGE One Precast Gel, 7.5-15%, 13wells	R	13065-54	10 Sheets
Extra PAGE One Precast Gel, 7.5-15%, 17wells	R	13066-44	10 Sheets
Extra PAGE One Precast Gel, 10-20%, 13wells	R	13067-34	10 Sheets
Extra PAGE One Precast Gel, 10-20%, 17wells	R	13068-24	10 Sheets
Extra PAGE One Precast Gel, 7.5%, 13wells	R	13069-14	10 Sheets
Extra PAGE One Precast Gel, 7.5%, 17wells	R	13070-74	10 Sheets
Extra PAGE One Precast Gel, 10%, 13wells	R	13071-64	10 Sheets
Extra PAGE One Precast Gel, 10%, 17wells	R	13072-54	10 Sheets
Extra PAGE One Precast Gel, 12.5%, 13wells	R	13073-44	10 Sheets
Extra PAGE One Precast Gel, 12.5%, 17wells	R	13074-34	10 Sheets
Extra PAGE One Precast Gel, 15%, 13wells	R	13075-24	10 Sheets
Extra PAGE One Precast Gel, 15%, 17wells	R	13076-14	10 Sheets

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

[Gel Buffer for Hand-made Gel]

WIDE RANGE Gel Preparation Buffer (4x) for PAGE

Gradient gels offer a much wider separation range of proteins than single percentage gels. However, casting gradient gels is more difficult and labor intensive. WIDE RANGE Gel Preparation Buffer offers a gradient gel-like separation on a single percentage gel by simply mixing it with acrylamide/ bisacrylamide gel casting solution. The gel can be used with the common sample buffers and running buffers. It is also suitable for standard staining methods including CBB and silver staining.

» Simple casting procedure

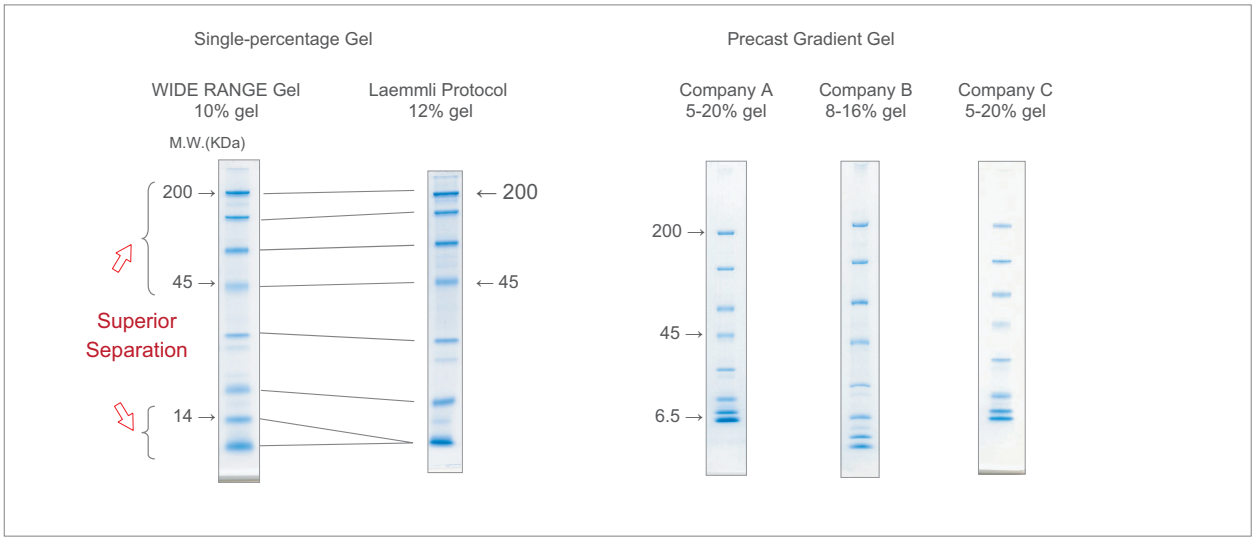
WIDE RANGE Gel Preparation buffer is a 4x concentrated neutral pH buffer. It can be used for preparation of both stacking gel and separation gel by replacing the Tris-HCl buffer in Laemmli buffer system.

» Improved stability and strength

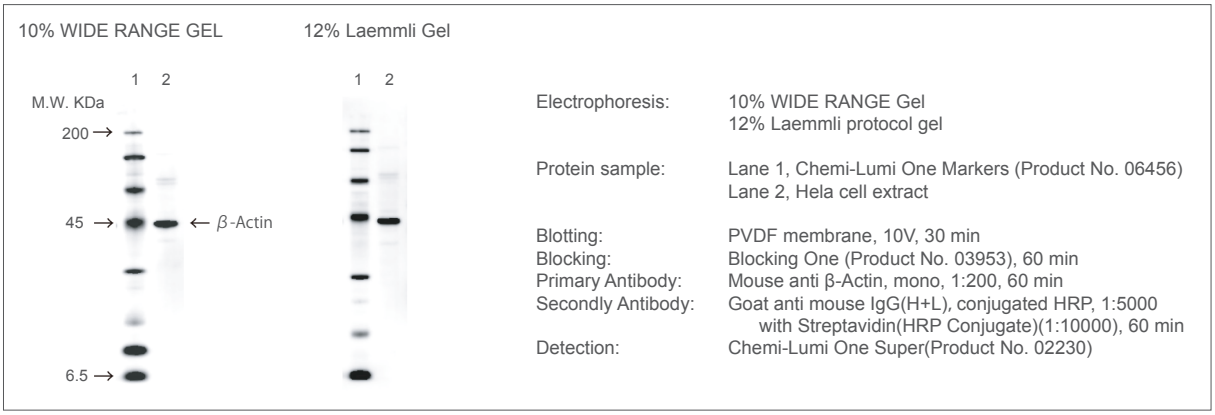
The incresed tensile strength allows easy handling even a low percentage gel. The neutral pH buffer improves the stability of gel resulting in a longer shelf life than the gel with Laemmli buffer system.

» A wide separation range

WIDE RANGE Gel provides a much greater separation range than the gel casted with a conventional Laemmli buffer system.



Applicable for Western blotting



Electrophoresis:	10% WIDE RANGE Gel 12% Laemmli protocol gel
Protein sample:	Lane 1, Chemi-Lumi One Markers (Product No. 06456) Lane 2, Hela cell extract
Blotting:	PVDF membrane, 10V, 30 min
Blocking:	Blocking One (Product No. 03953), 60 min
Primary Antibody:	Mouse anti β-Actin, mono, 1:200, 60 min
Secondly Antibody:	Goat anti mouse IgG(H+L), conjugated HRP, 1:5000 with Streptavidin(HRP Conjugate)(1:10000), 60 min
Detection:	Chemi-Lumi One Super(Product No. 02230)

Ordering Information

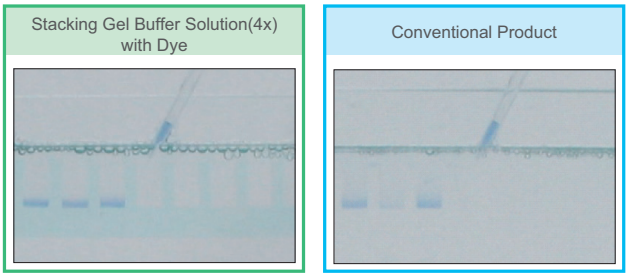
Product Name	Storage	Product No.	PKG Size
WIDE RANGE Gel Preparation Buffer (4x) for PAGE	R	07831-94	250 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

[Stacking Gel Buffer for Hand-made Gel]

Stacking Gel Buffer Solution (4x) with Blue Color

» **Easy to see well locations due to coloring stacking gel**



Wells are clearly confirmed on stacking gel prepared with Stacking Gel Buffer Solution with Dye.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Stacking Gel Buffer Solution (4x) with Dye for SDS-PAGE	R	09268-34	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

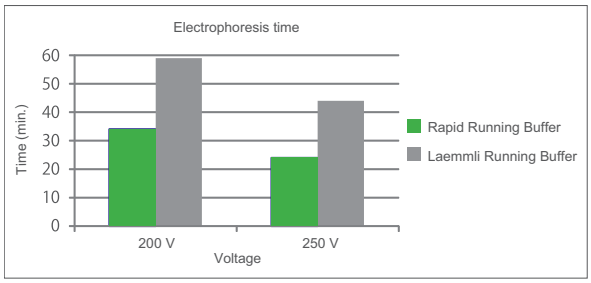
[Fast running buffer for Hand-made Gel]

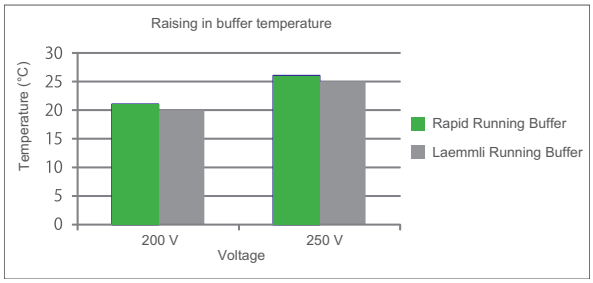
Rapid Running Buffer Solution

» **Approximately 25 min. electrophoresis time with mini-gel at 250 V**
» **Just replace the Laemmli running buffer with this product**
» **High protein transfer efficiency to western blotting membrane**

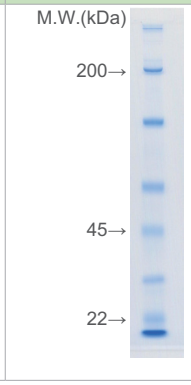
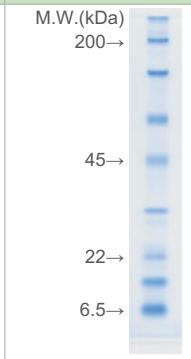
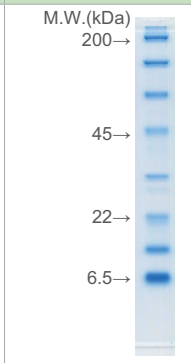
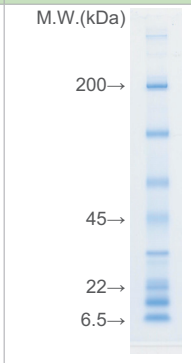
Comparison of electrophoresis duration and buffer temperature rise

Running proteins with this product shortens the electrophoresis duration to about 60% compared to Laemmli running buffer, and its rising in buffer temperature is the almost same as Laemmli running buffer's.





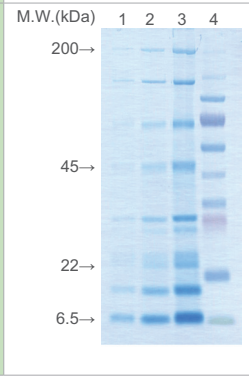
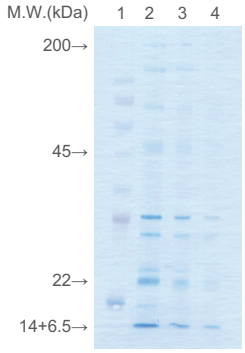
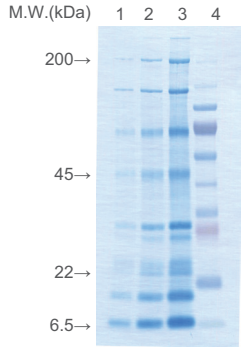
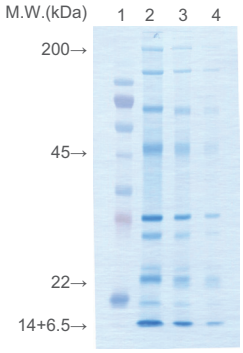
Separation patterns of Laemmli gel with Rapid Running Buffer

Gel concentration	6%	8%	10%	4-10%
Separation image				

Comparison of protein transfer efficiency to Western blotting membrane

Due to lower gel concentration when Rapid Running Buffer Solution is used[*1], its protein transfer efficiency to a membrane is higher than the Laemmli running buffer's.

[*1] By casting a gel with 4% lower gel concentration than usual, its separation patterns can be made similar to the original's, e.g. separation patterns of 8% gel with Rapid Running Buffer Solution is about the same as 12% gel with Laemmli running buffer.

Gel concentration	8%	12%	8%	12%
Running buffer	Rapid Running Buffer	Laemmli running buffer	Rapid Running Buffer	Laemmli running buffer
Blotting time	10 min.		30 min.	
Membrane staining image				

Ordering Information

Product Name	Storage	Product No.	PKG Size
Rapid Running Buffer Solution (20x) for SDS-PAGE	RT	12981-74	250 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Polyacrylamide Gel Casting Reagents

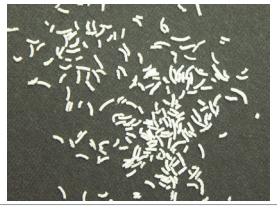
Ordering Information

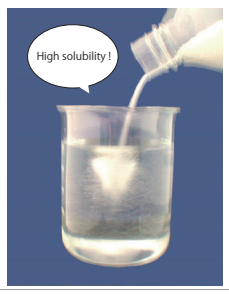
Product Name	Storage	Product No.	PKG Size
Acrylamides (monomer)			
Acrylamide (monomer), Purity, 99%	RT	00809-14	100 g
		00809-85	500 g
Acrylamide (monomer), Purity, 99%, Nuclease and Protease tested	RT	06114-24	100 g
		06114-95	500 g
		06114-11	1 kg
Crosslinking Agents			
N,N'-Methylenebisacrylamide, [BIS]	R	22402-02	25 g
N,N'-Methylenebisacrylamide, Purity, 99%, Nuclease and Protease tested	R	22407-52	25 g
Acrylamide/Bis Mixed Solutions			
30(w/v)%-Acrylamide/Bis Mixed Solution (37.5:1)	R	06144-05	500 mL
30(w/v)%-Acrylamide/Bis Mixed Solution (29:1)	R	06141-35	500 mL
40(w/v)%-Acrylamide/Bis Mixed Solution (37.5:1)	R	06121-95	500 mL
40(w/v)%-Acrylamide/Bis Mixed Solution (29:1)	R	06119-45	500 mL
Polymerization Initiators			
N,N,N',N'-Tetramethylethylenediamine [TEMED]	RT	33401-72	25 g
		33401-14	100 g
Polymerization Promotors			
Ammonium Peroxodisulfate [APS]	R	02627-21	1 g
		02627-34	10 g
10 (w/v)%-Ammonium Peroxodisulfate Solution	F	02634-34	10 mL
Gel Buffer Solutions			
Separating Gel Buffer Solution (4x) for SDS-PAGE, pH8.8 Filtrated by 0.45 µm filter Components：1.5M-Tris-HCl, 0.4 (w/v)%-SDS	RT	30651-05	500 mL
Stacking Gel Buffer Solution (4x) with Dye for SDS-PAGE, pH6.8 Filtrated by 0.45 µm filter Components：0.5M-Tris-HCl, 0.4(w/v)%-SDS, Dye	R	09268-34	100 mL
Stacking Gel Buffer Solution (4x) for SDS-PAGE, pH6.8 Filtrated by 0.45 µm filter Components：0.5M-Tris-HCl, 0.4 (w/v)%-SDS	R	09267-44	100 mL
		32158-25	500 mL

