SuperFemto ECL Master Mix

E433

Version 24.1

Product Description

SuperFemto ECL Master Mix is a sensitive, luminol-based enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots. This product is a novel single-component ready-to-use reagent, presenting a light yellow transparent appearance, breaking through the conventional mode of mixing equal volumes of solution A and solution B before use, making the operation more convenient, effectively avoiding reagent loss and cross-contamination during liquid absorption, and improving the consistency and reproducibility of results. In addition, SuperFemto ECL Master Mix has high sensitivity and enables femtogram detection of antigen by oxidizing luminol in the presence of HRP and peroxide. Blotting membranes (e.g. NC, PVDF) incubated with ECL working solution can be visualized on X-ray film or CCD imaging system.

Components

Components	E433-01 (100 ml)	E433-02 (500 ml)
SuperFemto ECL Master Mix	100 ml	2 × 250 ml

Storage

Store at 2 ~ 8°C and protect from light. Ship on ice pack.

Applications

It is applicable for blotting detection (e.g. Western Blot, EMSA) of HRP-labeled antibodies or nucleic acid probes.

Notes

1. Vazyme #E433 is quite sensitive. Recommended initial concentrations for most antibodies: 1:1,000 - 1:4,000 for primary antibody; 1:5,000 - 1:10,000 for secondary antibody. Antibody concentration that are too high may result in high background or white bands. The appropriate ECL luminescence detection kit can be selected according to the abundance of the target protein, such as SuperPico ECL Master Mix (Vazyme #E432).
2. The chemiluminescent substrate detects HRP, thus the detection system must be based on HRP-labeled antibodies or nucleic acid probes.
3. High quality plastic wrap is recommended. Unsatisfactory plastic wrap may contain impurities that can contaminate the blotting membrane, cause high background, or cause fluorescence quenching.
4. Exposure time should be adjusted according to the abundance of the target protein. Underexposure will cause the target band to be unclear, and overexposure will deepen the background.
5. Do not use sodium azide (NaN_3) in buffers, because it inhibits HRP.
6. Use a shaking or rocking platform during incubation steps for optimal results.
7. Wear a lab coat and disposable gloves for protection.

Experiment Process

1. Perform Western Blot experiments as required, and then wash the secondary antibody with PBST/PBS or TBST/TBS.
2. Remove the blotting membrane with flat tweezers, and drain the washing solution on the filter paper, but do not let the membrane dry completely. Evenly drop the ECL working solution onto the blotting membrane to ensure uniform coverage (The recommended volume of ECL working solution is about 70 μl/cm^2 membrane, which can also be adjusted according to personal habits), and incubate at room temperature for 1 min.
3. Clamp the blotting membrane with flat tweezers and place it vertically on the filter paper for a few seconds to remove excess ECL working solution.
4. Quickly place the blot between two layers of plastic wrap, trying to press out any bubbles.
5. Image the blot using the X-ray film: Place the blotting membrane with the protein side up in the X-ray film cassette. Secure the edges with scotch tape. Choose an appropriate exposure time according to the color development, or choose multiple exposures at different times to achieve better results.
6. Image the blot using the CCD imaging system: No need to put it in plastic wrap, just follow the instructions of the imager to take pictures.