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The quality for certainty.

High-speed transfer buffer for Bullet PAGE One Precast Gels

Bullet Semi-Dry Transfer One

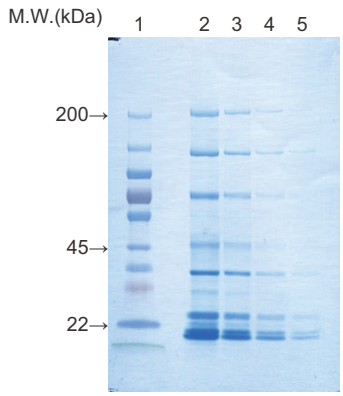
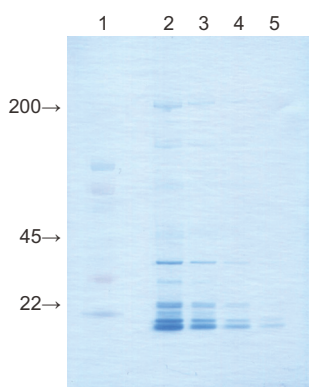
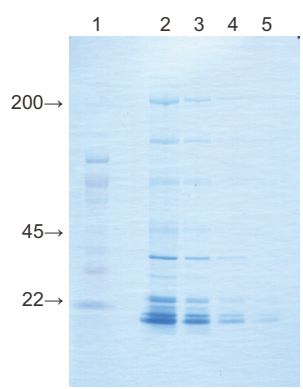
Nacalai Tesque has developed a transfer buffer for use with western blotting that allows for fast semi-dry protein transfer.
* This product is suitable for use with our Bullet PAGE One precast gel. It may not work as expected with other products.

Features

- ► Protein transfer in 10 min
- ► High transfer efficiency with a wide range of molecular weights
- ► No gel equilibration necessary
- ► Ready to use

Performance Comparison

Bullet Semi-Dry Transfer One was compared to the conventional methods by Kyhse-Andersen (1984) and Towbin (1979). Under the same conditions, it achieved higher transfer efficiency.

Transfer buffer	**Bullet Semi-Dry Transfer One**	Kyhse-Andersen	Towbin
Conditions	10V (constant), 10 min		
Membrane image			

**1 Kyhse-Andersen J.1984. J. Bio chem. Biophys. Methods 10:203-209*
Composition: 48 mM Tris, 39 mM Glycine, 20% Methanol, 0.04% SDS

**2 Towbin H., Staehelin T., and Gordon J. 1979. Proc. Natl. Acad Sci 76:4350-4354.*
Composition: 25 mM Tris, 192 mM Glycine, 20% Methanol

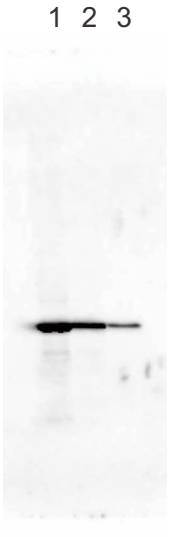
Conditions

Electrophoresis gel:	Bullet PAGE One Precast Gel 5-15% (#13079)
Samples:	1. Protein Ladder One (#09547), 5 µl added to each lane
	2. Protein Markers (10x) (#29458), 2 µl added to each lane
	3. Protein Markers (10x) (#29458), 3x diluted, 2 µl added to each lane
	4. Protein Markers (10x) (#29458), 10x diluted, 2 µl added to each lane
	5. Protein Markers (10x) (#29458), 30x diluted, 2 µl added to each lane
Membrane:	PVDF
Transfer conditions:	10V (constant voltage), 10 min
Detection:	CBB Stain One (#04543)

Application

Detection by antigen-antibody reaction

β-Actin's strong signal yielded a clear band using only 10V for 10 min to transfer proteins. A weaker signal, such as COX4's, may be detected more easily by increasing the voltage.

Protein	β-Actin	COX4	
Voltage	10V	10V	25V
Transfer time	10 min		
Chemiluminescence image			

Method

● Sample

Proteins extracted from HL-60 cells using RIPA Buffer (#08714).

Lane 1 : 10 µg
Lane 2 : 3 µg
Lane 3 : 1 µg

● Summary of procedures

The above results were obtained using our Bullet series to speed up electrophoresis and western blotting.

Protein	Products used	Time
Electrophoresis	Bullet PAGE One Precast Gel 5-15% (#13079)	10 min
Semi-dry protein transfer	Bullet Semi-dry Transfer One (#15353) *PVDF membrane	10 min
Blocking	Bullet Blocking One (#13779)	5 min
Primary antibody reaction	Anti β-Actin(C4)(Mouse), Monoclonal (Santa Cruz #sc-47778) diluted 1000x	60 min
	Anti-COX4(D-20)(Goat)(Santa Cruz #sc-69359) diluted 1000x	
Secondary antibody reaction	Anti-Mouse IgG(Goat), HRP Conjugate (Santa Cruz #sc-2005) diluted 10,000x	60 min
	Anti-Goat IgG(Bovine), HRP Conjugate (Santa Cruz #sc-2350) diluted 10,000x	
Chemiluminescence detection	Chemi-Lumi One Super (#02230)	5 min. exposure

