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# High Sensitivity ECL Kit

## 1 Components

| Component | HY-K2005-100 mL | HY-K2005-500 mL |
|---|---|---|
| Solution A | 50 mL | 50 mL × 5 |
| Solution B | 50 mL | 50 mL × 5 |

## 2 Introduction

MCE High Sensitivity ECL Kit enables low picogram detection of antigen by oxidizing luminol in the presence of HRP and peroxide. This reaction produces a prolonged chemiluminescence which can be visualized on X-ray film or digital imaging systems.

The Kit produces a strong, long-lived signal (6-8 hours), which, combined with very low background levels, allows for long exposure times and enables the detection of low-abundance proteins. In the Western Blot experiment, the storage solution of primary antibody (1 mg/mL) can be diluted at a ratio of 1:1,000-1:50,000 (the final concentration is about 0.2-1 μg/mL). The secondary antibody (1 mg/mL) storage solution can be diluted at a ratio of 1:20,000-1:100,000 (the final concentration is about 10-50 ng/mL).

## 3 General Protocol

1. Take the High Sensitivity ECL Kit out of the refrigerator and allow it to equilibrate at room temperature for about 20 minutes.
2. Prepare the ECL working solution.

Note: Approximately 0.125 mL of High Sensitivity ECL Reagent is required per $cm^2$ of membrane area.

a) Mix solutions A and B in a 1:1 ratio. Use a sufficient volume to ensure that the blot is completely wetted with the ECL working solution.

b) It is recommended to prepare the working solution just before use, although the mixed reagent solution is stable for several hours at room temperature.

3. Place the blot, protein-side up, in a clean container, and add ECL working solution.
4. Incubate the blot for 3-5 minutes at room temperature.
5. Drain the excess substrate and place the blot in a plastic membrane protector. Remove all air bubbles between the blot and the surface of the membrane protector.
6. Visualize the blot using X-ray film or a CCD-based imaging system.

## 4 Storage

4°C, 12 months.

Protect from light.

## 5 Precautions

1. To get the best results of Western Blot experiment, please optimize experimental reagents and process, including sample amount, gel type, transfer method, membrane type, blocking reagent, wash buffer, primary antibody concentration, secondary antibody concentration and incubation times.
2. Exposure to the sun or any other intense light can harm the working solution. Short-term exposure to laboratory lighting will not harm the working solution.
3. Do not use sodium azide ($NaN_3$) in any blocking buffers or wash solutions, as it inhibits HRP activity.
4. Use of blocking buffer to dilute antibodies may reduce background and increase sensitivity.
5. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.