Running Buffers

Ordering Information

Product Name	Storage	Product No.	PKG Size
Pre-mixed Buffers			
Running Buffer Solution (10x) for SDS-PAGE,Tris-Glycine, Filtrated by 0.45 µm filter Components: 0.25 mol/l-Tris, 1.92 mol/l-glycine, 10 g/l-SDS	RT	30329-61	1 L
		30329-74	5 L
Running Buffer Solution (10x) for PAGE,Tris-Glycine, Filtrated by 0.45 µm filter Components: 0.25 mol/l-Tris, 1.92 mol/l-glycine	RT	30340-91	1 L
Buffer Adjusting Reagents			
Tris(hydroxymethyl)aminomethane, Purity, 99%	RT	35410-34	100 g
Tris(hydroxymethyl)aminomethane, Purity, 99.9%, Nuclease and Protease tested	RT	35434-76	100 g
		35434-05	500 g
		35434-21	1 kg
Glycine	RT	17128-14	100 g
Glycine, Nuclease and Protease tested	RT	17141-24	100 g
		17141-95	500 g
Tricine {N-[Tris(hydroxymethyl)methyl]glycine}	RT	34713-62	25 g
		34713-04	100 g
Tricine {N-[Tris(hydroxymethyl)methyl]glycine} Nuclease and Protease tested	RT	02437-24	100 g
Sodium Lauryl Sulfate [Sodium Dodecyl Sulfate;SDS] Purity, 99%	RT	31607-52	25 g
		31607-94	100 g
		31607-65	500 g
Sodium Lauryl Sulfate granular [Sodium Dodecyl Sulfate;SDS] Purity, 99%, Solids (granular)	RT	02873-62	25 g
		02873-04	100 g
		02873-75	500 g
10%-SDS Solution [10%-Sodium Lauryl Sulfate Solution]	RT	30562-04	100 mL





[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Sample Buffer Solution for SDS-PAGE (6x)

» **Suitable for low concentration protein sample adjustment**
» **No precipitation in the refrigerator**
» **Two types of reagents (with and without reducing agent)**

Ordering Information

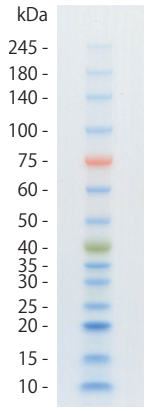
Product Name	Storage	Product No.	PKG Size
Sample Buffers			
Sample Buffer Solution with Reducing Reagent (6x) for SDS-PAGE pH6.8 Filtrated by 0.45 µm filter, Components: 0.375M-Tris-HCl, 0.03(w/v)%-BPB, glycerin, anion surface acting agent and reducing agent	R	09499-14	5 mL
Sample Buffer Solution without Reducing Reagent (6x) for SDS-PAGE pH6.8 Filtrated by 0.45 µm filter, Components: 0.375M-Tris-HCl, 0.03(w/v)%-BPB, glycerin and anion surface acting agent	R	09500-64	5 mL
Sample Buffer Solution with 2-ME (2x) for SDS-PAGE pH6.8 Filtrated by 0.45 µm filter, Components: 0.125M-Tris-HCl, 4(w/v)%-SDS, 20(v/v)%-glycerin, 0.01(w/v)%-BPB, 10(v/v)%-2-ME	R	30566-22	25 mL
Sample Buffer Solution without 2-ME (2x) for SDS-PAGE pH6.8 Filtrated by 0.45 µm filter, Components: 0.125M-Tris-HCl, 4(w/v)%-SDS, 20(v/v)%-glycerin, 0.01(w/v)%-BPB	R	30567-12	25 mL
Reducing Agent			
2-Mercaptoethanol	RT	21418-42 21418-84 21418-55	25 g 100 g 500 g
Dithiothreitol	R	14112-36 14112-81 14112-94 14112-52	100 mg 1 g 5 g 25 g
1mol/l-Dithiothreitol Solution	F	14130-41	1 mL
Tris (2-carboxyethyl) phosphine Hydrochloride (TCEP)	R	07277-61	1 g
Tracking Dyes			
Bromophenol Blue	RT	05808-61 05808-32	1 g 25 g
Others			
Glycerol Nuclease and Protease tested	RT	17045-94 17045-65	100 mL 500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Molecular Weight Markers

Protein Ladder One Plus

» **Excellent visibility with triple color**
» **Sharp bands**
» **Consists of 14 bands**
» **Low lot-to-lot variation**

Electrophoresis images	brand color
	blue
	blue
	blue
	blue
	red
	blue
	blue
	green
	blue
	blue
	blue
	blue(Dark)
	blue
	blue

Extra PAGE One Precast Gel, 5-20%, 13wells(#13063-74)
Load volume 5 µL

Notes
• There is a possibility of different molecular weight result depending on gel type (concentration, gradient, buffer, etc.). It may not be suitable for accurate molecular weight measurement.
• The product is ready-to-use type. Sample preparation, such as heating and reduction, is not required.

Protein	
14 kinds	
Composition	
Glycerol	15% (v/v)
SDS	2% (w/v)
DTT	0.2 mM
Urea	3.6 M
Tris-H3PO4	20 mM
pH 7.5	

Unstained Protein Markers (10x)

» **Contains 8 kinds of protein (M.W. 6,500 - 200,000 Da)**

Image

After CBB Staining

Composition of Unstained Protein Markers

MW (Da)	Proteins
200,000 ←	Myosin
116,250 ←	β-Galactosidase
66,200 ←	Albumin Bovine
45,000 ←	Ovalbumin
31,000 ←	Carbonic Anhydrase
21,500 ←	Trypsin Inhibitor
14,400 ←	Lysozyme
6,500 ←	Aprotinin

Composition

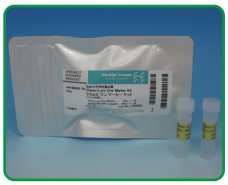
50(v/v)% Glycerol
0.3 M NaCl
0.1 M DTT, 2 mM EDTA・2Na
3 mM NaN_3
10 mM Tris-HCl (pH 7.0)

Chemi-Lumi One Markers Kit

Chemi-Lumi One Markers Kit consists of biotinylated proteins and HRP-conjugated streptavidin. Each band can be visualized on a western blotting by the same chemiluminescent reagents for the target protein.

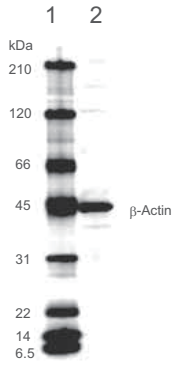
» **Contains 8 biotinylated proteins as molecular weight markers (M.W. 6,500 - 200,000 Da)**

» **Includes HRP-conjugated strepavidin to detect biotinylated proteins**

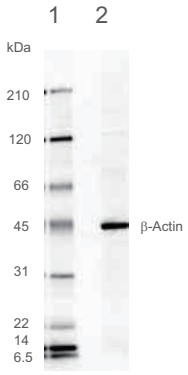


Western blotting for Detecting of β-Actin

Chemi-Lumi One Super
(Product No. 02230-30)



Chemi-Lumi One L
(Product No. 07880-70)



1. Chemi-Lumi One Markers
2. Detection of β-Actin from Hela cell protein extraction solution

Gel	: SDS-PAGE (4-20% gradient gel)
Marker	: Chemi-Lumi One Markers (5 µl)
Membrane	: PVDF ..... 10V, 30 min
Blocking	: Blocking One (Product No. 03953-95)..... 60 min
Primary Ab	: Mouse anti-β-Actin, mono (200x)..... 60 min
Secondly Ab	: Goat anti-mouse IgG, HRP (5000x)..... 60 min + Streptavidin (HRP conjugate)(10,000x)
Detection	: Chemi-Lumi One Super (Product No. 02230-30) Chemi-Lumi One L (Product No. 07880-70)

Components

Chemi-Lumi One Markers consists of 8 biotinylated proteins, 50 µl: 1 tube
Streptavidin (HRP conjugate), 250 µl: 1 tube

Note: The molecular weight of Chemi-Lumi One Markers may slightly differ from unmodified proteins because of biotinylation.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Protein Ladder One Plus, Triple-color for SDS-PAGE	F	19593-25	500 µL
Protein Markers(M.W. 6,500~200,000)(10x) for SDS-PAGE	F	29458-24	200 µL
Chemi-Lumi One Markers Kit	F	06456-70	1 KIT

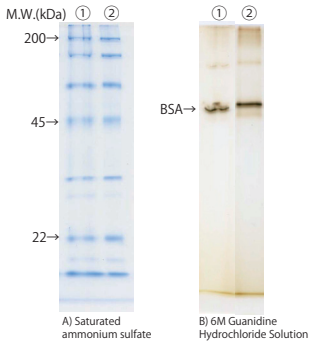
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

PAGE Clean Up Kit

This product is designed for sample preparation in two-dimensional electrophoresis and SDS-PAGE. This product is a combination of acetone, trichloroacetic acid, and coprecipitate optimized for protein precipitation. By resuspending the precipitated proteins in sample lysate according to the intended use, it is possible to concentrate the sample protein and remove interfering substances in the buffer.

» **Suitable for concentrating protein samples and removing analytical interferences**
» **Precipitation is possible even in the subsistence of high concentrations of urea, guanidine, and SDS**
» **Easier than dialysis and small volume processing at the µl level**

Removing electrolyte interfering substances



<Conditions>

[Sample]
(A) 10x dilution of Protein Markers(10x) #29458-24 (standard concentration)
(B) BSA (100ng: non-reducing)

[Precipitation treatment]
① without this product
② with this product

[Electrophoresis]
SDS-PAGE (12% gel)

[Staining]
(A) CBB Stain One #04543 (CBB staining)
(B) Sil-Best Stain One #06865-81 (silver staining)

When a sample containing components that affect the electrophoretic image was treated with this product, the electrophoretic image was improved.
Note) Yield may vary depending on the composition of the sample solution, protein concentration, and other factors. Please use after preliminary examination.

Ordering Information

Product Name	Storage	Product No.	PKG Size
PAGE Clean Up Kit	R	06441-50	1 KIT

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

CBB Stain Solution

CBB staining, Coomassie Briliant Blue staining, is a popular method for detectiong proteins in polyacrylamide gel. We offer four types of CBB staining solution.

Selection of CBB staining solution

	Bullet CBB Stain Lite (#21964)	CBB Stain One Super (#11642)	CBB Stain One (#04543)	Rapid Stain CBB Kit (#30035)
Dye type	CBB-G250	CBB-R250	CBB-G250	CBB-R250
Component	Single bottle (Ready-to-use) w/o acetic acid and methanol	Single bottle (Ready-to-use) w/o acetic acid and methanol	Single bottle (Ready-to-use) w/o acetic acid and methanol	Two bottles w/ acetic acid and w/o methanol
Gel rinsing	Unnecessary	Required 3 times for 5 min	Required 3 times for 5 min	Unnecessary
Staining period	15 min	30 min	60 min	20 min
Destaining period	Unnecessary	More than 1 h	More than 1 h	More than 1 h
Sensitivity	Up to tens of ng proteins			

Bullet CBB Stain Lite

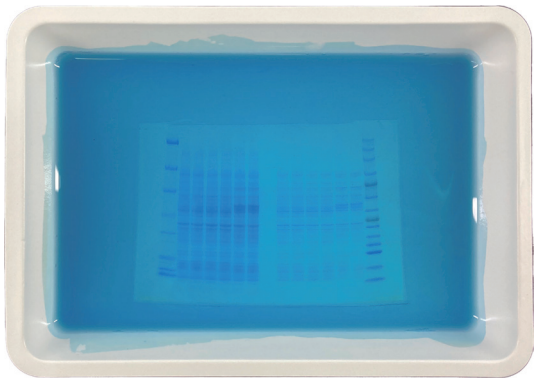
» **In one step, complete staining in 15 minutes**

No need for gel pre-staining washing

Clear staining image without the need for a destaining step

» **Free from strong irritants like acetic acid and methanol**

Observable staining image in the staining solution



<Conditions>

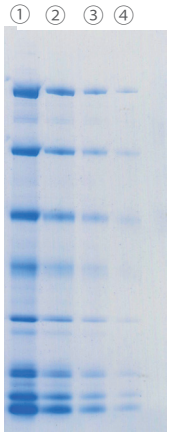
Sample : *E. coli* was prepared in Sample Buffer Solution with 2-ME(2x) for SDS-PAGE #30566-22

SDS-PAGE : Bullet PAGE Plus Precast Gel, 5-20%, 17wells (#21794-54) 400 V (constant voltage), for 10 minutes

CBB stain : Bullet CBB Stain Lite (#21964) at room temperature for 15 minutes

CBB Stain One

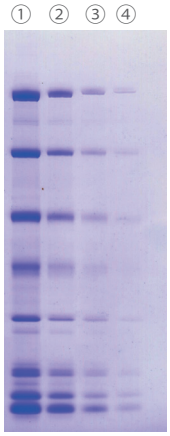
» **Single bottle**
» **CBB G-250 type**
» **Free from acetic acid and methanol**



CBB Stain One
(#04543)
Washing: 3 times x 5 mins.
Staining: 60 mins.
Destaining: Over night

CBB Stain One Super

» **Single bottle**
» **CBB R-250 type**
» **Free from acetic acid and methanol**



CBB Stain One Super
(#11642)
Washing: 3 times x 5 mins.
Staining: 30 mins.
Destaining: Over night

<Conditions>
Sample : Diluted Protein Markers (10x)(#29458-24) into ① 3x, ② 1x, ③ 1/3x, and ④ 1/10x, then added 2 µL.
SDS-PAGE : Extra PAGE One Precast Gel, 5-20%, 13wells **(**#13063-74**)** 400V **(**constant voltage**)**, for 30 mins.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Bullet CBB Stain Lite	R	21964-24	50 mL
		21964-95	500 mL
CBB Stain One Super (Ready-to-use)	RT	11642-31	1 L
CBB Stain One (Ready-to-use)	RT	04543-51	1 L
		04543-64	5 L
Rapid Stain CBB Kit (Coomassie R-250)	RT	30035-14	1 set (for 2 L)

Related Products

Product Name	Storage	Product No.	PKG Size
Coomassie Brilliant Blue G-250	RT	09409-42	25 g
Coomassie Brilliant Blue R-250	RT	09408-52	25 g
Ponceau S	RT	28322-72	25 g

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

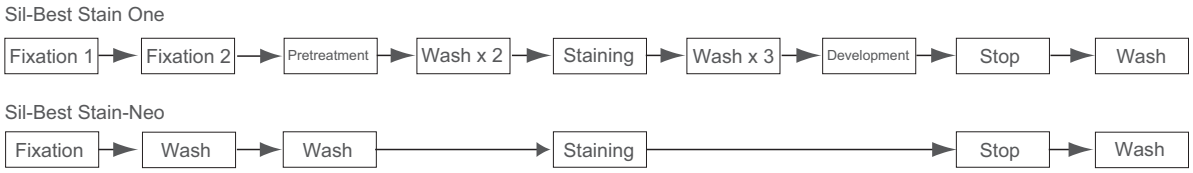
Silver Staining Kit

Silver staining method is a high sensitive method for detecting nucleic acids and proteins in polyacrylamide gel. We offer two types of silver staining kits, each having unique features for your experimental needs.

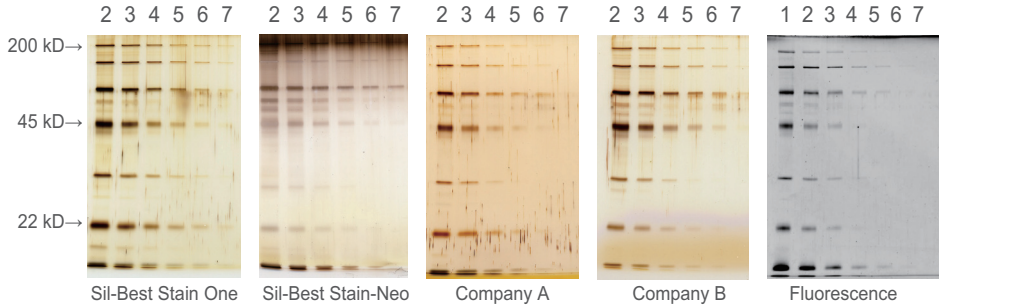
Selection of Silver Staining Kit

	Sil-Best Stain One	Sil-Best Stain-Neo
2-Dimensions	Excellent	Poor
SDS-PAGE	Good	Good
Nucleic acid	Poor	Good
Step	12	6
Staining time	80 min	60 min

Comparison of each procedure



Comparison of each staining image



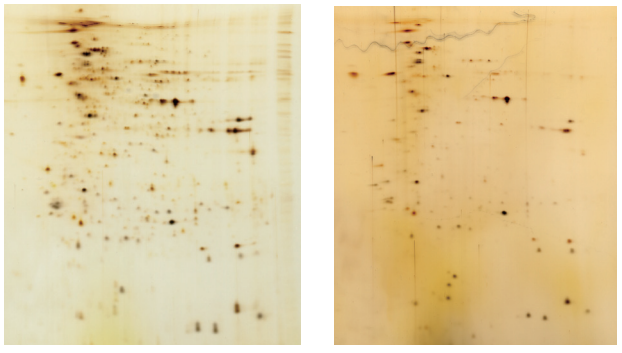
Gel : SDS-12% polyacrylamide gel
Sample : Protein Marker (Product No. 29458-24) 1: 510 ng 2: 255 ng 3: 130 ng 4: 50 ng 5: 26 ng 6: 13 ng 7: 5 ng

Sil-Best Stain One

Sil-Best Stain One is based on the silver staining method for protein detection. Its composition does not contain glutaraldehyde affects a result of mass spectrography.

