

Blindingly fast, blocking in 5 min!

# Bullet Blocking One for Western Blotting

Bullet Blocking One is a blocking reagent for western blotting, which exhibits excellent blocking efficiency in only 5 minutes due to the amphiphilic compound used.



## Features

- ▶ Fast blocking in 5 min.
- ▶ Ready to use

## Comparison Data: 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), 5 min. reaction time  
 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 min, while Bullet Blocking One performed well.

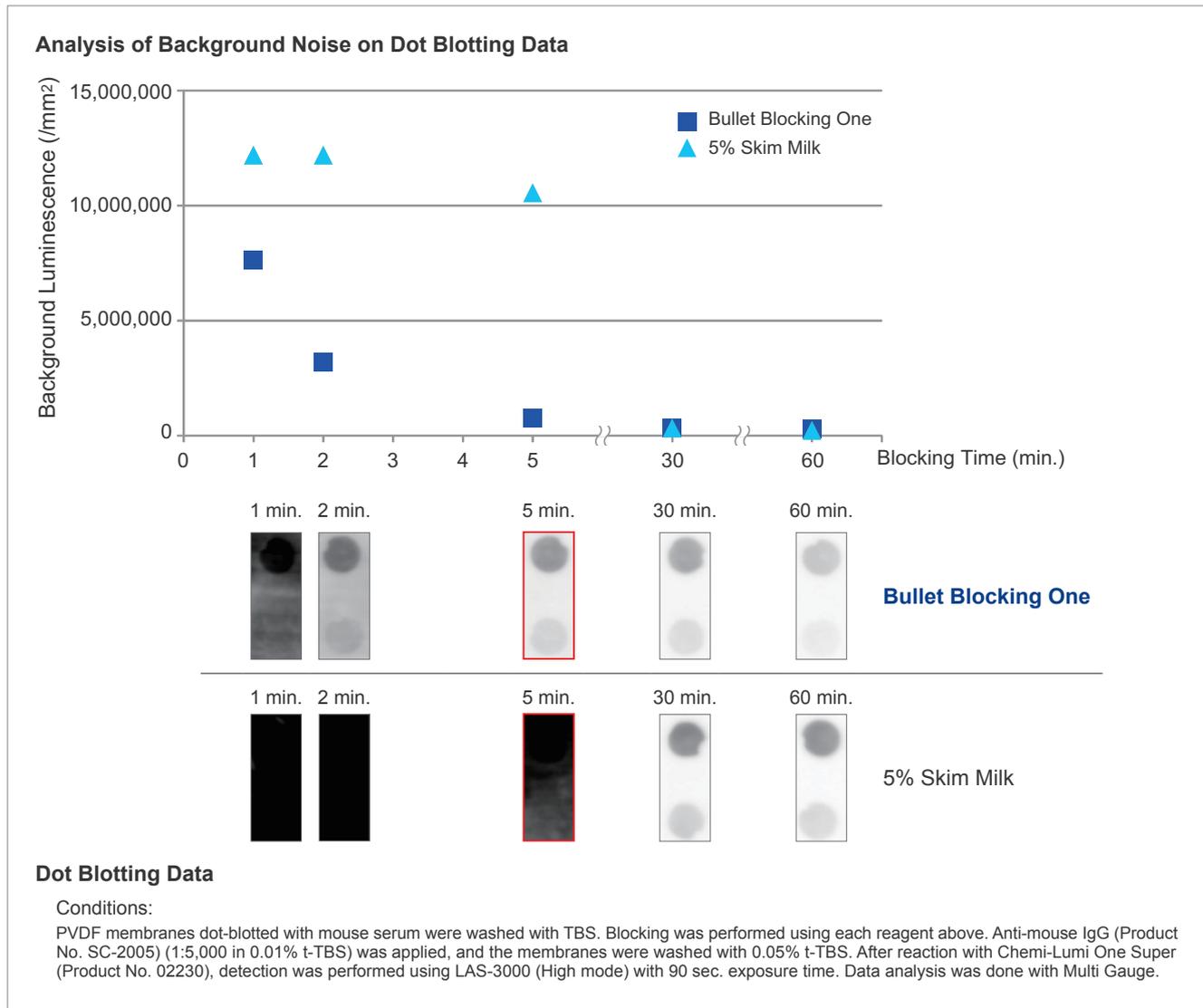
5 min. Blocking Time	Blocking Reagents	Manufacturers' Recommended Blocking Time
	<b>Bullet Blocking One</b>	
	Blocking One	30 min.
	Company A blocking reagent	60 min.
	Company B blocking reagent	30 min.
	Company C blocking reagent (protein-free)	60 min.
	5% Skim Milk in 0.05% Tween® 20-TBS	60 min.
	3% 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.



