IN0004 WB Enhanced ECL Luminescent Liquid

IN0005 WB Ultra Sensitive ECL Luminescent Liquid

Packing specification

Specifications: 100ml 、500ml

Storage conditions

Store in the dark at 4° C for 1 year; if not used for a long time, store at -20° C in dark; transport at 4° C or room temperature.

1. Product description:

This reagent detects protein or nucleic acid biological macromolecules by chemiluminescence method. After electrophoresis, the protein or nucleic acid biological macromolecules are transferred to the blotting membrane, and the primary antibody and the horseradish peroxidase (HRP) labeled secondary antibody are used to sequentially bind to the target protein on the membrane; or the target protein labeled with HRP The probe directly or indirectly binds the target nucleic acid on the membrane. After washing the membrane, incubate the membrane with a freshly prepared ECL working solution at room temperature in the dark for 2-3 min. Cover the blot membrane with plastic wrap and fix it on the X-ray film exposure cassette, then transfer it to the dark room to press the X-ray film on The exposure time on the film can be several seconds to several hours. After development and fixation, the protein or nucleic acid bands can be clearly displayed on the X-ray film, or the X-ray film exposure and development step can be omitted, and the blot film can be directly scanned by CCD.

Reagent components

Composition	IN0004、IN0005 (2×50 ml)	IN0004、IN0005 (2×250 ml)
ECL Substrate A	50 ml	250 ml
Peroxide Solution B	50 ml	250 ml

2. Operating instructions

1. Use appropriate methods to perform Western Blot experiments until the secondary antibody is washed with PBST or TBS/TBST.

2. Freshly prepared ECL working solution: ECL Substrate A and Peroxide Solution B are mixed 1:1 to become ECL working solution. Please use it as soon as possible after mixing the working solution. It can still be used after a few hours at room temperature, but the sensitivity is slightly reduced.

3. Use flat-tipped tweezers to take out the blotting membrane, put it on the filter paper and drain the dry-cleaning membrane solution but do not let the membrane dry completely. Add ECL working solution evenly onto the blotting membrane to ensure complete coverage, and incubate at room temperature for 2-3 min. The usage amount of ECL working fluid is about 0.125 ml/cm2 film or according to personal experimental habits.

4. Clamp the blotting membrane with flat-tipped tweezers, discard the luminescent working solution, and slightly absorb the excess working solution with absorbent paper.

5. Quickly place the blotting film between the two layers of cling film, and try to get rid of the bubbles.

6. Put the blotting membrane protein side up, put it in the X-ray film cassette, and fix the edge with transparent tape.

7. Put the X-ray film in the dark room, expose for an appropriate time according to the color development, develop and fix.

8. Adjust the exposure and development time appropriately according to the strength of the signal. You can also select multiple exposures at different times to achieve better results.

3. Matters needing attention

1. Be sure to replace the tip when drawing ECL Substrate A and Peroxide Solution B during the preparation of ECL working solution. The working solution should be used immediately after fresh preparation. It can still be used after being placed at room temperature for several hours, but the sensitivity is slightly reduced.

2. Peroxide Solution B contains oxidants, which are easily reduced and become invalid. After each solution is used, please close the cap tightly to prevent failure.

3. The ECL luminescent solution is the chromogenic substrate of HRP, so the detection system must be based on HRP enzyme-labeled antibodies or nucleic acid probes.

4. Try to avoid putting multiple membranes in the same membrane washing box to wash the membranes. Mutual absorption or friction may increase the background.

5. High-quality plastic wrap should be used. Poor quality cling film may quench the fluorescence or contaminate the blotting film due to impurities and cause high exposure background values.

6. Depending on the abundance of the target protein, the exposure time may be several seconds to several hours. Insufficient exposure time will cause unclear target bands, and too long exposure time will darken the background.

7. Sodium azide (NaN3) can inhibit the activity of HRP. If you recover HRP-labeled probes or antibodies, you should avoid using NaN3, if necessary, do not use more than 0.01%.

8. Because the luminescent fluid is extremely sensitive, it is recommended that the initial concentration of most imported antibodies is 1:1000-1:4000 for the primary antibody and 1:2000-1:5000 for the secondary antibody. Too high antibody concentration may cause high background or no bands.

9. ECL Substrate A and Peroxide Solution B are both harmful to the human body. Please be careful when handling and pay attention to effective protection to avoid direct contact with the human body or inhalation. For your safety and health, please wear laboratory clothes and disposable gloves operating

It can only be used for scientific research. It is forbidden to use it for human, animal or other purposes.