

## EasySee® Western Blot Kit

Cat. No. DW101

Storage: at 2-8°C protected from light for two years

### Description

EasySee® Western Blot Kit uses lumino-based chemiluminescence for the detection of horseradish peroxidase (HRP) conjugated antibodies and the corresponding antigens.

### Highlights

- High sensitivity.
- Easy to use, High stability.

### Kit Contents

Component	DW101-01	DW101-02
WB Solution A	50 ml	100 ml
WB Solution B	50 ml	100 ml

### Procedures

1. Perform standard protocols for electrophoresis, protein transfer to the membrane, incubation with HRP-conjugated antibodies and washing membrane.
2. After washing the membrane to remove nonbound HRP-conjugated antibodies, prepare chemiluminescence working solution by mixing equal volumes of WB Solution A and WB Solution B (e.g., add 1 ml of WB Solution A and 1 ml of WB Solution B). It is recommended to use freshly prepared working solution.
3. Take out the membrane with tweezers and remove excess wash buffer from the membrane. But do not completely dry it. Submerge the membrane with working solution completely (0.125 ml working solution per cm<sup>2</sup> of membrane). Incubate for 1 minute and prepare for immediate exposure to X-ray film. Longer incubation time will not improve sensitivity, but possibly lead to abnormal bands.
4. Take out the membrane with tweezers after incubation and drain excess chemiluminescence working solution.
5. Wrap the membrane in a clear plastic wrap. Remove the bubbles and wrinkles.
6. Place the membrane in the darkroom and expose to X-ray film or capture an image in a chemiluminescence instrument.

### Notes

- Chemiluminescence working solution should not be exposed to intense light for too long, otherwise the sensitivity may decline.
- Prolonged exposure to light or excess protein will increase background and lead to no linear relationship between protein amount and band intensity.
- To obtain the optimal exposure result, it is recommended to optimize the dilution ratios of primary antibodies and secondary antibodies.
- Avoid using sodium azide to recover HRP-conjugated antibodies, since sodium azide can inhibit the enzyme activity of HRP.

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