» **High sensitivity and low background**
» **No glutaraldehyde**

More visible protein spot numbers than Competitors' silver staining kit



Sil-Best Stain One Company A

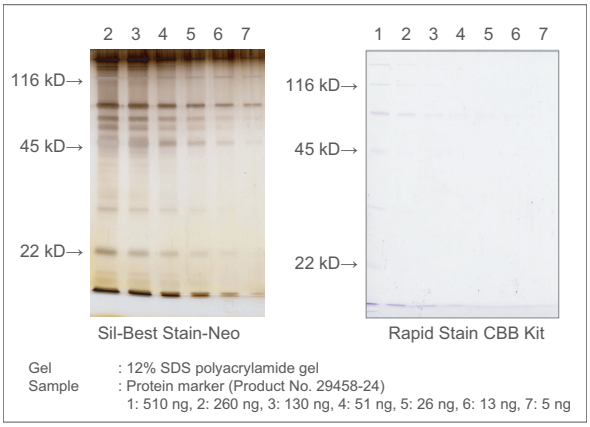
Gel 1-D: Immobiline pH 3-10 (Cytiva)
2-D: 12% Gel
Sample: HL-60 cell extracut

Sil-Best Stain-Neo

Sil-Best Stain-Neo is a highly sensitive method for detecting nucleic acids and proteins in polyacrylamide gel. It is 50-100 fold more sensitive than coomassie brilliant blue and ethidium bromide.

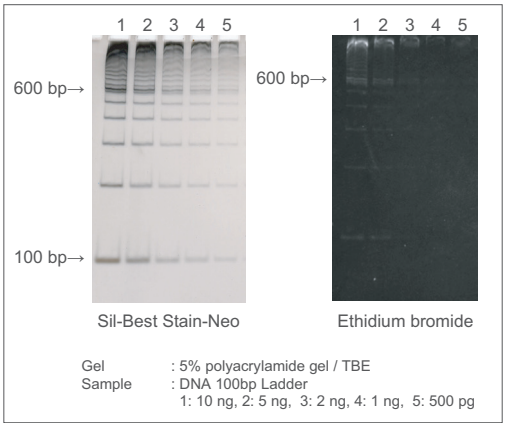
» **Only 6 steps within up to 1 hour**

Staining image of protein



Gel : 12% SDS polyacrylamide gel
Sample : Protein marker (Product No. 29458-24)
1: 510 ng, 2: 260 ng, 3: 130 ng, 4: 51 ng, 5: 26 ng, 6: 13 ng, 7: 5 ng

Staining image of nucleic acid



Gel : 5% polyacrylamide gel / TBE
Sample : DNA 100bp Ladder
1: 10 ng, 2: 5 ng, 3: 2 ng, 4: 1 ng, 5: 500 pg

Ordering Information

Product Name	Storage	Product No.	PKG Size
Sil-Best Stain One	R	06865-81	1 set
Sil-Best Stain-Neo for Protein and Nucleic Acid/PAGE	R	05773-11	1 set

Related Products

Product Name	Storage	Product No.	PKG Size
Dispotray-SS	RT	19386-74	40 pieces
Dispotray-S (for minigel staining)	RT	16526-82	25 pieces
Dispotray-M	RT	16551-84	20 pieces

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

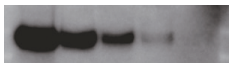
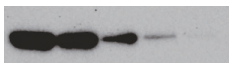
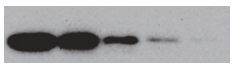
[Ultra-fast Blotting Buffer]

Bullet Semi-dry Transfer One

» **Transfer can be completed at high speed**
» **Efficient transfer a wide range of molecular weights**
» **No need for gel equilibration process**
» **Ready-to-use**

Application 1

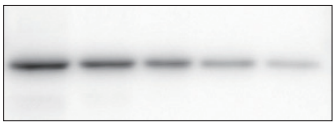
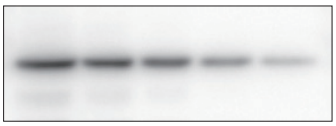
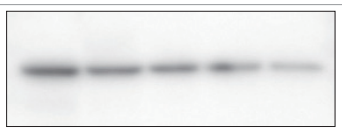
After electrophoresis on various gels, high speed transfer was performed by using this product. EGFR, β-Actin, and Histone H3 were detected through western blotting. Proteins ranging from low molecular weight (Histone H3) to high molecular weight (EGFR) have been successfully transferred by using this product and detected quantitatively.

Gel		Bullet PAGE One Precast Gel	Extra PAGE One Precast Gel	Laemmli method gel (Hand made gel)	WIDE RANGE Gel (Hand made gel)
Blotting images	EGFR (175 kDa)				
	β-Actin (42 kDa)				
	Histone H3 (17 kDa)				

(Conditions)
Samples : HeLa cell suspension (2 times dilution starting from 10 ug)
SDS-PAGE : Bullet PAGE One Precast Gel, 5-15%, 17wells (#13080-44) 400 V
Extra PAGE One Precast Gel, 5-15%, 17wells (#13062-84) 300 V
Laemmli method gel (EGFR; 6%, β-Actin; 12%, Histone H3; 15%) 200 V
WIDE RANGE Gel*(EGFR; 6%, β-Actin; 10%, Histone H3; 12%) 200 V
*Casted with WIDE RANGE Gel Preparation Buffer(4x) for PAGE (#07831-94)
Transfer : Bullet Semi-dry Transfer One (#15353-01) 25 V 10 min PVDF (poresize; 0.45 µm)
Blocking : Bullet Blocking One for Western Blotting (#13779) RT 5 min
Primary antibody (EGFR) : EGF Receptor (D38B1) XP Rabbit mAb (CST #4267) 2,000x diluted by Signal Enhancer HIKARI for Western Blotting and ELISA (#02270-81) Solution A RT 60 min
(β-Actin) : β-Actin (C4) (Santa Cruz #sc-47778) 5,000x diluted by Bullet ImmunoReaction Buffer (#18439) RT 30min
(Histone H3) : Histone H3 (D1H2) XP Rabbit mAb (CST #4499) 2,000x diluted by Bullet Blocking One for Western Blotting (#13779) RT 60 min
Secondary antibody (EGFR) : Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP](Pre-adsorbed) (Novus #NB7187) 50,000x diluted by Signal Enhancer HIKARI for Western Blotting and ELISA (#02270-81) Solution B RT 60 min
(β-Actin) : Goat anti-Rat, Mouse IgG (H+L) Secondary Antibody [HRP](Pre-adsorbed) (Novus #NB7574) 80,000x diluted by Bullet ImmunoReaction Buffer (#18439-85) RT 30 min
(Histone H3) : Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP](Pre-adsorbed) (Novus #NB7187) 30,000x diluted by Bullet Blocking One for Western Blotting (#13779) RT 60 min
Detection ChemiDoc Touch MP 3x3 binning (Bio-rad) (AUTO mode)
(EGFR) : Chemi-Lumi One Super (#02230)
(β-Actin) : Chemi-Lumi One L (#07880)
(Histone H3) : Chemi-Lumi One Super(#02230)

Comparison with conventional transfer method

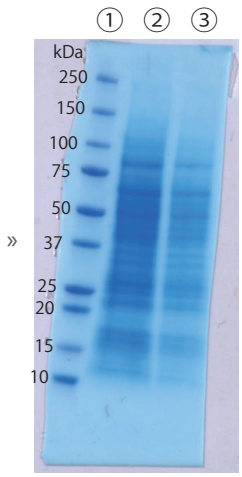
After electrophoresis on the Bullet PAGE Plus Precast Gel, rapid transfer (semi-dry) and conventional transfer (semi-dry and tank) were processed by this product, then detected GAPDH through western blotting. This product enables rapid transfer in just 10 minutes, achieving detection results comparable to the traditional method.

	Conditions	Blotting images
High-speed transfer	Bullet Semi-dry Transfer One(#15353-01) 25 V 10 mins Semi-dry transfer (no need for equilibration)	
Conventional transfer	Semi-dry Blotting Buffer Solution for Western Blotting 10 V for 45 min Semi-dry transfer (15 mins. equilibration performed)	
	Towbin method composition transfer buffer* 100 V tank transfer for 60 mins (15 mins equilibration performed) *25 mM Tris, 192 mM Glycine, 20%MeOH	

(Conditions)
Samples : HeLa cell suspension (2 times dilution started from 4 ug)
Transfer : Conditions listed above
Primary antibody : GAPDH Antibody (Novus #NB300-322) 10,000x diluted by Bullet ImmunoReaction Buffer (#18439) RT 30 min
Secondary antibody : Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP](Pre-adsorbed) (Novus #NB7187) 200,000x diluted
Detection : Exposure time 3 mins

Application 2

After running on Mini-PROTEAN TGX Gels, high-speed transfer was performed by this product and transfer was confirmed by CBB staining.



[Conditions]
Sample:
① Precision Plus Protein™ All Blue Prestained Protein Standards (Bio-rad #1610373)
② 293T Cell Suspension
③ 293T Cell Suspension (2x diluted by ②)
SDS-PAGE : 4-20% Mini-PROTEAN TGX Gels (Bio-rad #4561095)
Transfer : Bullet Semi-dry Transfer One (#15353-01) 25 V for 10 min
CBB Staining : Bullet CBB Stain One(Ready To Use) (#13542)

Data provided by a University researcher.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Bullet Semi-dry Transfer One	RT	15353-01	1 L

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

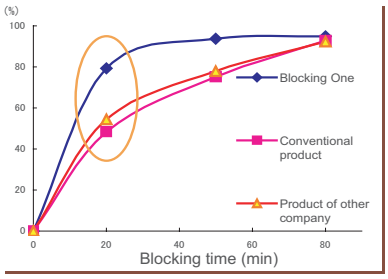
[Blocking Buffer]

Blocking One and Blocking One P

Blocking is indispensable in immunoassays to block non-specific binding reactions. As Blocking One contains high molecular weight compounds, casein and bovine serum protein, it is superior to conventional blocking solutions. Blocking One-P is an exclusive blocking solution, free of phosphate group and endogenous phosphatase for phosphorylated protein detection. The performance is superior compared with conventional blocking solutions such as 1% BSA. The preservative in our blocking solutions do not affect the enzyme activity of peroxidase (POD) or alkaline phosphatase (ALP). Only simple refrigerator storage is necessary, even after opening the bottle.

» **In many assays, a reduction of incubation time for blocking can be achieved**
» **Simple storage in a refrigerator even after opening the bottle**

Comparison of blocking efficiency



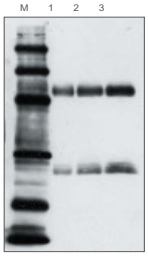
< Protocol >
Unlabeled antibody
↓ Unlabeled anti-human albumin antibody
Blocking
↓ Add Blocking One, 1:5 dilution
Add POD labeled antibody reaction
↓ Add anti-human albumin antibody, POD conjugated (1 h)
POD detection
↓ Add OPD (20 min)
Stop the reaction

The figure left shows the relationship between the reaction time and the effect of blocking in microplate assay.

In comparison with other products, Blocking One offers the strongest blocking efficiency along with faster blocking treatment time.

Western blotting

Blocking One



Incubation time: for 20 min.

Sample
M : Biotinylated Protein Marker (100 dilution, 5 µl)
1 : Mouse Serum (2.5 µl)
2 : Mouse Serum (5.0 µl)
3 : Mouse Serum (10 µl)

Mouse IgG (H+L) in Mouse Serum detected with biotinylated anti-Mouse IgG (H+L). Very little background occurs, indicating exceptional blocking performance.

Blocking One-P

Sample	: HeLa whole cell lysate (1.0 x 107) treated by RIPA Buffef (#08714-04) (1 ml)
Protein Conc.	: Lane 1. 5 µ Lane 2. 10 µ
Blocking	: Blocking One-P, 20 min.
1st antibody	: Anti-p-Tyr (PY20), Mono (Santa Cruz: #sc-508), 1:2000 dilution
2st antibody	: Anti-Mouse IgG-HRP (Santa Cruz: #sc-2005), 1:10000 dilution
WB Detection	: Chemi-Lumi One L (#07880-70)

Comparison with conventional blocking agents

	Composition	Treatment Time	Blocking Efficacy	Phospho-specific antibody applications
Bullet Blocking One	- Amphiphilic compound - High molecular weight compounds - BSA - Casein - refer to p75	5 min	+++	+
Blocking One	- High molecular weight compounds - BSA - Casein	20-30 min	+++	+
Blocking One-P	- High molecular weight compounds - BSA	20-30 min	+++	+++
Skim milk	- Casein	1 h	+	-
1% BSA	- BSA	1 h	+	++

Ordering Information

Product Name	Storage	Product No.	PKG Size
Blocking One	R	03953-95	500 mL
Blocking One-P	R	05999-84	200 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

[Ultra-fast Blocking Buffer]

Bullet Blocking One for Western Blotting

» **Fast blocking in 5 mins**
» **Ready-to-use**
» **Simple storage in a refrigerator even after opening the bottle**

Comparison data with Blocking One (our standard blocking reagent) and 5% skim milk

Blocking reagents	Bullet Blocking One	Blocking One	5% Skim Milk
Blocking time	5 min	30 min	60 min
Blotting image			

(Conditions)

Sample	: 20 µg of HeLa cell extract, 5 serial two-fold dilution series
SDS-PAGE	: Bullet PAGE One 5-15% (Product No. 13080) with SDS Running Buffer (Product No. 30329) at 400 V for 10 min
Blotting	: Semi-dry Blotting Buffer Solution (Product No. 30650) at 10 V for 30 min
Washing	: 0.1% t-TBS (Product No. 12750)
Blocking	: Refer to the figure above
1st antibody	: Anti-Vimentin (C-20) (Rabbit) (Product No. SC-7557-R) diluted 1:2,000, 1 hr. at RT
2nd antibody	: Anti-Rabbit IgG-HRP (Product No. SC-2004) diluted 1:100,000, 1 hr. at RT
Detection	: Chemi-Lumi One (Product No. 11644)
Detector	: LAS-3000 (High mode), 15 min. exposure time

Comparison data: Blocking efficiency in 5 minutes

The original Blocking One, the competitors' ready-to-use blocking reagents and the conventional blocking reagents did not show enough blocking efficiency in 5 mins, while Bullet Blocking One performed well.

5 min blocking time	Blocking reagents	Manufacturers' recommended blocking time	
	Bullet Blocking One		
	Blocking One		30 min
	Company A		60 min
	Company B		30 min
	Company C		60 min
	5% Skim Milk in 0.05 Tween® 20-TBS		60 min
	3% BSA in 0.05% Tween® 20-TBS		60 min

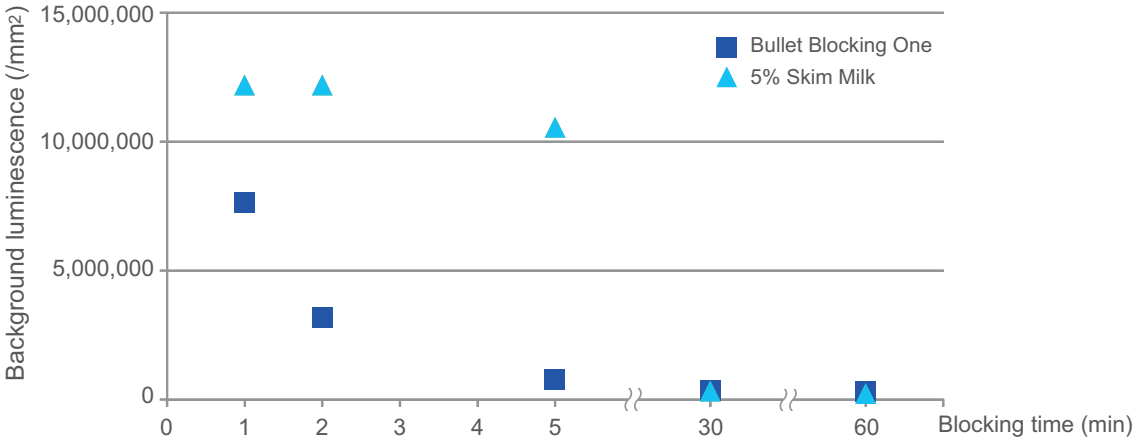
(Conditions)

PVDF membranes dot-blotted with mouse serum were washed with TBS. Blocking was performed using each reagent above. Anti-mouse IgG (Product No.SC-2005) (1:5,000 in 0.01% t-TBS) was applied, and the membranes were washed with 0.05% t-TBS. After reaction with Chemi-Lumi One Super (Product No. 02230), detection was performed using LAS-3000 (High mode) with 90 sec. exposure time.

