



## Cut antigen-antibody reaction time in half

# Bullet ImmunoReaction Buffer

When used for immunoblotting, Bullet ImmunoReaction Buffer may shorten antigen-antibody reaction time by half.

- **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**

### Application 1: Gly m4, a major soybean allergen

Western blotting	Saving-time protocol		Conventional protocol	
	(kDa)	(kDa)	(kDa)	(kDa)
Antibody diluent	Bullet ImmunoReaction Buffer	5% skim milk	Bullet ImmunoReaction Buffer	5% skim milk
Primary antibody: Anti-Gly m4 rabbit in-house polyclonal antibody(ST0225), X 1,000 dilution	<b>30 minutes</b> (Room temperature)		Overnight at 4°C	
Secondary antibody: Stabilized Peroxidase conjugated Goat anti-rabbit(H+L) manufactured by Thermo Fisher, X 400 dilution	<b>30 minutes</b> (Room temperature)		60 minutes (Room temperature)	

#### <Experimental details>

Sample : Commercially available soy milk  
 Blocking : Incubated for 60 minutes with 5% skim milk  
 Detection : ECL manufactured by Cytiva on X-ray film  
 Exposure time : 8 minutes for saving-time protocol / 1 minute for conventional protocol

Data courtesy of Professor Tatsuya Moriyama and Dr. Erika Yano, Faculty of Agriculture, Department of Applied Biological Chemistry, Kinki University

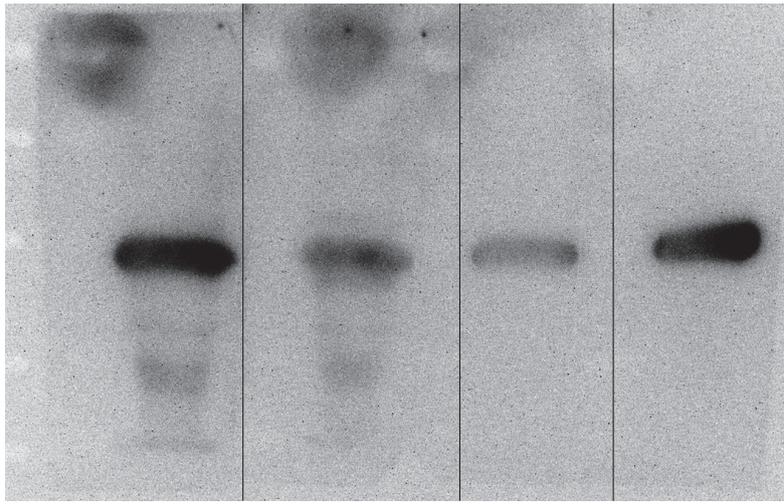
### Reference data: Comparison of sensitivity related to Ab-Ag reaction time with 0.1% tTBS

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

#### <Experimental details>

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

## Application 2: Comparison of detection of $\beta$ -Actin protein with 0.1% tTBS and 5% skim milk

Antibody diluent	Bullet ImmunoReaction Buffer	0.1%-tTBS	5% skim milk
Western blotting			
Primary antibody: Anti- $\beta$ -Actin rabbit manufactured by CST, X 5,000 dilution	<b>20 minutes</b>		60 minutes
Secondary antibody: anti-rabbit IgG(H+L) secondary antibody manufactured by Novous, X 5,000 dilution	<b>20 minutes</b>		30 minutes

<Experimental details>

Sample : White adipocyte cell extract; 10  $\mu$ g

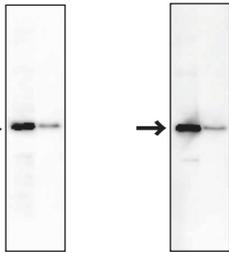
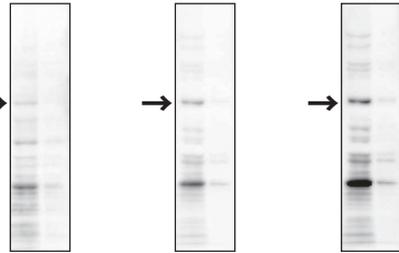
Blocking : Incubated for 60 minutes with 5% skim milk

Detection : ImmunoStar<sup>®</sup> LD manufactured by Fuji Film Wako, 15-minute exposure time

