

## Product Description

ECL Chemiluminescent Substrate Kit is a chemiluminescent detection kit that contains all reagents required for the detection of horseradish peroxidase (HRP) and is suitable for chemiluminescent detection in Western blot applications, enabling rapid detection of pg-level antigens. This product can be used to detect antibodies directly or indirectly labeled with HRP and their associated antigens. The principle is as follows: after electrophoresis, proteins or nucleic acids are transferred onto a blotting membrane. The target protein on the membrane is recognized by a primary antibody and an HRP-labeled secondary antibody, or nucleic acids are bound directly or indirectly by an HRP-labeled probe. HRP catalyzes the decomposition of hydrogen peroxide and the oxidation of luminol, generating an excited-state intermediate that emits photons during decay. This product can be used for the detection of proteins and other biomacromolecules immobilized on membranes and can be detected by X-ray film exposure or with a chemiluminescence imaging system.

## Components

Components	BR4D602-01 100 mL	BR4D602-02 500 mL
Solution A	50 mL	250 mL
Solution B	50 mL	250 mL

## Storage

Store at 2-8°C protected from light.

## Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. Use a sufficient volume of working solution to ensure complete coverage of the blotting membrane.
3. Excessive exposure time or protein overload will increase background and disrupt the linear relationship between band intensity and protein amount; insufficient exposure will result in faint bands.
4. It is recommended to use visible prestained protein markers and fluorescent/radiographic exposure markers to accurately determine the position and size of bands on the film.
5. For your safety and health, please wear a lab coat and disposable gloves when operating.

## Protocol

1. Remove the transferred membrane from the wash buffer, drain excess liquid, and keep the membrane moist.
2. Mix Solution A and Solution B at equal volumes to prepare the chemiluminescent working solution (approximately 0.1 mL working solution is required per cm<sup>2</sup> of blotting membrane).
3. Evenly dispense the chemiluminescent working solution onto the membrane. After reacting for 1-2 min, remove the membrane and drain off excess working solution. Do not allow the membrane to dry completely.
4. X-ray film exposure: expose the membrane to X-ray film in a darkroom. Exposure time may range from a few seconds to several minutes. Develop the film, and if necessary, adjust the exposure time based on the result and perform a second exposure.
5. Chemiluminescence imaging system: capture the image directly according to the operating instructions of the instrument.
6. Used membranes can be stripped and reprobed multiple times.