

EzWay™ DAB Substrate Kit

- 1. Catalog No.** K0335040
- 2. Quantity** 10 mini-blots
- 3. Storage** Store all components at 2-8°C. Do not freeze.
- 4. Description**
- Specific and accurate colorimetric protein detection using DAB
 - High sensitivity and clear background

5. Kit Contents

Description	Amount
30X DAB Solution	15 ml
Substrate solution (30% H ₂ O ₂)	1 ml

- 6. Cautions**
- Prepare all reagents as much as you need, because diluted solutions are not stable for longer storage.
 - This reagent may be sufficient for preparing about 20-30 ml per single assay.
 - Bring this reagent to room temperature prior to use.
 - Complete washing of the membrane after each incubation step is essential to obtain low background values.

7. Preparation of Reagents

- **DAB Solution (Working Solution)** : Add 1 volume of 30X DAB Solution to 29 volumes of PBS and add 30 ul Substrate Solution(30% H₂O₂) per 30 ml 1X DAB solution prior to use.

8. 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