ImmunoStar<sup>®</sup> LD is a trademark by Fuji Film Wako

Data courtesy of Assistant Professor Tsuyoshi Goto and Dr. Masaomi Komori, Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University

## Application 3: Flotillin-1, $\beta$ -Actin and Nup98 proteins detection

Antigen	Flotillin-1		$\beta$ -Actin		Nup98		
							
Antibody diluent	tTBS	Bullet ImmunoReaction Buffer	tTBS	Bullet ImmunoReaction Buffer	tTBS	Bullet ImmunoReaction Buffer	
Primary antibody	60 minutes	<b>30 minutes</b>	60 minutes	<b>30 minutes</b>	60 minutes	<b>30 minutes</b>	
	Flotillin-1 antibody(C-7) (Santa Cruz #sc-133153) x 1,000 dilution		Human anti Human Actin Beta:HRP (Bio-Rad #HCA147P) x 1,000 dilution		Nup98 antibody(C-5) (Santa Cruz #sc-74578) x 1,000 dilution		
Secondary antibody	60 minutes	<b>30 minutes</b>	-		60 minutes	<b>30 minutes</b>	
	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP] (Pre-adsorbed)(Novus #NB7574), x 40,000 dilution				Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP] (Pre-adsorbed)(Novus #NB7574), x 40,000 dilution		
Exposure time	15 minutes		15 minutes		15 minutes	<b>5 minutes</b>	15 minutes

<Experimental details>

Sample : Flotillin-1 from HeLa cell extract, (left) 2  $\mu$ g and (right) 0.4  $\mu$ g

$\beta$ -Actin and Nup98 from HeLa cell extract, (left) 10  $\mu$ g and (right) 2  $\mu$ g

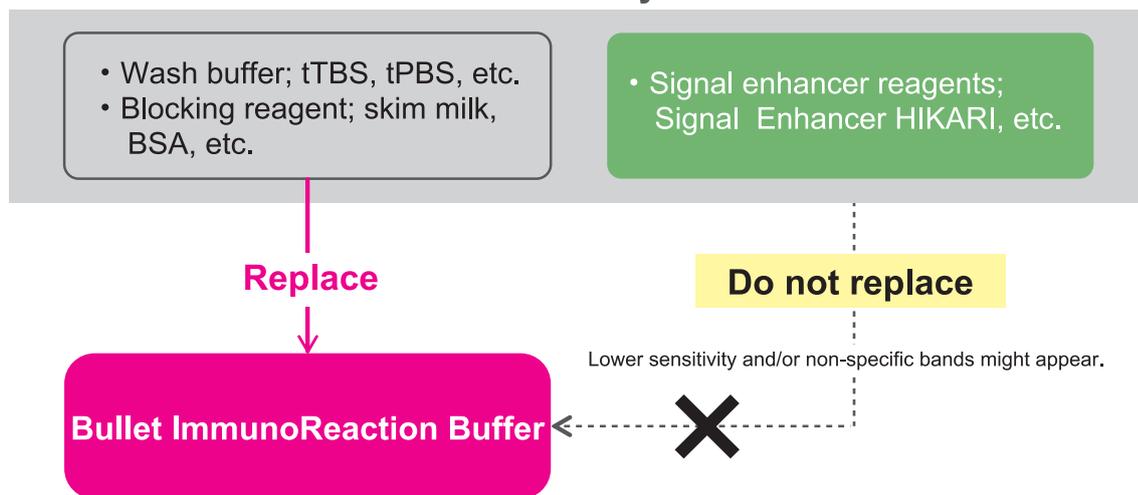
Blocking : Incubated for 5 minutes with Bullet Blocking One

Detection : Chemi-Lumi One Super

Detector : LAS-300 High mode

## Transition to saving-time protocol

### Current antibody diluent



Antibody diluent	Bullet ImmunoReaction Buffer	tTBS	HIKARI	A company	A company (Protein-free)	B company
Western blotting						
Primary antibody	30 minutes	60 minutes				
	EGF Receptor (D38B1)XP <sup>®</sup> Rabbit mAb(CST #4267), x 1,000 dilution XP is a trademark by Cell Signaling Technology, Inc.					
Secondary antibody	30 minutes	60 minutes				
	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP] (Pre-adsorbed)(Novus #NB7187), x 50,000 dilution					