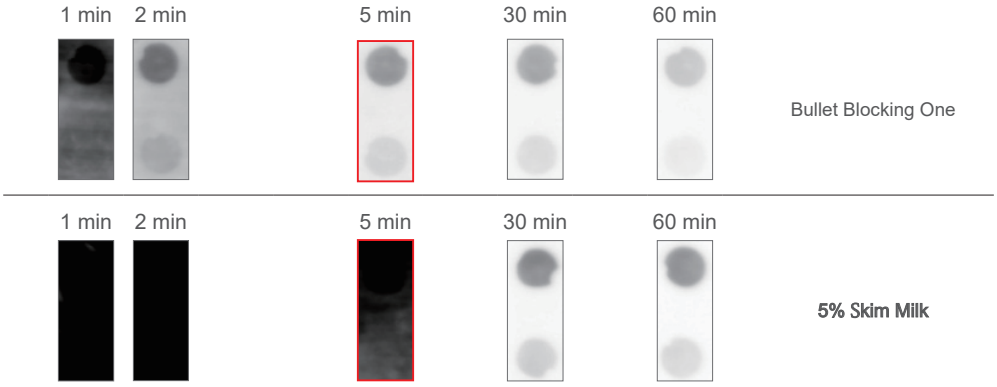
Comparison of blocking time and efficiency with 5% skim milk

Bullet Blocking One performed better than 5% skim milk in blocking time and efficiency.

Analysis of nackground noise on dot blotting data



Dot blotting data



(Conditions)

PVDF membranes dot-blotted with mouse serum were washed with TBS. Blocking was performed using each reagent above. Anti-mouse IgG (Product No. SC-2005) (1:5,000 in 0.01% t-TBS) was applied, and the membranes were washed with 0.05% t-TBS. After reaction with Chemi-Lumi One Super (Product No. 02230), detection was performed using LAS-3000 (High mode) with 90 sec. exposure time. Data analysis was done with Multi Gauge.

Use as antibody diluent

Diluting antibody with Bullet Blocking One resulted in less non-specific binding compared to using t-TBS as diluent.

Antibody diluent	Bullet Blocking One (undiluted)	0.1% t-TBS
Blotting image		

(Conditions)

Sample : 10 µg of HeLa cell extraction, 3 serial two-fold dilution series
SDS-PAGE : Bullet PAGE One 5-15% (Product No. 13080) with SDS Running Buffer (Product No. 30329) at 400 V for 12 min
Blotting : Semi-dry Blotting Buffer Solution (Product No. 30650) at 10 V for 30 min
Washing : 0.1% t-TBS (Product No. 12750)
Blocking : Bullet Blocking One, 5 min
1st antibody : Anti-Cox4 (D-20) (Goat) (Product No. SC-69359) diluted 1:500, 1 h at RT
2nd antibody : Anti-Goat IgG-HRP (Product No. SC-2350) diluted 1:5,000, 1 h at RT
Detection : Chemi-Lumi One Super (Product No. 02230), 1 min reaction time
Detector : LAS-3000 (High mode), 10 min. exposure time

(Please note)

For antibody dilution, use Bullet Blocking One undiluted, or dilute up to 20x with TBS or PBS containing 0.05-0.1% detergent, such as Tween 20. Since the appropriate dilution ratio depends on antibody conditions, such as type and concentration, pretest is required.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Bullet Blocking One for Western Blotting	R	13779-56	50 mL
		13779-14	200 mL
		13779-01	1 L

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

[Ultra-fast Antibody Dilution]

Bullet ImmunoReaction Buffer

» **Saving-time** : **Cut in half antigen-antibody reaction time**
» **Simple** : **Replace tTBS, tPBS, skim milk or BSA with Bullet ImmunoReaction Buffer**
» **Ready-to-use** : **Do not need to dilute**

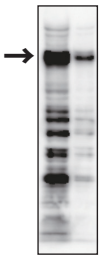
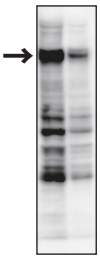
Reference data: Comparison of sensitivity related to Ab-Ag reaction time with 0.1% tTBS

Primary Ab incubation time	15 min	30 min	60 min	120 min	Overnight at 4°C
Secondary Ab incubation time	15 min	30 min	60 min	120 min	60 min
Bullet ImmunoReaction Buffer					
0.1%-tTBS					

(Conditions)
Sample : Hela cell extract; (left) 2 µg, (right) 0.4 µg
Blocking : Incubated for 5 minutes with Bullet Blocking One for Western Blotting manufactured by Nacalai, #13779
Primary antibody : β-Actin antibody manufactured by Santa Cruz, #sc-47778, x 1,000 dilution
Secondary antibody : Goat anti-mouse IgG(H+L) secondary antibody manufactured by Novous, NB7574, x 40,000 dilution
Detection : Chemi-Lumi One Super manufactured by Nacalai, #02230, 5-minute exposure time
Detector : LAS-300 High mode

Transition to saving-time protocol

If a signal enhancer reagent, such as HIKARI, is use, speeding up the process with this product is not recommended, as it may decrease sensitivity and increase non-specific reactions.

Antibody diluent	ImmunoReaction Buffer	tTBS	HIKARI →refer to p79	Company A	Company B	Company C
Western blotting						
Primary antibody	30 min	60 min				
	EGF Receptor(D38B1) XP® Rabbit mAb(CST #4267), x 1,000 dilution XP is a trademark by Cell Signaling Technology, Inc.					
Secondary antibody	30 min	60 min				
	Goat anti-Rabbit IgG(H+L) Secondary Antibody[HRP](Pre-adsorbed)(Novus #NB7187), x 50,000 dilution					

(Conditions)
Sample : Hela cell extract, (left) 10 µg and (right) 2 µg
Blocking : Incubated for 5 min with Bullet Blocking One
Detection : Chemi-Lumi One Super, #02230; 3-min exposure time
Detector : LAS-300 High mode

Ordering Information

Product Name	Storage	Product No.	PKG Size
Bullet ImmunoReaction Buffer	R	18439-85	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

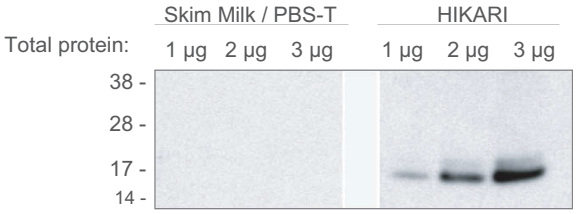
[High-performance Antibody Dilution]

Signal Enhancer HIKARI for Western Blotting and ELISA

Dilute your antibodies with Signal Enhancer HIKARI instead of conventional diluents such as PBS-t or TBS-t before performing your next western blotting detection protocol and witness a remarkable increase in the ability to detect the protein of interest and to eliminate undesired background. Signal Enhancer HIKARI was developed to resolve the problems of low sensitivity and high background often encountered during procedures such as western blotting and ELISA.

» **Enhances antigen-antibody reactions**
» **Removes background**
» **Works with any substrate and membrane**
» **Ready-to-use reagent**

Improved detection sensitivity

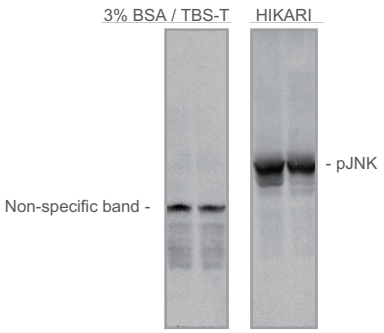


Treatment with Signal Enhancer HIKARI resulted in clear signal, whereas dilution with conventional PBS-T resulted in no signal.

Primary antibody	: Anti-Rac1 (1:1000)
Secondary antibody	: Anti-Mouse IgG-HRP (1:2500)
Sample	: Rat Cortical Primary Neuron
Detection	: ECL

Data courtesy of Dr. K. Ishizuka, Department of Psychiatry, Johns Hopkins University School of Medicine

Improved specificity



Treatment with Signal Enhancer HIKARI resulted in clear signal enhancement and background suppression compared with the conventional method using TBS-T.

Primary antibody	: Anti-pJNK (1:1000)
Secondary antibody	: Anti-Rat IgG-HRP (1:5000)
Sample	: Mouse Embryonic Fibroblast
Detection	: Super Signal West Pico

Data courtesy of Dr. S. Matsuzawa, Signal Transduction, NCI Cancer Center, Burnham Institute for Medical Research

References

1. Feng-Ming Yang *et al. FEBS* **276**, 425-436 (2009)
2. Jian-Bin Wang *et al. The Journal of Cell Science* **122**(12), 2024-2033 (2009)
3. Chunwei Huang *et al. Reproductive Toxicology* **27**, 103-110 (2009)
4. Sawako Yamashiro *et al. The Journal of Cell Science* **121** (Pt 23), 3867-3877 (2008)

Ordering Information

Product Name	Storage	Product No.	PKG Size
Signal Enhancer HIKARI for Western Blotting and ELISA Kit contents: Solution A for Primary Antibody Solution B for Secondary Antibody	R	02267-41 02270-81	1 set (50 mL each) 1 set (250 mL each)
Signal Enhancer HIKARI for Western Blotting and ELISA Solution A	R	02272-74	250 mL
Signal Enhancer HIKARI for Western Blotting and ELISA Solution B	R	02297-64	250 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

KyoBright488-conjugated Secondary Antibody

This product is a fluorescently conjugated secondary antibody that can be used for applications such as immunocytochemistry. Two affinity-purified antibody types are available: Anti-Mouse IgG and Anti-Rabbit IgG. KyoBright488, the fluorophore conjugated to this product, is a bright and highly photostable fluorescent dye. Like the widely used FITC and Alexa Fluor 488, it is excited by blue light and emits green fluorescence.

» **Detectable with a standard 488 nm filter set (Ex 495 nm / Em 520 nm)**
» **Affinity purified**
» **Cross-absorbed***

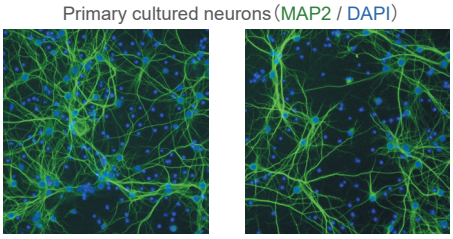
*Anti-Mouse IgG: Cross-absorbed with Human IgG, Rat IgG, and Rabbit IgG
Anti-Rabbit IgG: Cross-absorbed with Human IgG, Rat IgG, and Mouse IgG

Performance comparison with competitor products in immunocytochemistry staining

Fluorescence microscopy observation

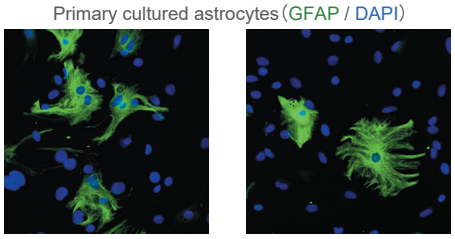
MAP2 or GFAP was detected using primary cultured mouse neurons and primary cultured mouse astrocytes. This product showed results comparable to those of Company A's product.

Primary cultured neurons (MAP2 / DAPI)



This product (Anti-Mouse IgG) | Company A (Anti-Mouse IgG)

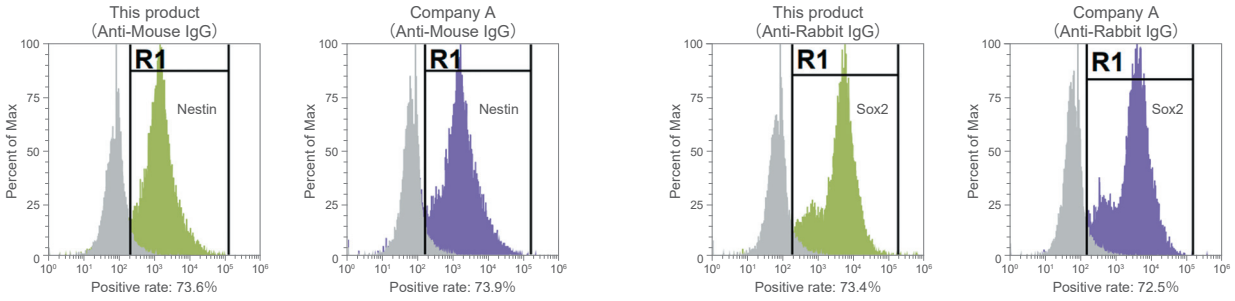
Primary cultured astrocytes (GFAP / DAPI)



This product (Anti-Rabbit IgG) | Company A (Anti-Rabbit IgG)

Detection by flow cytometry

Expression of Nestin or Sox2 was confirmed using mouse neural stem cells. This product showed results comparable to those of Company A's product.

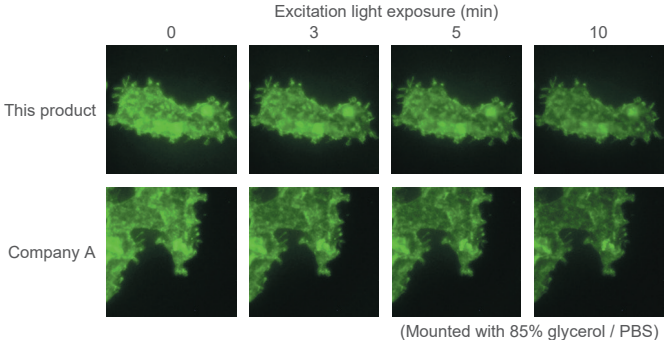


(Experimental conditions)
Please refer to our website (https://www.nacalai.com/global/reagent/kyobright488-conjugated_secondary_antibody.html).

Evaluation of fluorophore stability

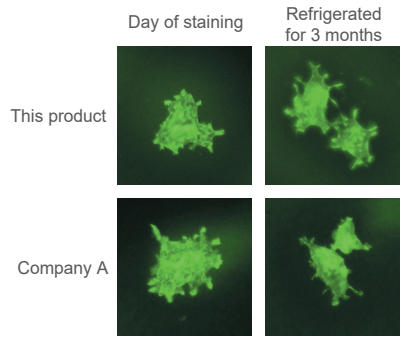
Using immunocytochemistry staining with HEK293 cells, the stability of the fluorophore under excitation light exposure and long-term storage was evaluated. This product was shown to exhibit dye stability equivalent to that of Company A's product.

Comparison of dye stability under excitation light exposure



(Mounted with 85% glycerol / PBS)

Comparison of dye stability after long-term storage

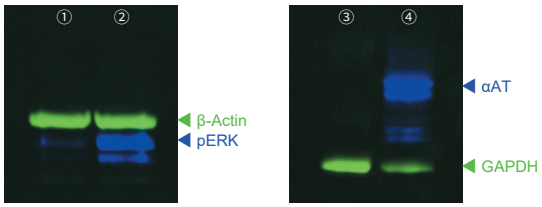


(Mounted with Fluoro-KEEPER)

Images taken on the day of staining and after 3 months of refrigerated storage were captured from specimens on the same slide.

