EasyBlot Kit User's Manual

☐ GTX225856-01 EasyBlot anti-Rabbit IgG Kit (Optimized for Prot A/G)
☐ GTX225857-01 EasyBlot anti-Mouse IgG Kit (Optimized for Prot A/G)

Background:

EasyBlot anti-rabbit/mouse IgG is an HRP-conjugated secondary antibody that specifically reacts with the native, non-reduced form of rabbit IgG and does not bind to the reduced, denatured form. Using Easyblot as a second step reagent decreases the interference caused by the heavy (~50 kDa) and light chains (~25 kDa) of the IgG used for immunoprecipitation (IP).

The protein A/G beads used in the IP procedure sometimes bind to IgG and produce contaminating signals at approximately 50 kDa and 25 kDa. This kit also includes an **EasyBlocker** (Cat. No.: GTX425858) that can minimize the background caused by Protein A/G contamination. GeneTex's EasyBlot kit is simple to use and detects only the target band in your IP experiments, resulting in cleaner western blots.

Kit contents:

1. EasyBlocker, lyophilized. (Cat. No.: GTX425858)
2. GTX225856-01 EasyBlot anti-Rabbit IgG Kit (Optimized for Prot A/G) contains EasyBlot anti-Rabbit IgG (HRP) (Cat. No.: GTX221666-01)
3. GTX225857-01 EasyBlot anti-Mouse IgG Kit (Optimized for Prot A/G) contains EasyBlot anti-Mouse IgG (HRP) (Cat. No.: GTX221667-01)

Preparation of EasyBlocker reagent:

1. Keep the EasyBlocker powder at RT for 10mins before dissolving it.
2. Dissolve the blocking powder in TBST buffer (0.5 g powder in 10 ml TBST buffer). Shaking it for 30~40 mins at RT to make sure that it is dissolved completely.

Note: In order to conserve the EasyBlocker reagent, we recommend diluting the antibody in 2-3 ml of EasyBlocker reagent and incubating the transferred membrane in a sealed bag. Make sure to remove as much air from the bag as possible.

3. We recommend that you reconstitute the EasyBlocker reagent immediately before use.

Protocol:

1. After transferring the IP sample to a PVDF or NC membrane, block the membrane with **5 ml** of **EasyBlocker reagent** for 1 hr at room temperature.

2. Dilute the primary antibody with **2 ml** of **EasyBlocker reagent**. The incubation conditions will depend on the conditions used for the primary antibody.
3. Wash the membrane with TBST buffer 3 times for 5 min each time.
4. Dilute the **EasyBlot anti-rabbit/mouse IgG (HRP)** with **2 ml** of **EasyBlocker reagent** and incubate for 1 hr at room temperature.
5. Wash the membrane with TBST buffer 3 times for 5 min each time.
6. Develop the signal with ECL/ECL+ reagent and detect the signal using either X-ray films or a digital camera.

Note: The recommended dilution ratio for the EasyBlot anti-Rabbit/Mouse IgG (HRP) is 1:1000. Use 2-5 µg of the IP antibody; do not exceed 5 µg. Completely reduce all IP samples by 100mM DTT.

Recipe:

20X TBS buffer (1 L)	
Tris	48.4 g
NaCl	160 g
Add ddH_2O to 1 L. Mix to dissolve and adjust the pH to 7.0 using concentrated HCl.	

TBST buffer (1 L)	
20X TBS buffer	50 ml
Tween-20	2 ml
Add ddH_2O to 1 L. Adjust pH to 7.0 if necessary.	

Storage instructions:

1. EasyBlocker : store at 4℃.
2. EasyBlot anti-rabbit IgG (HRP): Aliquot and store as a concentrated solution at -20ºC or below. Avoid multiple freeze-thaw cycles.