## 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:**

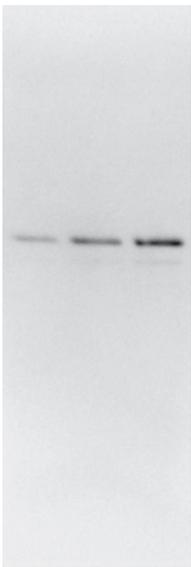
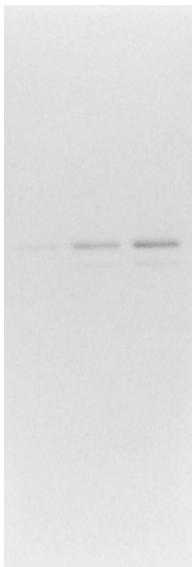
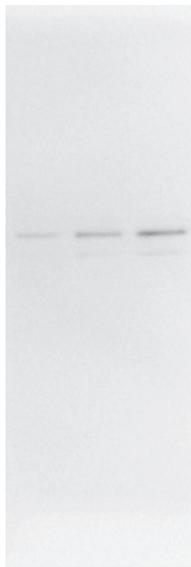
Sample: 10 µg of HeLa cell extraction, 3 serial two-fold dilution series  
 SDS-PAGE: Bullet PAGE One 5-15% (Product No. 13080) with SDS Running Buffer (Product No. 30329) at 400 V for 12 min.  
 Blotting: Semi-dry Blotting Buffer Solution (Product No. 30650) at 10 V for 30 min.  
 Washing: 0.1% t-TBS (Product No. 12750)  
 Blocking: Bullet Blocking One, 5 min.  
 1st antibody: Anti-Cox4 (D-20) (Goat) (Product No. SC-69359) diluted 1:500, 1 hr. at RT  
 2nd antibody: Anti-Goat IgG-HRP (Product No. SC-2350) diluted 1:5,000, 1 hr. 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. The appropriate dilution ratio depends on antibody conditions, such as type and concentration, pretest is required.

### Comparison with 5% Skim Milk

Bullet Blocking One showed good blocking efficiency in 5 min. Although optimizations of dilution ratio to types of antibody are required beforehand, in this experiment, diluting the antibody with Bullet Blocking One resulted in the strongest signal.

	Condition 1			Condition 2			Condition 3					
Blotting Image	protein (µg)	0.5	1	2	protein (µg)	0.5	1	2	protein (µg)	0.5	1	2
		UCP1→				UCP1→				UCP1→		
Blocking Reagents	Bullet Blocking One			Bullet Blocking One			5% Skim Milk					
Blocking Time	5 min.			5 min.			60 min.					
Antibody Diluent	Bullet Blocking One Diluted 20x			5% Skim Milk			5% Skim Milk					
1st Antibody	Anti-UCP1 Diluted 2,000x Room temperature, 1 hr.											
2nd Antibody	Anti-Rabbit IgG Diluted 2,000x Room temperature, 1 hr.											

Data courtesy of Associate Professor Goto Tsuyoshi / Kim Minji, Functions of Food that Support Everyone's Health, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University

### Protocol

Immerse a membrane thoroughly (about 50 ml for a 10 x 10 cm membrane) and shake for 5 min.

*Please note that over-blocking may cause signal reduction.*

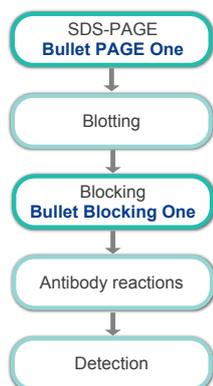
### Ordering Information

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

(Storage) R : Refrigerator

## 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 hours from electrophoresis to detection

	Bullet Series	Conventional Products
Combination	• Bullet PAGE One Precast Gel • Bullet Blocking One	Laemmli SDS-PAGE Gel/ 5% skim milk
Electrophoresis	10 min. under 400V	1 hr. under 200V
Blotting	45 min.	
Blocking	5 min.	1 hr.
Antibody reactions and detection	2 hrs. and 40 min.	
Total	<b>About 3 hrs. and 40 mins.</b>	About 5 hrs. and 30 mins.

## 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

### Performance Comparison

Bullet PAGE One gel can produce an excellent separation image with the shortest electrophoresis.

Product Name	Bullet PAGE One	Company A	Company B
Electrophoresis Time	<b>11 min.</b>	33 min.	50 min.
Electrophoresis Images			

### Ordering Information

Product Name	Well	Product No.	Product Name	Well	Product No.
Bullet PAGE One Precast Gel, 5-11%	13	13077-04	Bullet PAGE One Precast Gel, 6%	13	13081-34
	17	13078-94	Bullet PAGE One Precast Gel, 6%	17	13082-24
Bullet PAGE One Precast Gel, 5-15%	13	13079-84	Bullet PAGE One Precast Gel, 8%	13	13083-14
	17	13080-44	Bullet PAGE One Precast Gel, 8%	17	13084-04

Storage: Refrigerator, PKG Size: 10 Sheets

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

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