Application to fluorescent Western blotting

Using HEK293 cells treated to activate Protein Kinase C and iPS cell-derived hepatocytes, multiplex detection was performed for housekeeping proteins as well as proteins involved in phosphorylation and differentiation.



(Experimental conditions)
① HEK293 cells (10 µg)
② HEK293 cells treated to activate Protein Kinase C (10 µg)
③ iPS cells (10 µg)
④ iPS cell-derived hepatocytes (10 µg)

Green: KyoBright488-conjugated secondary antibody
Blue: StarBright Blue 700-conjugated secondary antibody

(Experimental conditions)
Please refer to our website (https://www.nacalai.com/global/reagent/kyobright488-conjugated_secondary_antibody.html).

All product names, trademarks, and registered trademarks are the property of their respective owners. Use of these names does not imply any affiliation or endorsement.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Anti-Mouse IgG(Goat), KyoBright488-conjugated, Pre-absorbed	R	22844-06	100 µL
		22844-64	0.5 mL
Anti-Rabbit IgG(Goat), KyoBright488-conjugated, Pre-absorbed	R	22849-56	100 µL
		22849-14	0.5 mL

Related Product

Product Name	Storage	Product No.	PKG Size
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type	R	12593-64	2 X 5 mL
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type with DAPI	R	12745-74	2 X 5 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

HRP-conjugated Secondary Antibody

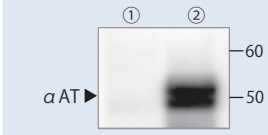
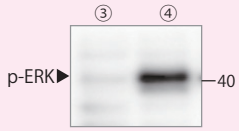
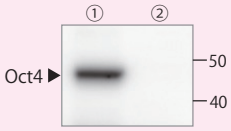
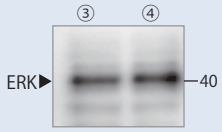
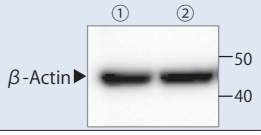
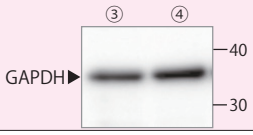
» **Affinity-purified**

» **Cross-absorbed***

*Anti-Mouse IgG: Cross-absorbed with Human IgG, Rat IgG, and Rabbit IgG
Anti-Rabbit IgG: Cross-absorbed with Human IgG, Rat IgG, and Mouse IgG

Application data

We detected target proteins in liver cells derived from induced pluripotent stem (iPS) cells, as well as in cells treated for Protein Kinase C activation. These proteins are related to cellular differentiation and phosphorylation processes. We employed three different chemiluminescent reagents with varying sensitivities to carry out the detection.

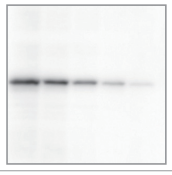
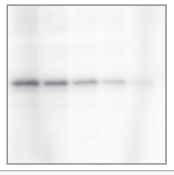
Chemiluminescent reagent (Recommended dilution rate for secondary antibodies)	Actual dilution rate	iPS cell-derived liver cells	PKC-activated HEK293 cells
Chemi Lumi One Ultra (1:50,000-1:500,000)	100,000x		
Chemi Lumi One Super (1:10,000-1:100,000)	20,000x		
Chemi Lumi One L (1:2,500-1:25,000)	5,000x		

Blue: Anti-Mouse IgG(#21860) Pink: Anti-Rabbit IgG(#21858)

(Conditions)
Sample: ① Control (l iPS cells 10 µg) ② iPS cell-derived liver cells 10 µg ③ Control (l HEK293 cells 10 µg) ④ PKC-activated HEK293 cells 10 µg
Primary antibody: Diluted with Bullet ImmunoReaction Buffer (#18439-85) and incubated for 30 mins at room temperature.
[αAT] (Proteintech #66135-1-Ig), [Oct4] (Abcam #ab19857), [β-Actin] (MBL #M177-3), [pERK] CST #4370S
[ERK] (Santa Cruz #sc-514302), [GAPDH] (Novus Biologicals #NB300-322)
Secondary antibody: Diluted with Bullet ImmunoReaction Buffer (#18439-85) and incubated for 30 mins at room temperature.

Comparison of detection sensitivity by Western blotting

We detected samples that were serially diluted two-fold, from 16 µg/well using Anti-Mouse IgG and Anti-Rabbit IgG antibodies sourced from various companies at a 20,000-fold dilution. Our product demonstrated equal or higher sensitivity compared to products from other companies.

	This product	Company A	Company B
Anti-Mouse IgG (Primary antibody: Anti-β-Actin)			
Anti-Rabbit IgG (Primary antibody: Anti-PCNA)			

(Conditions)
Reference (https://www.nacalai.co.jp/products/entry/d005021.html)

Ordering Information

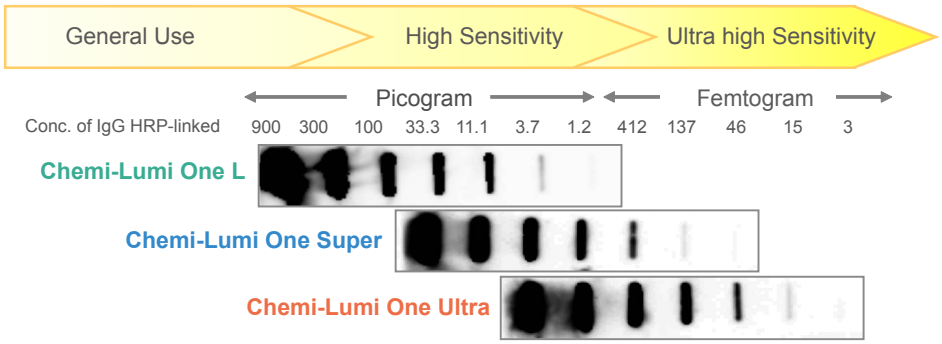
Product Name	Storage	Product No.	PKG Size
Anti-Mouse IgG(Goat), HRP-conjugated, Pre-absorbed	R	21860-74	100 µL
		21860-61	1 mL
Anti-Rabbit IgG(Goat), HRP-conjugated, Pre-absorbed	R	21858-24	100 µL
		21858-11	1 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

[Detection]

Chemiluminescent Western Blotting Substrates

Chemi-Lumi One is a series of high sensitive luminol-based chemiluminescence assay Kits for western blotting.Three types of chemiluminescent substrates are available for western blotting detection with horseradish peroxidase enzyme (HRP).

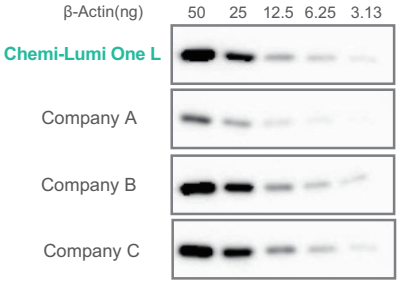
Product Name	Chemi-Lumi One L	Chemi-Lumi One Super	Chemi-Lumi One Ultra
Product No.	07880	02230	11644
Lower Detection Limit	Low-picogram	Mid-femtogram	Low-femtogram
Required Working Solution	Approx. 0.125 mL / cm^2	Approx. 0.1 mL / cm^2	Approx. 0.1 mL / cm^2
Suggested Antibody Dilution Ratio	Primary: 1:1,000-1:5,000 Secondary: 1:20,000-1:100,000	Primary: 1:1,000-1:20,000 Secondary: 1:20,000-1:200,000	Primary: 1:5,000-1:100,000 Secondary: 1:100,000-1:500,000
Reaction Period	1 min.	1 min.	5 min.
Comparable to	ECL (Cytiva) ECL Start (Cytiva) SuperSignal Pico (Thermo)	ECL Prime (Cytiva) SuperSignal Dura (Thermo) SuperSignal PLUS (Thermo) Clarity (Bio-Rad)	ECL Select (Cytiva) SuperSignal Femto (Thermo) Clarity Max (Bio-Rad)
Sensitivity <Conditions> Antigen : Anti-Mouse IgG (Goat), HRP Conjugated (Santa Cruz, sc-2005) Detection : L (1 min.) Super (1 min.) Ultra (5 min.) Detector : LAS-3000 Super mode (Analyze 3 min. later after reaction with each substrate) Ex. time : 30 min.			

Chemi-Lumi One L

» **Suitable for optimization of target proteins**
» **Reasonable price**
» **Detect wide range of protein concentration**

Comparison of sensitivity with competitors

Chemi-Lumi One L offers similar sensitivity to competitors' and higher sensitivity than Company A's products.



<Conditions>

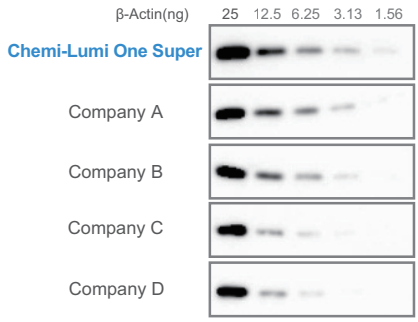
Gel	: 10% PAGE gel
Wash	: 0.1% t-TBS(1x), pH7.4
Blocking	: Blocking One
1st Ab	: Anti-β-Actin Mouse Monol Antibody, (Santa Cruze sc-47778), 1:1,000
2nd Ab	: Anti-Mouse IgG(Goat)-HRP, (Santa Cruze sc-2005), 1:20,000
Detection	: 1min.
Detector	: LAS-3000 High mode
Ex. time	: 5 min.

Chemi-Lumi One Super

» **Extendable of exposure time**
» **Detects proteins at mid-femtogram level with low background**
» **Rapid substrate processing of blot**

Comparison of sensitivity with competitors

Chemi-Lumi One Super offers the highest sensitivity out of competitors' substrates even though its exposure time is 1 minute, while others require 5 minutes.



<Conditions>

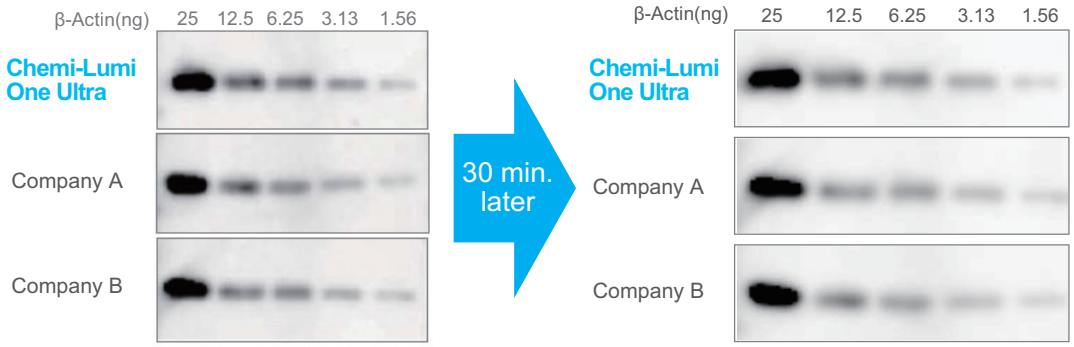
Gel	: 10% PAGE gel
Wash	: 0.1% t-TBS, pH7.4
Blocking	: Blocking One
1st Ab	: Anti-β-Actin Mouse Mono, (Santa Cruze sc-47778), 1:2,000
2nd Ab	: Anti-Mouse IgG (Goat)-HRP, (Santa Cruze sc-2005), 1:30,000
Detection	: Chemi-Lumi One Super; 1 min. Competitor A, B, C and D; 5 min
Detector	: LAS-3000 High mode
Ex. time	: 5 min

Chemi-Lumi One Ultra

» **The most sensitive in Chemi-Lumi One series**
» **Longer signal duration**
» **Wider range of experimental conditions due to low background**

Comparison of sensitivity with competitors

Western blotting of identical transferrin samples. The membranes were incubated with substrate that was prepared according to the manufacturers' instructions.The membranes were exposed to film for 2 minutes.



<Conditions>

Gel	: 10% acrylamidegel
Wash	: 0.1% t-TBS(x1), pH7.4
Blocking	: Blocking One
1st Ab	: Anti-β-Actin Mouse Monoclonal Antibody, (Santa Cruze sc-47778), 1:300
2nd Ab	: Anti-Mouse IgG (Goat), HRP Conjugated, (Santa Cruze sc-2005), 1:1,000
Detection period	: 5 min
Detector	: LAS-3000 High mode
Expose time	: 3 min

Ordering Information

Product Name	Storage	Product No.	PKG Size
Chemi-Lumi One L, Luminol 250 mL and Peroxide 250 mL Sufficient substrate for 4,000 cm² of blotting membrane	R	07880-70	1 KIT
Chemi-Lumi One Super, Luminol 50 mL and Peroxide 50 mL Sufficient substrate for 1,000 cm² of blotting membrane	R	02230-30	1 KIT
Chemi-Lumi One Ultra, Luminol 50 mL and Peroxide 50 mL Sufficient substrate for 1,000 cm² of blotting membrane	RT	11644-40	1 KIT

[Storage] RT = Room Temperature, R = Refrigerator, F = Freeze

Colorimetric Western Blotting Substrates

Colorimetric detection enables detection of a target protein on a membrane by a simple procedure without usage of detection equipment. Depending on the enzyme type conjugated to the antibody, some detection kits are available.

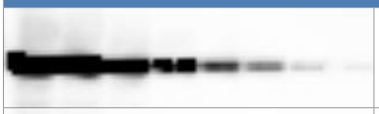
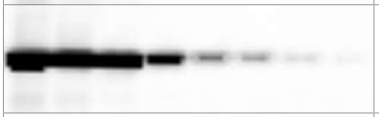
TMB Solution for Western blotting

TMB (tetramethyl benzidine) solution is used with horseradish peroxidase (HRP)-based western blotting procedure, but not applicable for ELISA or immunohistochemistry.

- » **Convenience : No CCD-camera-equipped imager or X-ray film needed**
- » **Sensitivity : Low picogram level**
- » **Safety : Does not contain DAB or o-Dianisidine**
- » **Ready-to-use : Does not need to dilute**

Reference data: Comparison with other picogram-level chemiluminescence kits and colorimetric substrates

TMB Solution for Western blotting; incubation time,10 min	
	TMB Solution for Western blotting

Chemiluminescence Kits / exposure time,10 min		Other colorimetric substrates / incubation time,10 min	
	Chemi-Lumi One L (#07880)		Peroxidase Stain Kit (#26652-70)
	Company A		Peroxidase Stain DAB Kit (#25985-50)
	Company B		Peroxidase Stain DAB Kit +Metal Enhancer for DAB Stain (#07388-24)

Sample : Hela cell extraction, 2 times dilution started from 12 ug
Primary antibody : Anti-β-actin mAb manufactured by MBL, #177-3, x 5,000 dilution
Secondary antibody : Anti-mouse IgG, HRP-linked whole Ab Sheep manufactured by GE, #NA931, x 10,000 dilution
Detector for chemiluminescence : LAS-3000 Super mode

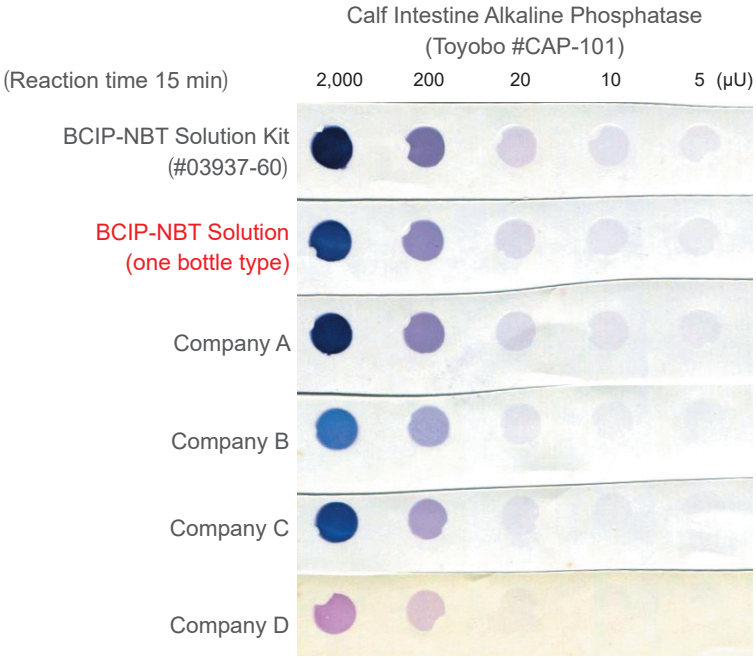
BCIP-NBT Solution (Ready-to-use)

A ready-to-use solution of chromogenic substrate of alkaline phosphatase (AP). It can be used in a wide range of applications, such as detection of alkaline phosphatase (AP) bound by antigen-antibody reaction and endogenous alkaline phosphatase (AP) of stem cells and osteoblasts, in blotting and tissue or cell staining.