How To Use

[Materials required]

Western blotting filter paper (ex: Whatman® 3MM Chr , 70mm×85mm) 12 sheets
Transfer membrane (ex: PVDF membrane, 70mm×85mm) 1 sheet

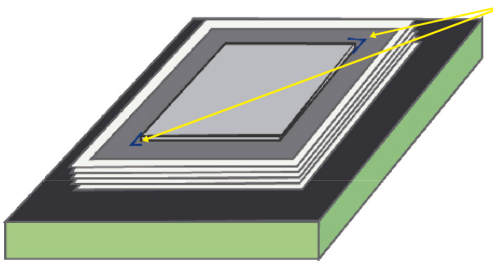
[Pre-wetting the membrane]

1. Soak the membrane with methanol (or ethanol) in tray.
2. Remove methanol (or ethanol) from the tray, add 100 mL of water and shake.

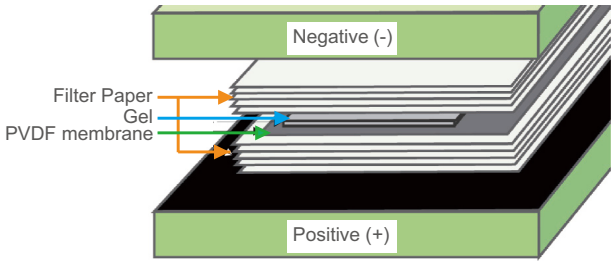
[Transfer]

> Note:
> 1) Use 40-50 mL of transfer buffer for one Bullet PAGE One Precast Gel.
> 2) Carefully assemble the gel, membrane and filter papers to avoid bubbles.

1. Soak 6 sheets of filter paper in transfer buffer and place on anode plate of a blotting apparatus.
2. Place membrane on filter papers.
   Note: Remove excess water with a paper towel.
3. Wash the gel with water for 10 seconds (within 20 seconds) and place on the membrane.
   Note: Remove excess water with a paper towel.



4. Soak 6 sheets of filter paper in transfer buffer and place on the gel.
5. Set the blotting apparatus and connect to a power supply.
6. Transfer proteins from the gel to the membrane with constant voltage (10-25V) for 10 minutes (refer to the below table).



Transfer condition for one Bullet PAGE One Precast Gel

Constant voltage	Time	Maximum current
25V	10 min	approx. 1.2A
10V	10 min	approx. 330mA

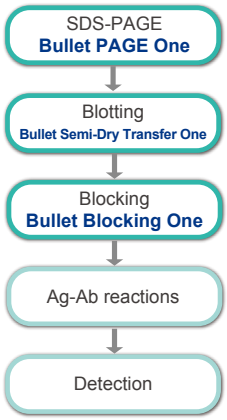
In case of insufficient transfer at 10V for 10 minutes, transfer at 25V (10 minutes), or extend the transfer time.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Bullet Semi-Dry Transfer One	Room Temp.	15353-01	1 L

Related Bullet Series Products

By combining Bullet PAGE One Precast Gel and Bullet Blocking One, protein electrophoresis and western blotting can be done quickly and efficiently.



Save nearly 2.5 hours from electrophoresis to detection

	Bullet Series	Conventional Products
Combination	• Bullet PAGE One Precast Gel • Bullet Semi-Dry Transfer One • Bullet Blocking One	• Laemmli SDS-PAGE Gel • Blocking reagent (5% skim milk)
Electrophoresis	10 min. under 400V	1 hr. under 200V
Blotting	10 min.	45 min.
Blocking	5 min.	1 hr.
Ag-Ab reactions Detection	2 hrs. and 40 min.	
Total	About 3 hrs.	About 5 hrs. and 30 mins.

10 min Bullet PAGE One Precast Gel

Features

- ► Only 10 minutes with 400 V
- ► High transfer efficiency of proteins on western blot membrane
- ► Works well in conventional Laemmli running buffer and sample buffer
- ► 17-well gel is usable with multichannel pipet for consistent loading

Ordering Information

Product Name	Wells	Product No.
Bullet PAGE One Precast Gel, 5-11%	13	13077-04
	17	13078-94
Bullet PAGE One Precast Gel, 5-15%	13	13079-84
	17	13080-44

Product Name	Wells	Product No.
Bullet PAGE One Precast Gel, 6%	13	13081-34
	17	13082-24
Bullet PAGE One Precast Gel, 8%	13	13083-14
	17	13084-04

Storage: Refrigerator, PKG Size: 10 Sheets

5 min Bullet Blocking One for Western Blotting

Features

- ► Fast blocking in 5 min.

Ordering Information

Product Name	Grade	Storage	Product No.	PKG Size
Bullet Blocking One for Western Blotting	SP	4-8°C	13779-14	200 ml
			13779-01	1 L

For research use only, not intended for diagnostic or drug use.

NACALAI TESQUE, INC.

Nijo Karasuma, Nakagyo-ku, Kyoto 604-0855 JAPAN
TEL : +81-(0)75-251-1730
FAX : +81-(0)75-251-1763
Website : www.nacalai.com
E-mail : info.intl@nacalai.com