

Anti-Mouse IgG (Goat) , HRP-conjugated, Pre-absorbed Anti-Rabbit IgG (Goat) , HRP-conjugated, Pre-absorbed

Features

- Affinity purified
- Cross-absorbed*
 - * Anti-Mouse IgG antibody absorbed against Human IgG, Rat IgG, Rabbit IgG.
 - Anti-Rabbit IgG antibody absorbed against Human IgG, Rat IgG, Mouse IgG

Composition

HRP-conjugated antibody, D-PBS(-), 1%BSA, and a preservative

Required reagents

- Wash buffer:
TBS or PBS with 0.05-0.1%Tween20
- Blocking buffer:
One of the followings suggested.
 - a) Bullet Blocking One (Product No. 13779) : Fast blocking in 5 mins.
 - b) Blocking One (Product No. 03953)
 - c) Blocking One P (Product No. 05999) : Recommended for phosphoprotein detection.
 - d) 1-5% skim milk in tTBS or tPBS
 - e) 1-5% BSA in tTBS or tPBS
- Dilution buffer for antibody:
One of the followings suggested.
 - a) Wash buffer like tTBS or tPBS
 - b) Blocking buffer : Use blocking buffer undiluted or diluted the appropriated times with wash buffer.
Undiluted Bullet Blocking One is recommended for reducing high background.
 - c) Bullet ImmunoReaction Buffer (Product No. 18439) : Cut in half antigen-antibody reaction time.
 - d) Signal Enhancer HIKARI for Western Blotting and ELISA (Product No. 02770): Recommended for increasing weak signal.
- Detection reagent suggested:
 - a) Chemi-Lumi One L (Product No. 07880) : General sensitive.
 - b) Chemi-Lumi One Super (Product No. 02230) : Highly sensitive.
 - c) Chemi-Lumi One Ultra (Product No. 11644) : Ultra highly sensitive
 - d) TMB Solution for Western Blotting (Product No. 18186) : For colorimetric detection. The detected band can be observed without any equipment.

Protocol

(The following protocol is an example. Modification may be necessary.)

1. After electrophoresis, transfer protein samples from the gel to a membrane, and wash the membrane with wash buffer for 5 mins. Repeat once.
2. Block the membrane with blocking buffer. Blocking time varies depending on blocking buffer you use.
3. Rinse the membrane with wash buffer.
4. Dilute a primary antibody with an antibody diluent. Incubate the membrane with the diluted antibody for 30 mins to 2 hours at room temperature or overnight at 4°C.

5. Wash the membrane with wash buffer for 5 mins. Repeat twice.
6. Dilute this product with an antibody diluent. Incubate the membrane with the diluted antibody for 30 mins to 2 hours at room temperature.

Antibody dilution concentration varies depending on a detection reagent you use. Please refer to the table below.

< HRP-conjugated secondary antibody recommended concentration >

Detection Reagent	Recommended antibody concentration	
TMB Solution for Western Blotting	1:500 - 1:5,000	: 1:1,000 is recommended at first
Chemi-Lumi One L	1:2,500 - 1:25,000	: 1:2,000 is recommended at first
Chemi-Lumi One Super	1:10,000 - 1:100,000	: 1:20,000 is recommended at first
Chemi-Lumi One Ultra	1:50,000 - 1:500,000	: 1:100,000 is recommended at first

7. Wash the membrane with wash buffer for 5 mins. Repeat twice.
8. Detect bands with detection reagent.

Attention

- Optimize a primary antibody concentration and reaction time by preliminary experiments.
- If high background is observed, it is often caused by high concentration of the secondary antibody. To reduce the background, decreasing the concentration of the secondary antibody or diluting 1st and 2nd Ab with blocking buffer or Signal Enhancer HIKARI can be helpful. In addition, the high background can be reduced by rinsing the membrane with wash buffer several times after the secondary antibody reaction.

Additional information about troubleshooting, tips, and other details can be found in the 'Protocol for Western Blotting'(in Japanese), which is available at the following address.

<https://www.nacalai.co.jp/ss/Contact/pdf/WesternBrotting.pdf>



Storage

Refrigerator

Expiration

Expiration date is stated on the product label.

Packing

Anti-Mouse IgG(Goat), HRP-conjugated, Pre-absorbed
1 mL (Product No. 21860-61), 100 µL (Product No. 21860-74)

Anti-Rabbit IgG(Goat), HRP-conjugated, Pre-absorbed
1 mL (Product No. 21858-11), 100 µL (Product No. 21858-24)