- » **Ready-to-use**
- » **Can be used in wide range of applications such as immunochemical staining or endogenous AP staining**
- » **Can be used in situ hybridization (ISH), as it is tested for DNase and RNase**

Comparison: Detection sensitivity by dot blotting

Compare with other brands, BCIP-NBT Solution has equal or better sensitivity.



Ordering Information

Product Name	Storage	Product No.	PKG Size
TMB Solution for Western Blotting	R	18186-24	200 mL
BCIP-NBT Solution (Ready To Use)	R	19880-84	100 mL

Related products

Product Name	Storage	Product No.	PKG Size
Peroxidase Stain DAB Kit (Brown Stain)	R	25985-50	1 KIT
Metal Enhancer for DAB Stain	RT	07388-24	100 mL

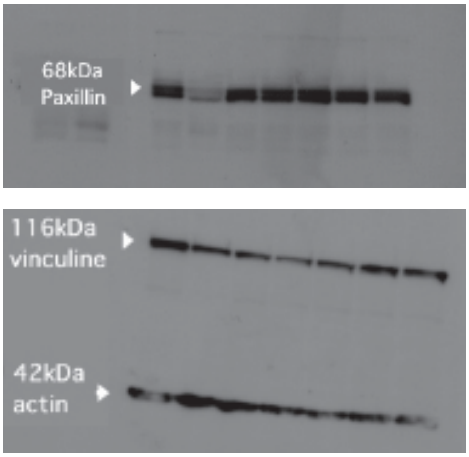
[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

WB Stripping Solution

WB Stripping Solution removes antibodies from blotting membrane, enabling subsequent detections with different antibodies on the very same blot. After the first antigen-antibody reaction and following chemiluminescent visualization, the antibodies can be removed by the WB Stripping Solution. A second antigen-antibody reaction can be conducted on the same blot. The same blot can be probed 2-5 times if chemiluminescent detection is employed.

- » **No heating : Reaction at room temperature**
- » **No odor : Does not contain 2-mercaptoethanol**
- » **Fast : Stripping time 5-15 minutes**
- » **Ready-to-use : One solution in one bottle**

Applications

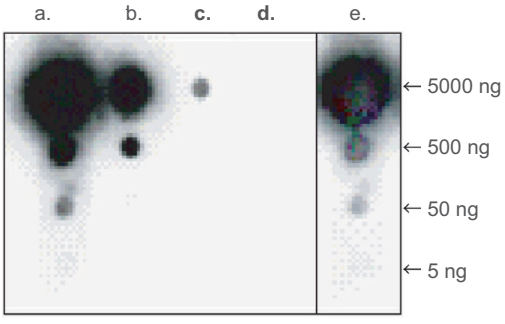


First antigen-antibody reaction
- Blocking : Blocking One (Product No.: 03953-95), 30 min
- Wash : t-Tris Buffered Saline
- Primary ab : Anti-Paxillin (mouse IgG)
- Secondary ab : Anti-mouse IgG-POD
- Detection : Chemiluminescence Detection Kit (commercially available product)

Stripping
- Condition: RT, 15 min for conventional protocol

Second, different antigen-antibody reaction
- Blocking : Blocking One (Product No.: 03953-95), 30 min
- Wash : t-Tris Buffered Saline
- Primary ab : Anti-Vinculin (mouse IgG) / Anti-Actin (mouse IgG)
- Secondary ab : Anti-mouse IgG-POD
- Detection : Chemi-Lumi One

Comparison of WB Stripping Solution and WB Stripping Solution Strong



Apply HPR-labeled anti-GST antibody to 5000 ng, 500 ng, 50 ng, or 5 ng (as desired) of c-Myc-GST antigen on a PVDF membrane, then remove the antibody by agitating gently for 10 minutes using one of the following stripping solutions.

a: 0.05%(v/v) t-TBS
b: 2%(w/v) SDS, 100mM 2-Meraptoethanol
c: WB Stripping Solution
d: WB Stripping Solution Strong

After stripping the antibodies and washing the membrane with t-PBS for 2 min, use the chemiluminescence method to detect the HPR-labeled anti-GST antibody remaining on the membrane.

*Image “e” is a result that shows detection of the antigen with HPR-labeled anti-GST antibody on the “d” . The similar result is marked with “a” . Therefore, WB Stripping Solution Strong only stripped antibodies, not antigens.

Ordering Information

Product Name	Storage	Product No.	PKG Size
WB Stripping Solution	RT	05364-55	500 mL
WB Stripping Solution Strong	R	05677-65	500 mL
WB Stripping Solution Trial Set (WB Stripping Solution: 40 ml, WB Stripping Solution Strong: 40 ml)	R	05680-21	1 set

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

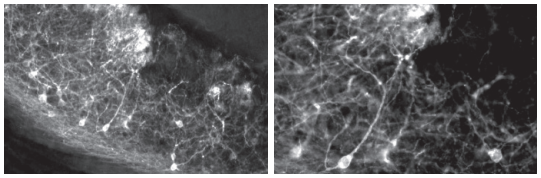
Epitope Tag Antibody

A family of epitope tag antibodies available for the detection and purification of the recombinant proteins. Most of our tag antibodies are highly specific mouse and rat monoclonal antibodies.

Anti-GFP (Rat IgG2a), Mono (GF090R)

Clone	: GF090R	
Isotype	: IgG2a (Rat)	
Product form	: Liquid	
Immunogen	: His-GFP (full length) fusion protein	
Application	: Immunohistochemstry	1:1000-1:2000
	Western blotting	1:1000-1:2000
	ELISA	1:2000-1:20000

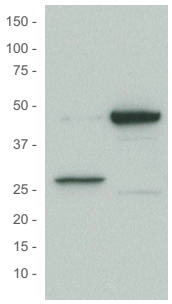
Immunohistochemistry



Sample : Mouse brain (nerve cell)
Primary antibody : Anti-GFP(Rat IgG2a), Mono (1:1000) RT, O/N
Secondary antibody : Anti-Rat IgG-Cy3 (1:300) RT, 1 hr
Blocking 5% Normal goat serum/0.2% TritonX-100 in PBS
Fixing method : 4% Paraformaldehyde

Data courtesy of Dr. Y. Yoshihara, RIKEN Brain Science Institute

Western blotting



Sample : 1; GFP, 2; GFP-14-3-3 (293 cell lysate 50 µg)
Gel : 5-20% Gradient gel
Primary antibody : Anti-GFP (Rat IgG2a), Mono (3 µg/ml), O/N
Secondary antibody : Anti-Rat IgG-HRP (1:10000)

Data courtesy of Dr. K. Yoshida, Department of Molecular Genetics, Medical Research Institute, Tokyo Medical and Dental University

Reference

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13. Ogata M. *et al. Molecular and Cell Biology* **26**(24), 9220-9231 (2006)
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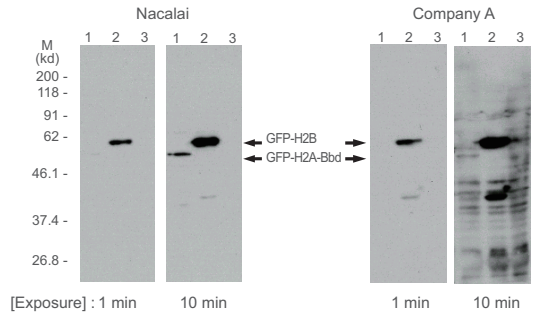
Anti-GFP (Mouse IgG1-k), Mono (GF200)

Clone	: GF200	
Isotype	: IgG1-k (Mouse)	
Product form	: Liquid	
Immunogen	: His-GFP (full length) fusion protein	
Application	: Western blotting	1:1000-1:2000
	ELISA	1:2000-1:20000

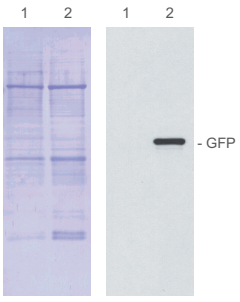
Western blotting



[Exposure] : 1 min 10 min 1 min 10 min
[Primary antibody] :
mouse monoclonal anti-GFP (3.6 µg/ml) rabbit polyclonal anti-GFP (1:1000)
[Secondary antibody] :
anti-mouse IgG (1:1000) anti-rabbit IgG (1:1000)
[Sample]: 1. Hela: GFP-H2A-Bbd
2. Hela: GFP-H2B
3. Hela

Data courtesy of Dr. H. Kimura, Horizontal Medical Research Organization, Kyoto University Faculty of Medicine

Western blotting



CBB Staining Western blotting

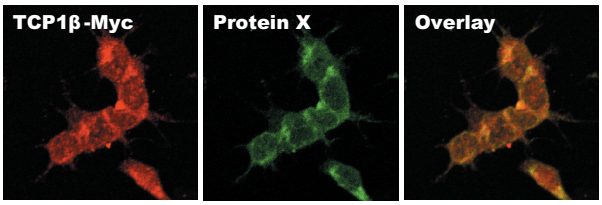
Sample : Protein extract from seedlings of Arabidopsis (10 mg)
1. Non-transformants (Control)
2. Transformants
Primary antibody : Anti-GFP (Mouse IgG1-κ) (1:5000)
Secondary antibody : Anti-Mouse IgG-HRP (1:2000)

Data courtesy of Dr. T. Shimada, Department of Botany, Graduate School of Science, Kyoto University

Anti-c-Myc (Mouse IgG1-k), Mono (MC045)

Clone : MC045
Isotype : IgG1-k (Mouse)
Product form : Liquid
Immunogen : c-Myc synthetic peptide [EQKLISEEDL] conjugated with KLH
Application : Western blotting 1:1000-1:2000
Immunoprecipitation 1:400-1:1000
Immunocytochemstry 1:400-1:1000
ELISA 1:2000-1:20000

Immunocytochemistry



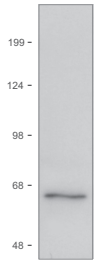
Sample SH-SY5Y cell in which c-Myc-tagged TCP1β is expressed
Primary antibody : Anti-c-Myc (Mouse IgG1-κ) (2.5 µg/ml)
Anti-Protein X
Secondary antibody : Anti-Fluor 546-conjugated antibody (1:400)
Anti-Fluor 488-conjugated antibody (1:400)

Data courtesy of RIKEN Brain Science Institute

Anti-6xHis (Mouse IgG1a-k), Mono (HI192)

Clone : HI192
Isotype : IgG1-k (Mouse)
Product form : Liquid
Immunogen : 6xHis synthetic peptide [HHHHHH] conjugated with KLH
Application : Western blotting 1:1000-1:2000
ELISA 1:2000-1:20000

Western blotting



Sample:
Lysate from Sf9 cells in which His-tagged Drosophila PTEN is expressed

Filter : FluoroTrans [PALL]
Blocking : 5% non-fat dry milk/PBS (30 min)
Primary antibody : Anti-6xHis (Mouse IgG1κ), Monoclonal (HI192)
Wash : 0.25% Tween-20/TBS (10 min x 3)
Secondary antibody : Anti-Mouse IgG-HRP (1:1000) /2% BSA/0.25% Tween-20 /TBS (30 min)
Wash : 0.25% Tween-20/TBS (10 min x 5)
Detection : Luminol Reagent (Santa Cruz)

Data courtesy of Dr. A. Maehama, The Tokyo Metropolitan Institute of Medical Science (RINSHOKEN)

Ordering Information

Product Name	Application	Storage	Product No.	PKG Size
Anti-c-Myc (Mouse IgG1-k), Monoclonal (MC045), AS	WB, IP, ICC, ELISA	R	04362-76	50 µg
			04362-34	200 µg
Anti-c-Myc (Mouse IgG1-κ), Monoclonal (MC045), AS, Agarose Conjugate	IP	R	04145-55	500 µg
Anti-GFP (Mouse IgG1-k), Monoclonal (GF200), AS	WB, ELISA	R	04363-66	50 µg
			04363-24	200 µg
Anti-GFP (Rat IgG2a), Monoclonal (GF090R), CC	WB, IHC ELISA	R	04404-26	50 µg
			04404-84	200 µg
Anti-GFP (Rat IgG2a), Monoclonal(GF090R), CC, Agarose Conjugate	IP	R	06083-05	500 µg
Anti-6xHis (Mouse IgG1-k), Monoclonal (HI192), AS	WB, ELISA	R	04428-26	50 µg
			04428-84	200 µg

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

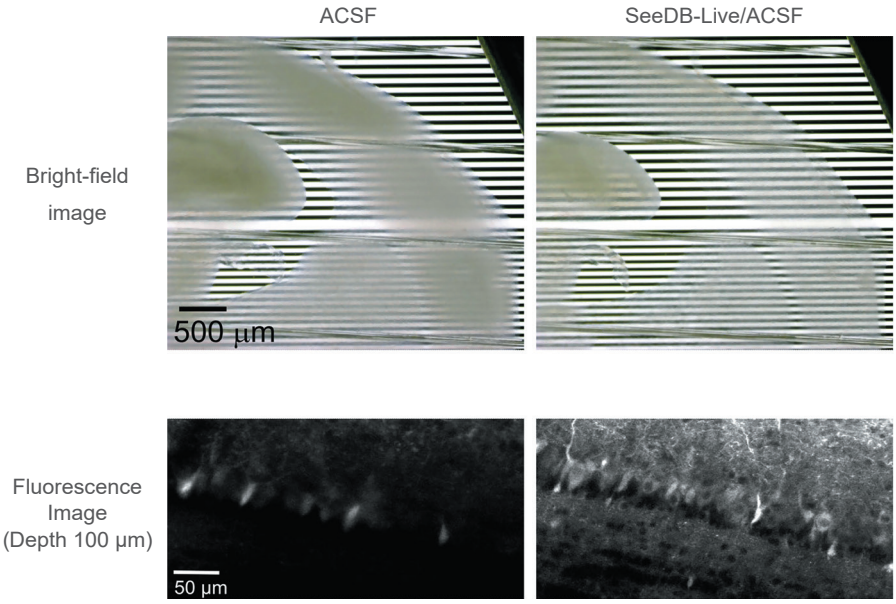
SeeDB-Live/ACSF

This product allows fluorescent observation of neuronal activity while maintaining normal physiological functions by rendering the tissue transparent. Application of this product to acute brain slices has enabled, for the first time worldwide, non-invasive optical clearing of living tissue. This product is manufactured and sold by NACALAI TESQUE, INC. under a license from Kyushu University.