<Experimental details>

Sample : HeLa cell extract, (left) 10 µg and (right) 2 µg

Blocking : Incubated for 5 minutes with Bullet Blocking One

Detection : Chemi-Lumi One Super manufactured by Nacalai, #02230; 3-minute exposure time

Detector : LAS-300 High mode

### Procedure

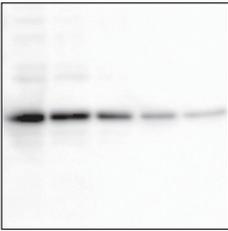
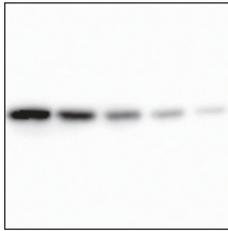
- Replace a current antibody diluent with Bullet ImmunoReaction Buffer
- Ab-Ag incubation time should be by half at a first trial; for a transition from overnight incubation protocol, 30–60-minute incubation time should be used.
- The instruction is downloadable through website, e-nacalai.

### Ordering information

Product Name	Grade	Storage	Catalog Number	PKG Size
Bullet ImmunoReaction Buffer	SP	Refrigerator	18439-85	500 mL

# Bullet series

The Bullet series is designed to shorten the time required for western blotting, including SDS-PAGE, semi-dry blotting, blocking and Ab-Ag reaction as below figure.

	Bullet series			Conventional method	
Western blotting					
Sample	Hela cell extract; 2-time dilution series started from 5 µg				
Electrophoresis	Bullet PAGE One Precast Gel, 5-15%, 17wells	400 V	<b>10 mins.</b>	Laemmli gel 10%, 18 wells	200 V 60 mins.
Transfer	Bullet Semi-Dry Transfer One	25 V	<b>10 mins.</b>	Semi-dry transfer buffer for Western blotting	10 V 45 mins.
Blocking	Bullet Blocking One		<b>5 mins.</b>	5% skim milk	60 mins.
Primary antibody GAPDH Antibody (Novous #NB300-322), x 10,000 dilution	Diluted with Bullet ImmunoReaction Buffer		<b>30 mins.</b>	Diluted with 0.1% tTBS 60 mins.	
Secondary antibody Goat anti-rabbit IgG(H+L) secondary antibody(Novous #MB7187), x 20,000 dilution	Diluted with Bullet ImmunoReaction Buffer		<b>30 mins.</b>	Diluted with 0.1% tTBS 60 mins.	
Detection	Chemi-Lumi One Super / 5-minute exposure time / LAS-3000 High mode				
Total required time	<b>About 2 hrs.</b>			About 5 hrs. and 30 mins.	

## Ordering information

Category	Product Name	Grade	Storage	Catalog Number	PKG Size
Precast gel	Bullet PAGE One Precast Gel, 5-11%, 13wells	SP	R	13077-04	10 sheets
	Bullet PAGE One Precast Gel, 5-11%, 17wells	SP	R	13078-94	10 sheets
	Bullet PAGE One Precast Gel, 5-15%, 13wells	SP	R	13079-84	10 sheets
	Bullet PAGE One Precast Gel, 5-15%, 17wells	SP	R	13080-44	10 sheets
	Bullet PAGE One Precast Gel, 6%, 13wells	SP	R	13081-34	10 sheets
	Bullet PAGE One Precast Gel, 6%, 17wells	SP	R	13082-24	10 sheets
	Bullet PAGE One Precast Gel, 8%, 13wells	SP	R	13083-14	10 sheets
	Bullet PAGE One Precast Gel, 8%, 17wells	SP	R	13084-04	10 sheets
Semi-dry transfer buffer	Bullet Semi-dry Transfer One	SP	RT	15353-01	1 L
Blocking reagent	Bullet Blocking One for Western Blotting	SP	R	13779-56	50 mL
				13779-14	200 mL
				13779-01	1 L
Antibody diluent	Bullet ImmunoReaction Buffer	SP	R	18439-85	500 mL
Gel staining solution	Bullet CBB Stain One(Ready To Use)	SP	R	13542-94	50 mL
				13542-65	500 mL
				13542-81	1 L

(Storage) RT: Room temperature, R: Refrigerator

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

