Enhanced ECL Chemiluminescent Substrate Kit

Product Information

Product name	Cat#	Size
Enhanced ECL Chemiluminescent Substrate Kit	36222ES60	100 mL
	36222ES76	500 mL

Product Description

Enhanced ECL Chemiluminescent Substrate Kit is designed to detect antibodies and associated antigens directly or indirectly labeled with horseradish peroxidase (HRP). The principle of Enhanced ECL Chemiluminescent Substrate Kit is that, proteins or nucleic acids were transferred to the imprinted membrane after electrophoresis, and the target proteins on the membrane were bound by primary antibody and secondary antibody labeled with HRP, or the nucleic acids on the membrane were bound directly or indirectly by probes labeled with HRP. After washing the membrane, the ECL working solution prepared by the product was used to incubate the membrane at room temperature for several minutes. The imprinted membrane was wrapped with plastic wrap and fixed to the X-ray exposure Cassette. Then the X-ray film is pressed on the membrane in a darkroom and exposed for several seconds to several hours. After development and fixing, protein or nucleic acid bands can be clearly displayed on the X-ray film.

This kit has a unique luminescent substrate system, reducing the exposure background while introducing a new oxidant greatly improves the stability of the kit, allowing it to be stable at room temperature for up to one year. In addition to X-ray, fluorescent CCD scan can also be directly used mainly for WB detection and chemiluminescence immune detection system.

Product components

Component		36222ES60 (100 mL)	36222ES76 (500 mL)
36222-A	Reagent A	50 mL	250 mL
36222-B	Reagent B	50 mL	250 mL

Shipping and Storage

The products are shipped with ambient temperature and can be stored at room temperature for one year. If not used for a long time, it is recommended to store at 4℃ to extend the validity period.

【Notes】: Reagent A (36222-A) should be stored away from light!

Instructions (X-ray film, for example)

1. Routine electrophoresis, transfer membrane, antibody labeled with HRP or nucleic acid probe labeled with HRP incubation and washing membrane.

【Notes】: ECL luminescent solution is the color substrate of HRP, so the detection system must be based on HRP enzyme-labeled antibody or nucleic acid probe.

2. At the same time of washing the film for the last time, the luminescent working solution was prepared fresh (100-200 μL luminescent working solution/cm^2 membrane): take the same volume of Reagent A and B, mix well for using later.

【Notes】: Take Reagent A and Reagent B must use different tips. It is recommended to use the working fluid immediately. It can still be used after a few hours at room temperature, but the sensitivity is slightly reduced.

3. Use flat tweezers to take out the membrane, put it on filter paper to drain the lotion, do not make the membrane completely dry. Completely immerse the membrane in luminescent working solution, sufficient contact with luminescent working fluid. Incubate at room temperature for 1-2 minutes and prepare for tablet exposure immediately.

4. Pick up the membrane with tweezers and lay it on filter paper to drain the luminescent working fluid. But do not wash off the luminescent fluid.

5. Lay a piece of plastic wrap larger than the membrane on the inner surface of the X-ray film obscura. Attach the imprinted membrane to the plastic wrap, fold the plastic wrap completely around the imprinted membrane, and remove the gas bubble and fold, can cut off the edge of excess plastic wrap. Use filter paper to suck up excess luminous working fluid. Fix the plastic wrap covering the imprinted membrane in the obscura with tape, with the protein band facing upwards.

6. A piece of X-ray film was taken from the darkroom and placed on the wrapped membrane. The film was pressed and exposed for 30 secs-2 mins. Then the film was developed and fixed.

【Notes】: The exposure time should be adjusted according to the exposure intensity. If the background is too high, use two X-ray films at the same time.

Other exposure methods

If the use of CCD photography: the membrane can be placed in the working fluid, after the boot according to the use of the instructions, remove the film, take photos. In addition, the measurement parameters of the machine can be adjusted according to the situation to improve the signal-to-noise ratio.

Cautions

1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. Bubbles should be avoided in membrane transfer, sealing and incubation. In addition, wearing gloves will do avoid leaving fingerprints on the membrane and keep it clean.
3. Prolonged exposure or excessive protein will deepen the background and make the change of band strength lose linear relation. Underexposure will blur the bands.
4. Some plastic wrap may quench the fluorescence when wrapping the imprinted membrane. High quality plastic wrap should be selected.
5. Avoid placing multiple membranes in the same washing membrane box, as mutual adsorption or friction may cause a deep background.
6. The position and size of the bands on the film can be accurately determined using visible prestained protein markers and fluorescent-autoradiography exposure tags.
7. Use a biotin-avidin system and avoid using milk closure, which may cause the background to be too high.
8. Metal oxide particles may cause granular spots on the membrane. Avoid using rusty scissors and tweezers. Use plastic flat tweezers.
9. Sodium azide (NaN_3) can inhibit HRP activity, if the recovery of HRP labeled probe or antibody should avoid using NaN_3, if necessary, not more than 0.01%.
10. This product has no special toxicity, according to common chemical treatment.
11. For research use only!