» **Transparent brain tissue with preserved normal neuronal activity**
» **Enabling deeper fluorescence imaging**
» **Non-cytotoxic**

Application

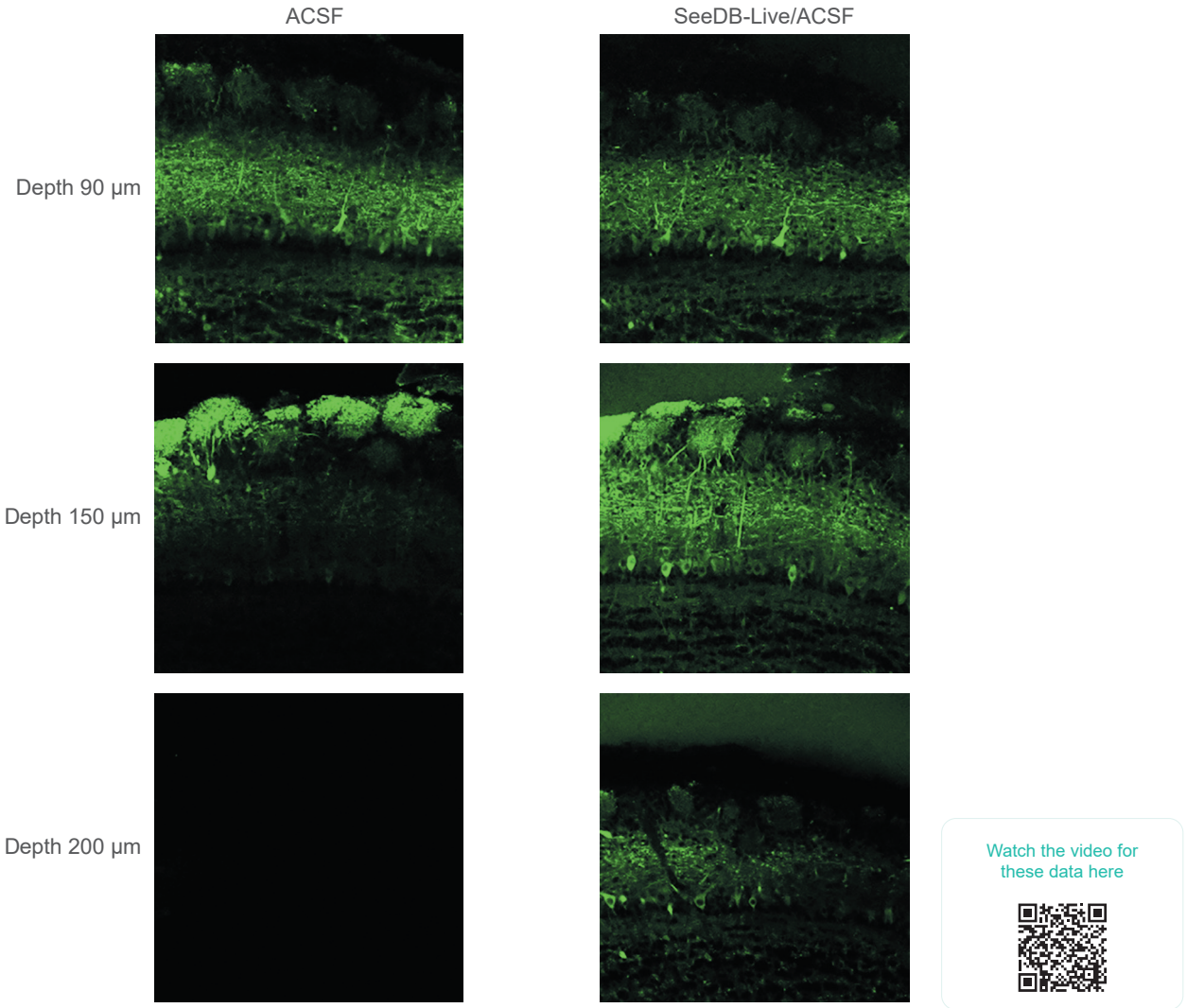
Acute brain slices (300 µm thick) were prepared from P5 mice. After perfusion with ACSF and SeeDB-Live/ACSF, transmitted-light images were acquired. In a separate experiment, olfactory bulb slices from P13 Thy1-GCaMP6f mice were imaged using confocal microscopy.



These data were provided by Professor Takeshi Imai and Assistant Professor Shigenori Inagaki, Faculty of Medical Sciences, University of Kyushu.

Calcium imaging in acute olfactory bulb slice from Thy1-GCaMP6f mice

Acute olfactory bulb slices from Thy1-GCaMP6f mice were optically cleared using either SeeDB-Live/ACSF or ACSF and imaged by two-photon microscopy. The results demonstrated that SeeDB-Live/ACSF enables deep imaging of live tissue while preserving normal cellular function.



Watch the video for these data here

These data were provided by Professor Takeshi Imai and Assistant Professor Shigenori Inagaki, Faculty of Medical Sciences, University of Kyushu.

Ordering Information

Product Name	Storage	Product No.	PKG Size
SeeDB-Live/ACSF	R	23041-44	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

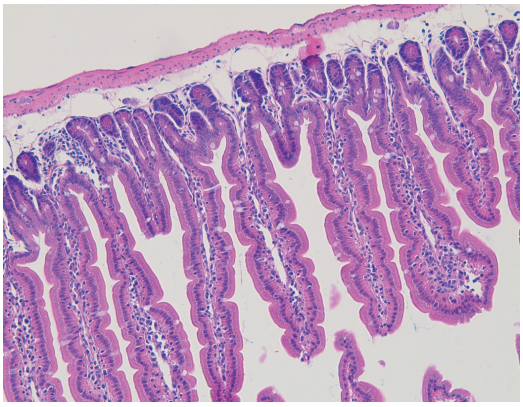
4% - Paraformaldehyde Phosphate Buffer Solution

We offer a 10% formalin neutral buffer solution, which contains about 4% formaldehyde, designed for use as a general fixation buffer in histological specimen preparations. Since this product is based on commonly available formalin, methanol is used as a stabilizer.
If a methanol free formalin solution is required, paraformaldehyde is used instead of formaldehyde. However, paraformaldehyde is extremely toxic and can cause injury if scattered. To deal with this hazard, additional work, such as making the solution alkaline with heating when dissolving the paraformaldehyde, is required. Our product is available in two volume types: 500 mL and a 5 x 10 mL package set.

- **Small unit volume**
- **Enables to immerse histological specimens directly into the solution (5 x 10 mL)**
- **Low cost for waste**
- **Ready-to-use**
- **Storable in refrigerator**

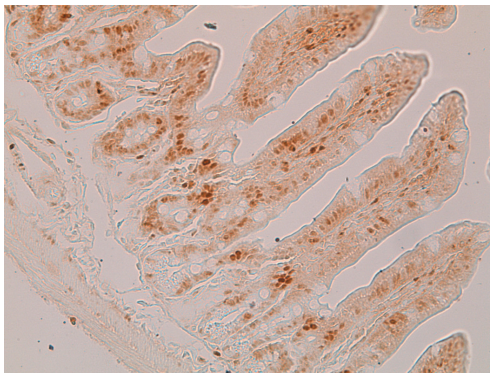
Usage examples

Hematoxylin-eosin staining



Sample	: Mouse small intestine
Fixation method	: Immersion fixation with this product (over night at 4ºC)
Deparaffinization	: Limonene and ethanol

Immunohistological staining



Sample	: Mouse small intestine
Fixation method	: Immersion fixation with this product (overnight at 4ºC)
Deparaffinization	: Limonene and ethanol
Primary antibody	: Anti-PCNA (FL-261) (rabbit)
Staining	: Peroxidase Stain DAB Kit (brown stain) (Product No. 25985-50)

Ordering Information

Product Name	Storage	Product No.	PKG Size
4% - Paraformaldehyde Phosphate Buffer Solution	R	09154-14	5 x 10 mL
		09154-85	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

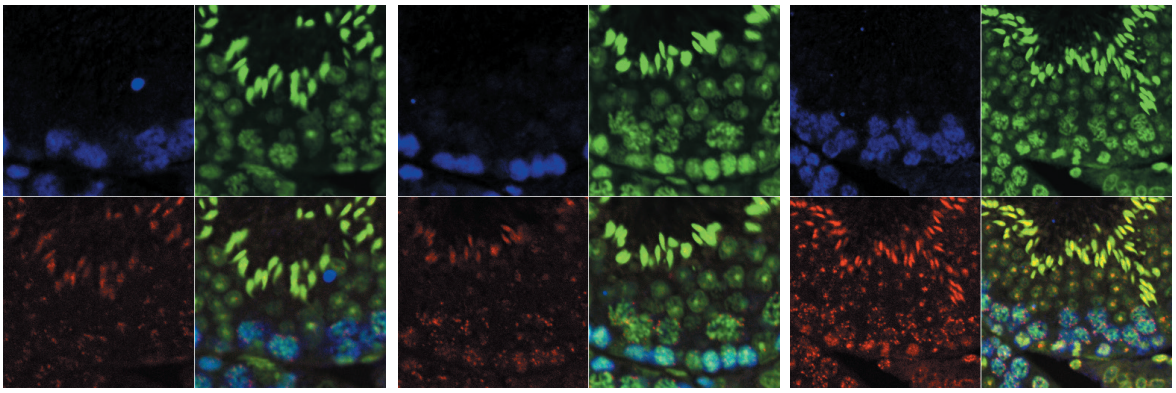
HistoVT One (10x, pH 7.0)

HistoVT One is an antigen retrieval solution for immunohistochemistry and *in situ* hybridization. This product can unmask antigenic sites without damage to antigen from formalin-fixed, frozen or paraffin-embedded tissue sections.

- » **Enhancing antigen-antibody reaction**
- » **Usable with frozen or paraffin-embedded tissue section**
- » **High reproducibility**

Application 1

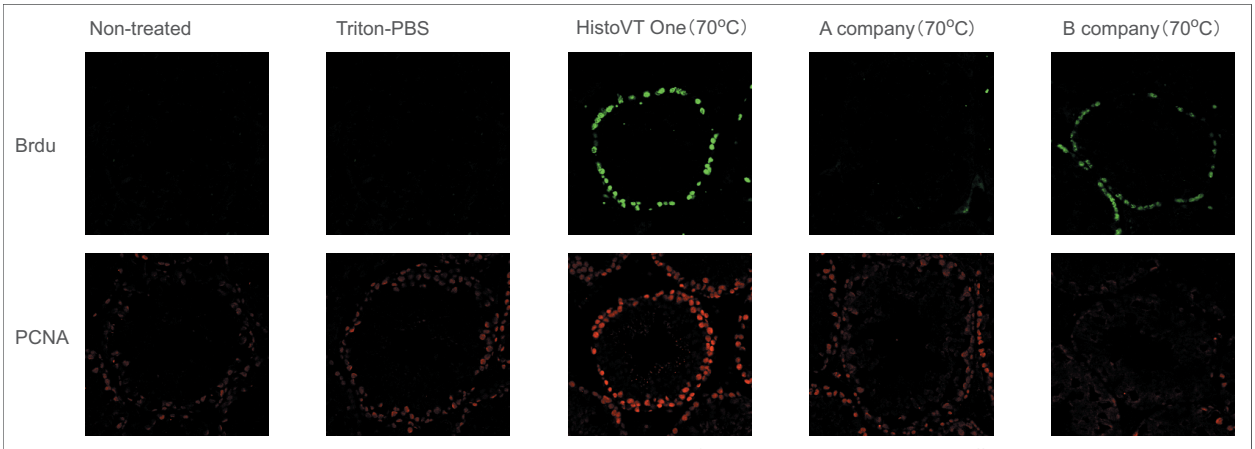
Formalin-fixed, paraffin-embedded tissue sections



Blue: PCNA (Immunohistochemistry) Green: Nuclear staining (Cytogreen staining) Red: Telomere DNA (FISH)

Application 2

Frozen sections



HistoVT One can unmask antigen more effectively than other treatments.

Data courtesy of RIKEN Brain Science Institute, Brain Development Research Group

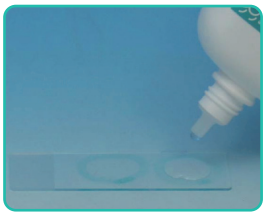
Ordering Information

Product Name	Storage	Product No.	PKG Size
HistoVT One (10x, pH 7.0)	RT	06380-05	500 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

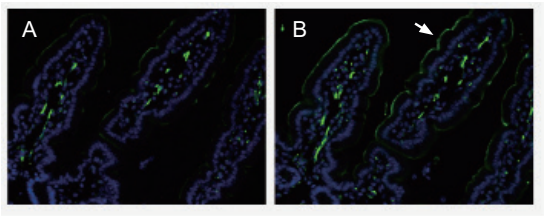
Blocking One Histo

Blocking One Histo is a blocking solution to prevent non-specific binding of antibodies in immunohistochemistry (IHC). The product is designed for immunohistochemistry application based on Blocking One (refer to Western Blotting Section).



- » **Eye-drop bottle**
- » **Can be used for immunofluorescence staining**
- » **The preservative does not affect the activity of alkaline phosphatase (AP) or horseradish peroxidase (HRP)**

Comparison of blocking efficiency with 10% goat serum (Immunofluorescence)

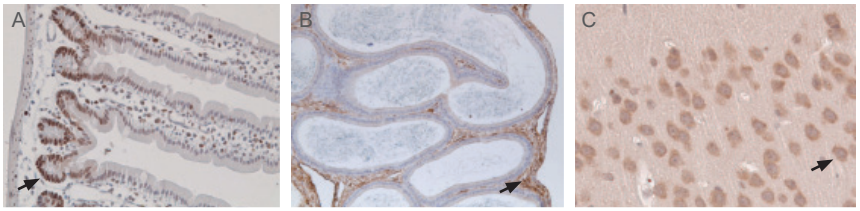


Blocking One Histo (10 min.) 10% Goat Serum (10 min.)

Sample : Mouse small intestine (Paraffin-embedded section)
Antigen Retrieval : Histo VT One, 90°C, 20 min.
Primary antibody : Anti-Vimentin rabbit polyclonal antibody (Santa Cruz: #sc-7557R)
Secondary antibody : CF™ 488A Goat anti-Rabbit IgG(H+L), $F(ab')_2$ Fragment (Biotium: #20013)

In both panels, mouse small intestine tissue section was stained with secondary antibody conjugated with CF™ 488A (green) and counter stained with DAPI (blue). In the panel B with 10% Goat Serum, the stained white arrow along the lines of shape of small intestine show non-specific staining. Blocking One Histo is more effective at reducing non-specific background staining than normal serum.

Applications



A: Mouse small intestine (PCNA) x5
B: Mouse epididymis (Vimentin) x25
C: Mouse brain (GluR) x100

Antigen Retrieval : Histo VT One, Room temp., 10 min.
Primary antibody : A: Anti-PCNA rabbit pAb (Santa Cruz: #sc-7907)
B: Anti-Vimentin rabbit pAb (Santa Cruz: #sc-7557R)
C: Anti-GluR-1 goat pAb (Santa Cruz: #sc-7608)
Secondary antibody : A: Goat anti-rabbit IgG (H+L), biotin conjugated (Vector, #BA-1000)
B: Goat anti-rabbit IgG (H+L), biotin conjugated (Vector, #BA-1000)
C: Bovine anti-goat IgG (H+L), biotin conjugated (Santa Cruz: #sc-2347)
Detection : Streptavidin Biotin Complex Peroxidase Kit (Product No. 30462)
Peroxidase Stain DAB Kit (Brown Stain) (Product No. 25985)

Blocking treatment of each tissue section had been performed by Blocking One Histo. Mouse small intestine (panel A) was stained with anti-PCNA and DAB (3,3'-Diamino Benzidine) to stain nuclear (black arrow), Mouse epididymis (panel B) was stained with anti-Vimentin and DAB to stain muscle (black arrow), Mouse brain (panel C) was stained with anti-GluR and DAB to stain membrane proteins (black arrow) and counter stained with hematoxylin.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Blocking One Histo	R	06349-64	50 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

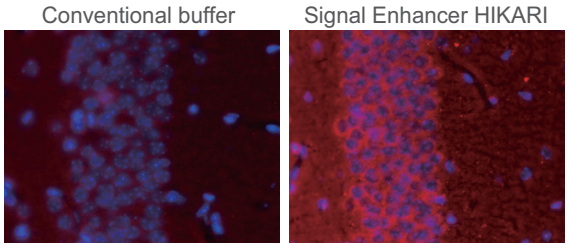
Signal Enhancer HIKARI for Immunostain

Signal Enhancer HIKARI for Immunostain was developed to resolve the problems of low sensitivity and high background often encountered during immunostain procedures such as immunohistochemistry (IHC) and immunocytochemistry. Dilute your antibodies with Signal Enhancer HIKARI for Immunostain instead of conventional diluents such as PBS or TBS before performing your next IHC experiment and witness a remarkable increase in the ability to detect the protein of interest and to eliminate unwanted background.

» **Enhances signals**
» **Reduces background**
» **Ready-to-use reagent**
» **Works with any detection system**

* The kit can also be used in combination with sensitizing systems such as the ABC or polymer complex method.

