# Western Blot Kit

**BS392-XX: with NC membrane**
**BS393-YY: with PVDF membrane**

| Component | BS392-XX | BS393-YY |
|---|---|---|
| Transfer Mixture (ready-pack) | 5 ready-packs | 5 ready-packs |
| Ponceau S 10 x buffer | 20 ml | 20 ml |
| Block reagent | 5 packs | 5 packs |
| Tween-20 | 2 ml | 2 ml |
| 20x PBS | 250ml | 250ml |
| 20x TBS | 250ml | 250ml |
| HRP-conjugated secondary IgG | 0.1 ml | 0.1 ml |
| DAB | 5 packs | 5 packs |
| 10x DAB Development solution | 10 ml | 10 ml |
| Hydrogen peroxide H2O2 | 1 ml | 1 ml |
| Heat-sealable Bag | 20 | 20 |
| Membranes | NC 5(10x 10cm) | PVDF 5(10x 10cm) |

**Storage Condition:**

- Store HRP-conjugated secondary IgG and DAB at $-20^0$ C, and store the rest contents of the kit at 4°C,

**Transferring Protein to Membrane**

1. Prepare transfer buffer: add one ready-pack Transfer Mixture and 200ml methanol (if needed), add deionized water to 1,000ml;
2. Cut filter paper and membrane, float the membrane (pretreated PVDF membrane with methanol) on the surface of a dish containing deionized water until it wets completely from beneath;
3. Once the gel electrophoresis is completed, remove the gel from the electrophoresis chamber, cut the stacking gel. Make a small cut on the bottom right corner of the gel;
4. Immerse the gel in the transfer buffer for 20minutes; immerse the membrane and the filter papers in the transfer buffer;
5. Place one holder with sponge, 3 sheet of filter paper, smooth out all air bubbles with a glass rod.
6. Invert the gel onto the filter paper, smooth out all air bubbles with the rod.
7. Place the membrane on the top of the gel. Make a small cut in the right bottom corner of the membrane to match the corner of the gel; smooth out air bubbles with the rod.
8. Place 3 pieces of filter paper on the membrane and press out any air bubbles.
9. On top of the filter paper, place the sponge; close the holder.
10. Place the sandwich in the transfer chamber, filled with transfer buffer, make sure that the membrane is facing the red electrode.
11. Run the transfer according to the membrane area and protein MW.
12. Open the cassette, remove the gel from the membrane (the gel can be dyed with coomassie brilliant blue to see the transferring efficiency). The membrane can be stained with Ponceau S for 10min and destained with deionized water

**Developing the Blot**

1. Prepare block solution: add a bag of block reagent to 100ml 1x TBS;
2. Place the membrane in heat sealable bag; add block solution 0.1-0.2ml for each square centimeter of membrane; squeeze out air as much as possible; seal the bag, place on a shaking platform for 1 hour at RT.
3. Cut the bag at one corner and drain out the blocking solution;

4. Add block solution 0.1-0.2ml for each square centimeter of membrane; add appropriate concentration antibody, squeeze as much air as possible from the bag; seal the bag, place on a shaking platform for 1 hour at RT (or overnight at 4°C);
5. Wash membranes in PBST (0.05% Tween in PBS) on a shaking platform for 5 minutes. Repeat 3 times total.
6. Wash the membrane with TBS for 10min; add block solution 0.1-0.2ml for each square centimeter of membrane; add appropriate concentration HRP conjugated-secondary antibody (1:2,500–1:50,000), squeeze as much air as possible from the bag; seal the bag, place on a shaking platform for 1 hour at RT;
7. Wash membrane in TBST (0.05% Tween) on a shaking platform for 5 minutes. Repeat 3 times total; Wash again in TBS;
8. Prepare the DAB substrate solution:
    a. Dissolve one pack of DAB in 10 ml 1x Development solution;
    b. Add 10ul hydrogen peroxide;
9. Develop the color of the blot rocking;
10. .Stop reaction after the expected band appears by pouring out the substrate and rinsing with distilled water repeatedly. Dry the membrane and place in the dark.

**Notes:**

1. For BS392 and BS393, use 5% pig serum as block reagent to reduce the background, when HRP- conjugated anti-goat IgG react with components in milk.
2. Use appropriate gloves, a fresh scalpel and blunt-ended forceps to handle the membrane. Membrane and paper should be cut approx.1 mm larger than the gel in both dimensions
3. PVDF membrane should be treated with methanol first.
4. Smooth out any air bubbles with a glass rod every step in setting up the transfer cassette.
5. The recommend dilution of HRP conjugated-secondary antibody is 1:2,500 to 1:50, 000. If the blot will be visualized with ECL or high backgrounds are observed, secondary antibody should be diluted further (up to 1:100,000)
6. Air should be squeezed out as much as possible from the sealable bag in the blocking and blotting step.
7. Use of sodium azide as a preservative in HRP-conjugated IgG will substantially inhibit the enzyme activity of horseradish peroxidase.
8. Any membranes not used immediately in blotting should be thoroughly dry, and stored at RT.

***Storage:*** *With the exception of the RNase A, the kit may be stored at room temperature. The RNase A should be stored at 4ºC. The kit is stable for 12 months at room temperature. For longer storage, keep all contents cold.*

*For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol : volume of Wash Solution = 4:1).*

*Storage: All contents of the kit should be kept in freezer after usage. The Kit is stable for 9 months.*

*Storage: With the exception of the Proteinase K, the kit may be stored at room temperature. The proteinase K should be stored at 4ºC. The kit is stable for 12 months at room temperature. For longer storage, keep all contents cold.*