

Product Data Sheet

Western Blot Reagents with Anti-Mouse & Anti-Rabbit HRP

Catalog number : WB-110-10, WB-110-25

Product Description

This pack contains HRP conjugated anti-mouse, anti-rabbit IgG secondary antibodies and enhanced chemiluminescence substrate reagents for Western Blotting or other immunoblotting tests.

Intended Use

For Research Use Only

Kit Includes

Cat. No. WB-110-10 : sufficient for 10 blots (8 x 10 cm)

- Anti-Mouse IgG HRP stock (Part No. 5201) 50 µl
- Anti-Rabbit IgG HRP stock (Part No. 2201) 50 µl
- Chemiluminescence Solution A (Part No. 401-A): 110 ml (1X)
- Chemiluminescence Solution B (Part No. 401-B): 3 ml (40X)

Cat. No. WB-110-25: sufficient for 25 blots (8 x 10 cm)

- Anti-Mouse IgG HRP stock (Part No. 5201) 125 µl
- Anti-Rabbit IgG HRP stock (Part No. 2201) 125 µl
- Chemiluminescence Solution A (Part No. 401-A): 250 ml (1X)
- Chemiluminescence Solution B (Part No. 401-B): 7 ml (40X)

Storage and Stability

Store at 2-8 °C, stable for 12 months from the date of shipment.

Preparation of Washing Buffer and Blocking Solution

- Prepare 100 ml of 1 M Tris-HCl pH 7.5 buffer

12.1 g Tris base

6.8 ml HCl

Add H₂O to 100 ml after adjust pH to 7.5

- TBS (1000 ml)

100 ml 1 M Tris-HCl pH7.5

9 g NaCl

Add H₂O to 1000 ml

- 5% non-fat milk in TBS (200 ml)

10 g Non-fat milk powder

200 ml TBS

-TBST (500 ml)

add 0.5 ml of Tween 20 to 500 ml TBST to make 0.1% (v/v) of Tween 20 in TBS.

The preparation volumes should be determined by each user based on the numbers of blots.

Procedure for Western Blot Detection

1. After blotting, block the membrane with 5% non-fat milk in TBS for 30 minutes at room temperature.
2. Remove blocking solution, briefly rinse membrane blot with TBST.
3. For each blot, prepare 15 ml of working solution of primary antibody (mouse or rabbit IgG) in 5% non-fat milk.
4. For primary antibody binding to target protein, incubate antibody diluted solution with blot for 1 hour at room temperature (or use antibody supplier's instruction).
5. Remove primary antibody solution, and wash blot 3 times with TBST (5 minutes for each wash.) on the rocker platform.
6. For each blot, prepare 15 ml of working dilution of secondary antibody HRP conjugate.
7. To make 1:1000 or 1:3000 dilution, add 15 µl (or 5 µl) of anti-mouse HRP conjugate (or anti-rabbit HRP conjugate if primary antibody is rabbit antibody) to 15 ml of 5% milk in TBS.
8. Incubate secondary antibody HRP conjugate working solution with blot for 30 minutes at room temperature.
9. After incubation, remove secondary antibody solution and wash each membrane blot 3 times with TBST (5 minutes for each wash on rocker platform).
10. After washing, transfer blot to a tray containing 10 ml of TBS to keep the membrane blot from drying.
11. For each membrane blot (8 x10 cm), prepare 5 – 10 ml of chemiluminescence detection working solution. For 10 ml volume, add 330 µl of Reagent 1 to 10 ml of Reagent 2.
12. Apply chemiluminescence detection solution onto the membrane blot to cover the entire membrane blot. Incubate for 1 minute.
13. Remove the chemiluminescence solution by press filter paper on the membrane to adsorb excess solution.
14. Perform film exposure or semi-quantitative analysis using CCD imager.