EzWay™ DAB Western Blot Kit

1. Catalog No. K03350

2. Quantity 10 mini-blots

3. Storage Store at 2-8°C. Do not freeze.

4. Description

- Specific and accurate colorimetric protein detection using DAB
- Optimized all reagents supplied for quick and convenient assay
- High sensitivity and clear background

5. Kit Contents

Description	Amount
25X Transfer Buffer (Tris-Glycine)	100 ml x 2
6X Blocking Solution (3% Casein/PBS)	120 ml x 2
PBS Powder	Pouch for 1L x 5
Tween-20 (50%)	1 ml (50%) x 5
30X DAB Solution	15 ml
Substrate solution (30% H_2O_2)	1 ml

6. Materials needed

- Primary antibody and Secondary antibody
- Transfer membrane (Nitrocellulose, PVDF or Nylon)
- Deionized water
- Orbital shaker

7. Cautions

- Prepare all reagents as much as you need, because diluted solutions are not stable for longer storage.
- Each reagent may be sufficient for preparing about 20-30 ml per single assay.
- Bring all reagents to room temperature prior to use.
- Complete washing of the membrane after each incubation step is essential to obtain low background values.
- Individual solution of the kit contains no preservatives except Blocking Solution and DAB solution. Blocking Solution contains 0.01% Sodium Azide and DAB solution Thimerosal for longer storage. Sodium Azide and Thimerosal are caustic materials.

8. Preparation of Reagents

- **Transfer buffer (1X) :** Add 1 volume of 25X Transfer Buffer to 19 volumes of D.W. and add 5 volumes of Methanol.
- **Diluent (PBS) :** Add 1 pouch of PBS powder to 1L DW and solve it well.
- **Washing solution (PBST) :** Add 1vial (1 ml) of Tween-20(50%) to 1L PBS and mix well.
- **Blocking solution :** Add 1 volume of 6X Blocking solution (3% casein/PBS) to 5 volumes of PBS and mix it well. If necessary, it can be slightly heated.
- **Color Reaction Mixture :** Add 1 volume of 30X DAB Solution to 29 volumes of PBS and add 30 ul Substrate Solution(30% H_2O_2) per 30 ml 1X DAB solution prior to use.
- **Dilution of Primary and secondary antibody :** Dilute your primary/secondary antibody in PBS according to the proper dilution factor. Or use PBST (Washing solution) or Blocking solution instead of PBS to prevent non-specific binding.

9. Procedure

1. Transfer to a membrane
- Perform SDS-PAGE and transfer the gel to a nitrocellulose (or other) membrane.

2. Rinsing
- Rinse the membrane with DW.
- If desired, the membrane may be stained with Ponceau S to visualize protein bands to confirm the protein transfer before antibody binding and detection process.

3. Blocking
- Incubate the membrane in Blocking Solution for 1 hour at room temperature or at 37°C (or overnight at 4°C) with slight agitation.

4. Washing
- All washing step is the same but time can be changed if necessary.
- Rinse the membrane with Washing Solution for 3-5 minutes each, repeating 3 times.
- After the last wash, it is recommended to rinse the membrane with DW.

5. Reacting with Primary Antibody
- Incubate the membrane in the primary antibody solution for 1 hour at room temperature or at 37°C with slight agitation.

6. Washing (Repeat step 4)

7. Reacting with Secondary Antibody
- Incubate the membrane in the secondary antibody solution for 1 hour at room temperature or at 37°C with slight agitation.

8. Washing (Repeat step 4)

9. Color Reaction
- Incubate the membrane in the color reaction mixture for several minutes at room temperature or at 37°C.
- After sufficient color development, wash the membrane with DW in order to stop the color reaction