

Blindingly fast, blocking in 5 min!

# Bullet Blocking One for Western Blotting


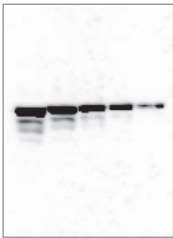

Bullet Blocking One is a blocking reagent for western blotting, which exhibits excellent blocking efficiency in only 5 minutes due to the amphiphilic compound used.

## 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.

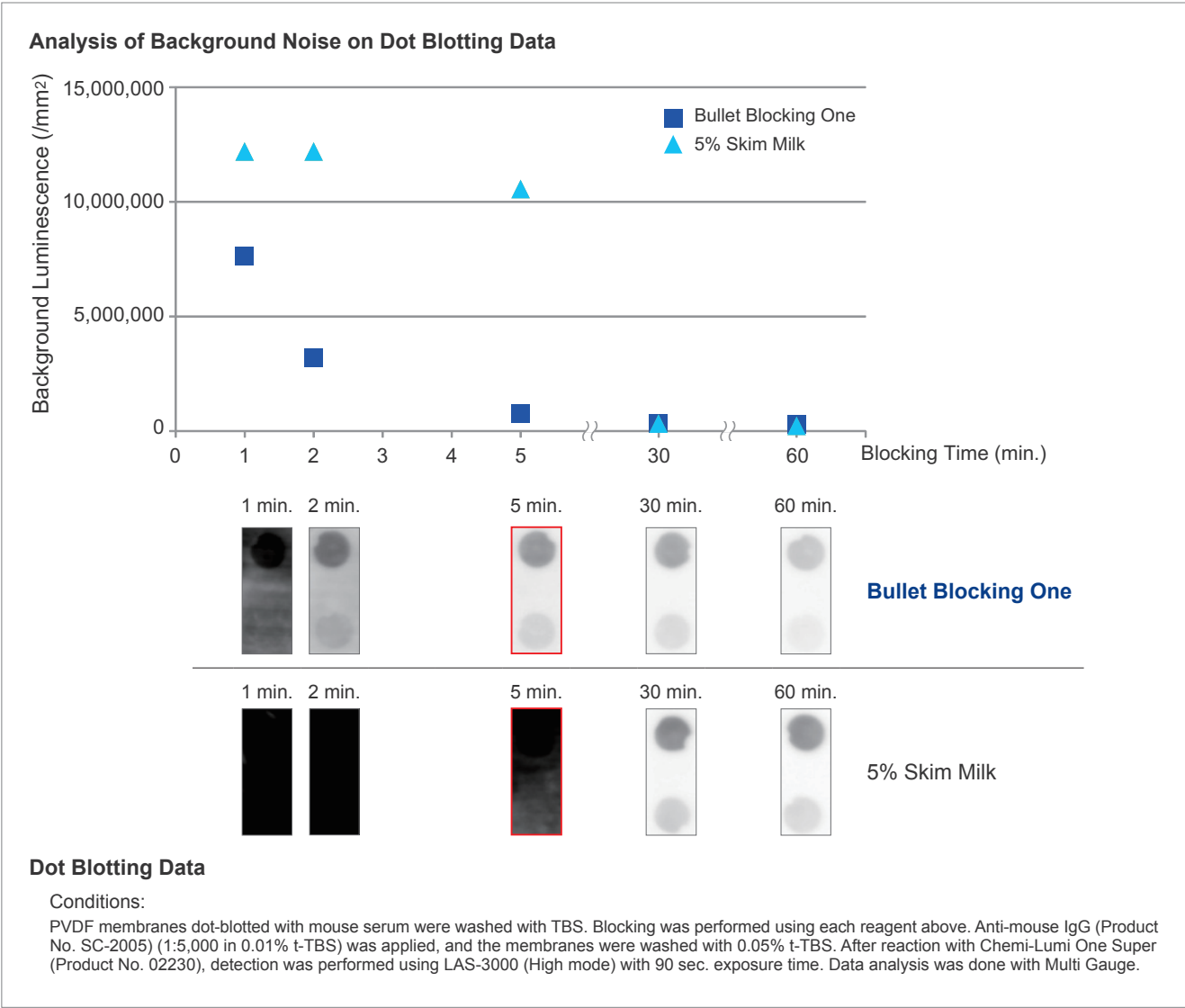
| 5 min. Blocking Time  | Blocking Reagents                               | Manufacturers' Recommended Blocking Time   |
|---|---|--|
|  | <b>Bullet Blocking One</b>                      |  |
|  | Blocking One                                    |  30 min. |
|  | Company A blocking reagent           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

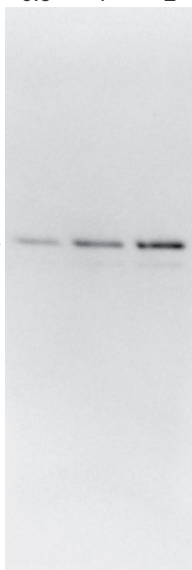
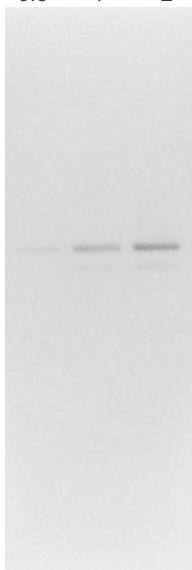
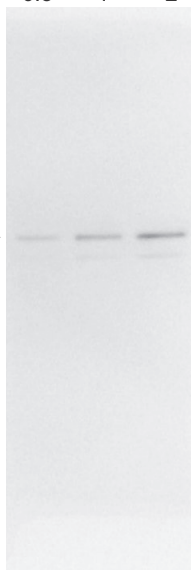
| Antibody Diluent | Bullet Blocking One (Undiluted) | 0.1% t-TBS |
|------------------|---------------------------------|------------|
| Blotting Image   |                                 |            |

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<br>( $\mu$ g)   | 0.5 | 1 | 2                   | protein<br>( $\mu$ g)   | 0.5 | 1            | 2     | protein<br>( $\mu$ g)   | 0.5 | 1 | 2 |
|                   |  |     |   | 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<br>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.*

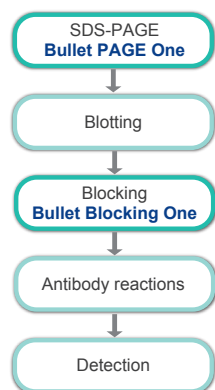
### Ordering Information

| 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<br>• 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                            | About 3 hrs. and 40 mins.                              | About 5 hrs. and 30 mins.          |

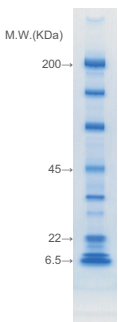
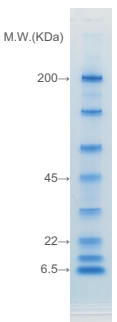
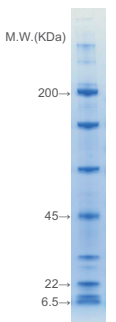
## Bullet PAGE One Precast Gel

### 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   | 11 min.   | 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    |                                 | 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    |                                 | 17   | 13084-04    |

Storage: Refrigerator, PKG Size: 10 Sheets

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

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