Data Invention Biotech

ECL Plus Chemiluminescent Substrate Manual

Product number: DIB049-100ml DIB049-500ml

Product specifications: 100ml 500ml

Storage: Transport at room temperature, store reagents at 4° C and protected from light after receipt

1. Product description:
Super ECL Plus is used to detect antibodies that directly or indirectly label horseradish peroxidase HRP and their associated antigens. Due to the unique luminescent substrate system, Super ECL Plus is the most sensitive commercial fluorescent ECL detection reagent at present:
(1) A higher antibody dilution factor (1:2000～1:10000) can be used, which saves the antibody extremely.
(2) Simple and easy to use-can replace other companies' ECL luminescent substrates, and the operation steps do not need to be specially optimized
(3) Higher sensitivity-can detect low picogram proteins
(4) Signal duration is longer-light signal lasts Time is up to 5 hours
(5) More imaging methods — suitable for X-ray film, CCD or laser imager
(6) More economical — Compared with similar products of other brands, it not only has high quality and performance, but also the price Lower

2. Uses: Used for Western Blot of HRP-labeled antibody and nucleic acid hybridization of HRP-labeled probe.

3. How to use:
①. Perform routine SDS-PAGE, transfer and Western Blot procedures. Note the use of HRP-labeled lgG or primary antibody-streptavidin-biotin-HRP clip method. Operation overview
Note: Optimize the concentration of antigen and antibody. The recommended antibody dilution must be used to ensure a positive result.
1) Dilute the concentration of the primary antibody to 0.05～1ug/ml
2) Dilute the concentration of the secondary antibody to 0.005～0.04ug/mL
3) Mix the two substrate components in a ratio of 1:1 to prepare the working substrate solution. Note: Exposure to sunlight or any other strong light may damage the working fluid. For best results, store this working fluid in an amber bottle and avoid long-term exposure to any strong light. Exposure to routine lighting in the laboratory for a short time will not damage the working fluid.
4) Incubate the blot in the ECL substrate working solution for 5 minutes.
5) Aspirate excess reagent. Cover the blotting membrane with a clean plastic film.
6) Expose the blot film on X-ray film.
②. Freshly prepare the luminescent working solution while washing the membrane for the last time by Western Blot: Take equal volumes of solutions A and B, and mix them in a clean container. It is recommended to use the working solution immediately, it can still be used after a few hours at room temperature, but the sensitivity is slightly reduced.
③. Remove the membrane with tweezers and place it on the filter paper to drain the dry cleaning solution but do not let the membrane dry completely. The film is completely immersed in the luminescent working fluid (0.125mL luminescent working fluid/cm2 film) and fully contacted with the luminescent working fluid. Incubate at room temperature for 3 minutes, ready to press and expose immediately. Too long incubation time will not increase sensitivity and sometimes cause abnormal exposure bands. The essence of the luminescence process is an enzymatic reaction. Using too little luminescence working fluid will adversely affect the progress of the reaction, which will also cause uneven exposure of the bands on the film and significantly reduce sensitivity. In order to achieve the purpose of saving, the film can be cut small but do not reduce the amount of luminescent liquid.
④. Use tweezers to pick up the membrane and place it on the filter paper to drain the luminous working solution. But do not wash off the luminous liquid.
⑤. Open the X-ray film cassette, and spread a fresh-keeping film larger than the film on the inner surface of the cassette. Place the Western Blot film on the plastic wrap, fold the plastic wrap to completely wrap the Western Blot film, remove bubbles and wrinkles, and cut off the excess plastic wrap at the edges. Use filter paper to absorb excess luminescent working fluid. Fix the fresh-keeping film covering the Western Blot film in the cassette with tape, with the protein band facing up.
⑥. Press the X-ray film in the darkroom and expose for different time such as several seconds to several minutes. Develop and rinse.

4. Storage: sealed and protected from light at 4 oC for more than one year. Can be placed at room temperature for a short time.

5. Safety: No special toxicity, treat as ordinary chemicals.

6. Matters needing attention:
(1) Steps 1 to 5 can be operated under a fluorescent lamp; however, the sensitivity may be slightly reduced when the luminescent liquid is exposed to strong light for too long, and it can be avoided by moving to a darkroom. Wearing gloves can avoid leaving fingerprints on the membrane.
(2) Long-term exposure or excessive protein will deepen the background and make the band strength change lose the linear relationship. Underexposure will blur the bands.
(3) The strips on the membrane glow after incubating for about 3 minutes in the luminescent working solution. The strong band luminescence is visible to the naked eye in the darkroom, and the low-abundance protein band luminescence is weak or even invisible to the naked eye, but it can be exposed to X-ray film. The light emission time of the strip cannot be judged simply by visual observation. Fluorescence invisible to the naked eye can actually last for several hours and sensitize the X-ray film, so the weak band can be exposed for 1-10 hours. If the band is not good after exposure, wash the membrane with membrane washing buffer, re-incubate the secondary antibody, and then re-illuminate and expose with ECL.
(4) As the ultra-sensitive luminescent solution is extremely sensitive, it is strongly 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 will cause high background or no bands, leading to failure.
(5) Some plastic wrap may quench the fluorescence when wrapping the blotting membrane, so high-quality plastic wrap should be selected.
(6) Pre-stained protein Marker and fluorescence-autoradiography exposure label can be used to accurately determine the position and size of the band on the film.
(7) NaN3 can inhibit the activity of HRP. The use of NaN3 should be avoided when recovering the secondary antibody. If necessary, use no more than 0.01%.