Applications



Brain tissue section was stained with secondary antibody conjugated with AF555 (red) and counter stained with Hoechst dye (blue).

Data courtesy of Dr. R. Ishimura, The Jackson Laboratory

Ordering Information

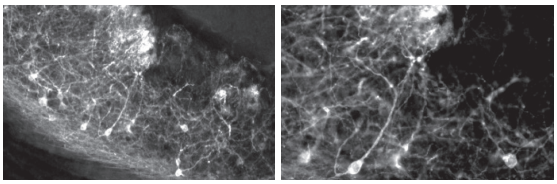
Product Name	Storage	Product No.	PKG Size
Signal Enhancer HIKARI for Immunostain Trial Set	R	02363-71	1 set (5 mL each)
Signal Enhancer HIKARI for Immunostain Solution A	R	02373-54	20 mL
Signal Enhancer HIKARI for Immunostain Solution B	R	02375-34	20 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Anti-GFP (Rat IgG2a), Monoclonal (GF090R)

» **Immunohistochemical application**

» **Rat monoclonal antibody**



Sample : Mouse brain (nerve cell)
Primary antibody : Anti-GFP(Rat IgG2a), Mono (1:1000) RT, O/N
Secondary antibody : Anti-Rat IgG-Cy3 (1:300) RT, 1 hr
Blocking 5% Normal goat serum/0.2% TritonX-100 in PBS
Fixing method : 4% Paraformaldehyde

Data courtesy of Dr. Y. Yoshihara, RIKEN Brain Science Institute

References for Immunostaining

1. Takeshi Sasamura *et al. Development* **134**, 1347-1356 (2007)
2. Takashi Inoue *et al. The Journal of Neuroscience*, **27**(20), 5461–5473 (2007)
3. Anoop Kumar G. Velikkakath *et al. Mol Biol Cell.* **23**(5), 896-909 (2012)
4. Eisuke Itakura *et al. Molecular Biology of the Cell*, **19**, 5360–5372,
5. Keith N. Brown, *et al. Science,* **334**, 480 (2011)
6. Anna N. Rubin *et al. The Journal of Neuroscience*, **30**(36), 12050-12062 (2010)
7. Shinsuke Nakao *et al. J Cell Biol.,* **182**(2), 395–410 (2008)
8. Maiko Ogata *et al. Mol Cell Biol*., **26**(24), 9220–9231 (2006)
9. Matthew Fogarty *et al. The Journal of Neuroscience*, **27**(41), 10935-10946 (2007)
10. Takuya Sato *et al. Nature Communications,* **2** (472)
11. Toshiaki Nakashiba *et al. Science*, **319**(5867), 1260-4 (2008)
12. Hiromi Takanaga *et al. Stem Cells*, **27**(1), 165-74 (2009)
13. Akinori Yamasaki *et al. Mol. Biol. Cell ,* **17** (11), 4876-4887 (2006)
14. Natsumi Ageta-Ishihara *et al. The Journal of Neuroscience*, **29**(43), 13720-13729 (2009)
15. Naoyuki Asada *et al. Journal of Neuroscience*, **30**(26), 8852-8865 (2010)
16. Shizue Ohsawa *et al. Dev Cell,* **20**(3), 315-28 (2011).

Ordering Information

For more informations about Epitope Tag Antibody, please refer to p101.

Product Name	Storage	Product No.	PKG Size
Anti-GFP(Rat IgG2a), Monoclonal(GF090R), CC	R	04404-26	50 µg
Anti-GFP(Rat IgG2a), Monoclonal(GF090R), CC, Agarose Conjugate	R	06083-05	500 µg

Streptavidin Biotin Complex Peroxidase Kit

Streptavidin Biotin Complex Peroxidase Kit includes reagents for the "Avidin-Biotin Complex, ABC technique", a highly sensitive method for immunoblotting, immunohistochemistry, ELISA and *in situ* hybridization.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Streptavidin Biotin Complex Peroxidase Kit	R	30462-30	1 KIT

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

High Sensitivity Peroxidase DAB Stain

Peroxidase DAB Stain Kit and Metal Enhancer

Peroxidase Stain DAB Kit (Brown Stain) is used to detect horseradish peroxidase (HRP) activity and stain them brown in immunoblotting, immunohistochemistry and *in situ* hybridization.
Metal Enhancer for DAB Stain (Product No. 07388-24) is used to stain peroxidase purplish gray with Peroxidase Stain DAB Kit (Brown Stain)(Product No. 25985-50) in immunoblotting, immunohistochemistry and *in situ* hybridization. The sensitivity of Metal Enahcer for DAB Stain used with Peroxidase Stain DAB Kit (Brown Stain) is about two times higher than the product with Peroxidase Stain DAB Kit (Brown Stain) alone.

- **High sensitivity**
- **Metal Enhancer for DAB Stain stains peroxidase purplish gray**
  (Just change the solution mix from water to Metal Enhancer for DAB)
- **RNase, DNase free, applicable to *in situ* hybridization**
- **Eye drop bottle**

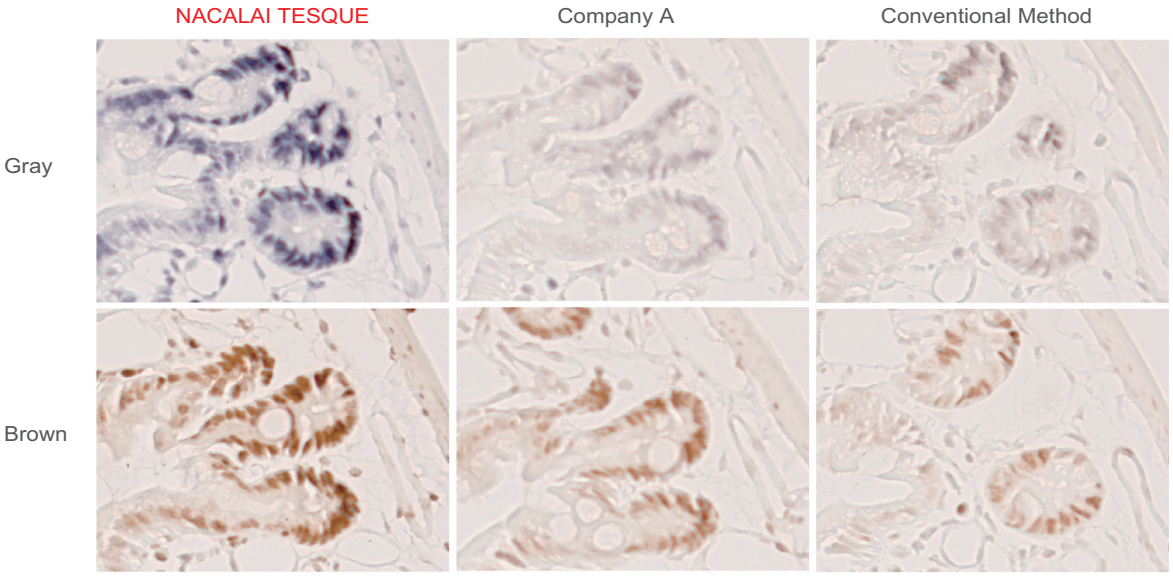

Procedure of combination Peroxidase Stain DAB Kit and Metal Enhancer Solution

Change the adjusting solution of Peroxidase Stain DAB Kit (Brown Stain) from water to Metal Enhancer for DAB.

Application

Immunohistostaining of mouse small intestines with anti-PCNA antibody (Serial membranes)

The sensitivity achieved when the Peroxidase Stain DAB Kit (Brown Stain) is used alone is higher than the competitors' products. However, when used in conjunction with Metal Enhancer for DAB stain, the sensitivity of Peroxidase Stain DAB Kit (Brown Stain) is about two times higher than what can be achieved by the Peroxidase Stain DAB Kit (Brown Stain) alone.



Reaction Time: 7 min.

NACALAI TESQUE : (Gray) Peroxidase Stain DAB Kit (Brown Stain) + Metal Enhancer for DAB
(Brown) Peroxidase Stain DAB Kit (Brown Stain)

Vector : (Gray) Kit (witth attached nickel solution)
(Brown) Kit (without attached nickel solution)

Basic method : (Gray) 0.6mg/mL DAB, 0.03%H_2O_2, 50mM Tris-HCl Buffer pH7.6, 0.03%$NiCl_2$
(Brown) 0.6mg/mL DAB, 0.03%H_2O_2, 50mM Tris-HCl Buffer pH7.6

Ordering Information

Product Name	Storage	Product No.	PKG Size
Peroxidase Stain DAB Kit (Brown Stain)	R	25985-50	1 KIT
Metal Enhancer for DAB Stain	RT	07388-24	100 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

Mounting Medium for Fluorescent Staining

Fluoro-KEEPER Antifade Reagent is a non-hardening mounting medium with a unique antifade reagent. It suppresses rapid photobleaching during fluorescence microscopy observation. The coverslipped slide with nail polish or other sealants can be stable for several weeks. There are two types of products available, with DAPI [4',6-Diamidino-2-phenylindole] and without DAPI, which counterstains nucleus blue.

» **Inhibits photobleaching of various fluorescent dyes**
» **Easy to use with eye-drop bottle**

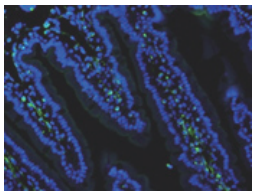
Application 1 (Fluoro-KEEPER with DAPI)

Fluorescent microscopy experiments

Fluoro-KEEPER with DAPI offers nuclear staining along with mounting.

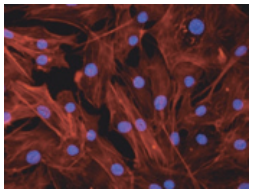
Mounting Medium : Fluoro-KEEPER with DAPI for 30 min. at room temperature protecting from light.
Microscopy : Olympus BX-50-34-FLA1

Mouse Small Intestine



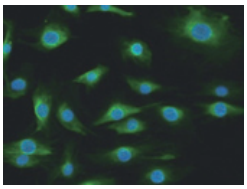
Antigen retrieval : HistoVT One (#06380)
Blocking : Blocking One Histo (#06349)
1st Ab : Anti-Vimentin Rabbit Polyclonal Antibody (Santa Cruz #sc-7557R)
2nd Ab : CFTM 488A Goat Anti-Rabbit IgG (H+L), Fragment (Biotium #20013)

MC3T3-E1 Cell



Blocking : Blocking One Histo (#06349)
Rhodamine-conjugated phalloidin (Cytoskeleton #PHDR1)

MC3T3-E1 Cell



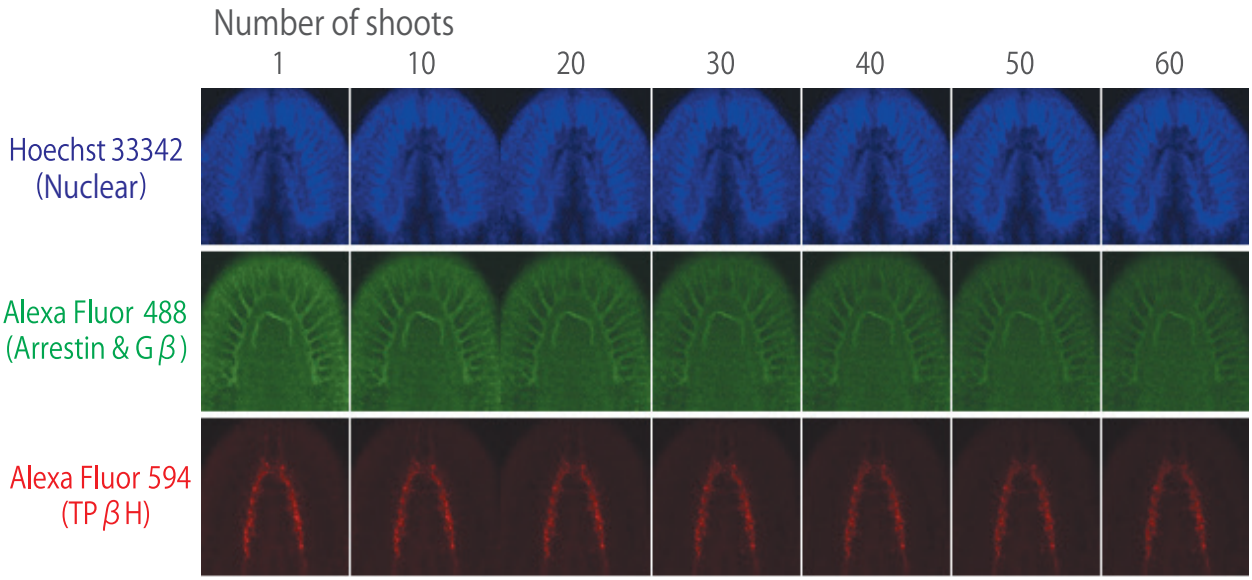
Blocking : Blocking One Histo (#06349)
1st Ab : Anti-Vimentin Rabbit Polyclonal Antibod (Santa Cruz #sc-7557R)
2nd Ab : Cy®2 Goat Anti-Rabbit IgG (H+L)(GENETEX #GTX26940)

Application 2 (Fluoro-KEEPER without DAPI)

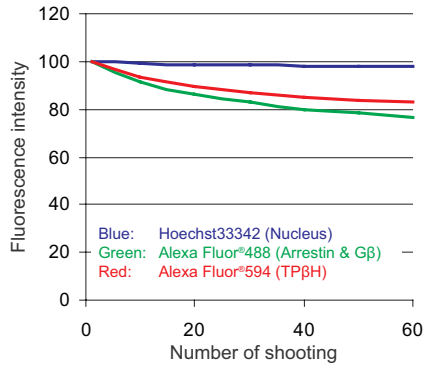
Fluorescent microscopy experiments: Planarian

Fluorescence intensities are shown as percentages of initial intensities remaining during repeated frame capture up to 60 times. The images were acquired by Olympus FV10. The samples mounted in the Fluoro-KEEPER Antifade Reagent were clearly detected after 60 times of frame capture.

■ Stain Image



■ Fluorescence intensity



Nuclear
Hoechst 33342
Arrestin and G Protein β Subunit(Gβ)
1st Ab: Mouse Anti-planarian Arrestin
Mouse Anti-planarian Gβ
2nd Ab: Alexa Fluor 488 Goat Anti-mouse IgG
Tryptophan β Hydroxylase(TPβH)
1st Ab: Rabbit Anti-planarian TPβH
2nd Ab: Alexa Fluor 594 Goat Anti-rabbit IgG

Samples were mounted in the Fluoro-KEEPER Antifade Reagent

Data courtesy of Agata Lab, Department of Biophysics, Kyoto University

Comparison of antifade effectiveness with different fluorescent dyes

Fluoro-KEEPER Antifade Reagent offers increased resistance to photobleaching of various fluorescent dyes.

	without DAPI		with DAPI	
Fluorescence dye	**Fluoro-KEEPER**	**Control**	**Fluoro-KEEPER**	**Control**
Hoechst 33258	98	96	—	—
Hoechst 33342	100	90	—	—
DAPI	99	93	—	—
Propidium Iodide	95	67	—	—
Fluorescein	97	49	96	49
Alexa Fluor 488	93	86	96	91
CF 488	93	82	91	82
Cy 2	99	83	98	81
Rhodamine	72	51	78	41
Alexa Fluor 555	98	81	97	87
CF 555	98	85	97	85
Cy 3	89	71	86	66

Cells stained by each fluorescent dye were mounted in Fluoro-KEEPER Antifade Reagent, 85% Glycerol containing PBS as a control. Samples were illuminated for 60 seconds. Each number indicates fluorescence intensity as percentage of initial intensity after 60 seconds exposure photobleaching.

Control condition: 85% Glycerol-PBS w/o DAPI
85% Glycerol-PBS w/ DAPI

Fluorescent microscopy: Olympus BX-50-34-FLA1

Exposure time: 60 seconds

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Ordering Information

Product Name	Storage	Product No.	PKG Size
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type	R	12593-64	2 x 5 mL
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type with DAPI	R	12745-74	2 x 5 mL

[Storage] RT = Room Temperature, R = Refrigerate, F = Freezer

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