

Ver.A261001

ECL PLUS Substrate

PE0010 (12.5mL)

Introduction

ECL Western Blotting Substrate directly or indirectly detects proteins or nucleic acid substrates associated with horseradish peroxidase (HRP). This unique luminescent substrate system is one of the most sensitive commercial ECL detection reagents available, offering extremely high sensitivity and high signal-to-noise ratio, capable of detecting trace antigens at 10-100 fg levels. It produces rapid luminescence, and the signal can be captured on X-ray film for more than 12 hours, making it particularly suitable for the detection of trace proteins or nucleic acids. In addition, the reagent allows the use of highly diluted antibodies (1:2000 to 1:10,000), greatly conserving antibody consumption.

Application

Western blotting using HRP-conjugated antibodies, and nucleic acid hybridization using HRP-labeled probes.

Storage conditions

Store at 2-8°C, Avoid light. Valid for 2 years.

Safety Information

No special toxicity, handle as a general chemical.

Kit Components

Reagents	Volume	Storage
ECL PLUS Substrate A	12.5mL	2-8°C
ECL PLUS Substrate B	12.5mL	2-8°C

Protocols (for reference only)

1. Routine electrophoresis, membrane transfer, incubation with HRP-conjugated antibodies or nucleic acid probes, and washing.
Note: Use the IgG antibody–streptavidin–biotin–HRP sandwich method for nucleic acid hybridization membranes, or hybridize the membrane with HRP-labeled probes and wash the membrane.
2. While washing the membrane with HRP-conjugated secondary antibody bound to it, prepare the fresh working luminescent solution. Mix equal volumes of Solution A and Solution B, and allow the mixture to reach room temperature, otherwise luminescence intensity will be reduced. Immediate use is recommended. The working solution can still be used after standing at room temperature for several hours, but with slightly decreased sensitivity.

3. Use forceps to remove the membrane and place it on filter paper to drain excess washing solution, without letting the membrane dry completely. Immerse the membrane fully to ensure thorough contact with the working luminescent solution (approximately 0.125mL. working solution per cm² of membrane). Incubate at room temperature for 3 minutes, then immediately perform film exposure. Prolonged incubation does not improve sensitivity and may occasionally cause abnormal bands on the exposed film. The luminescence is essentially an enzymatic reaction; insufficient working luminescent solution hinders the reaction and also leads to uneven band exposure and markedly reduced sensitivity. To conserve reagent, the membrane may be cut into smaller pieces, but the volume of luminescent solution should not be reduced.
4. Use forceps to lift the membrane and place it on filter paper to drain excess working luminescent solution. Do not wash away the luminescent solution.
5. Line the inside of an X-ray film cassette with a piece of plastic wrap larger than the membrane. Place the hybridization membrane onto the plastic wrap, fold the wrap to fully enclose the membrane, and remove air bubbles and wrinkles, excess plastic wrap at the edges may be trimmed. Blot away remaining working luminescent solution with filter paper and secure the plastic-wrapped membrane inside the cassette using tape, preferably with the protein band side facing upward.
6. In the dark, insert X-ray film and expose for various durations ranging from several seconds to several minutes. Develop and fix the film.

NOTE

1. Steps 1-5 may be performed under fluorescent room light, however, prolonged exposure of the luminescent substrate to strong light may slightly reduce sensitivity, which can be avoided by carrying out the procedure in a darkroom. Wear gloves to prevent fingerprints on the membrane and maintain its cleanliness.
2. Prolonged exposure or excessive protein loading will increase background and destroy the linear relationship of band intensity. Insufficient exposure results in blurred bands.
3. Luminescence on the membrane bands becomes visible approximately 3 minutes after incubation with the working luminescent solution. Strong bands emit light visible to the naked eye in a darkroom, while low-abundance protein bands produce weaker luminescence that is invisible to the eye but still sufficient to expose X-ray film. Do not judge exposure time based solely on visual observation. Invisible luminescence can actually persist for several hours and expose X-ray film; therefore, weak bands may be exposed for 1-10 hours. If bands are unsatisfactory after exposure, wash the membrane with washing buffer, re-incubate with secondary antibody, then repeat ECL detection and exposure.
4. Due to the high sensitivity of this ultra-sensitive luminescent reagent, it is strongly recommended that most imported antibodies be used at initial dilutions of 1:1000 to 1:4000 for primary antibody and 1:2000 to 1:5000 for secondary antibody. Excessively high antibody concentrations will cause high background or complete absence of bands, leading to assay failure.
5. Some commercially available plastic wraps may quench luminescence when wrapping blotting membranes. Use high-quality plastic wrap such as Cling Wrap brand.

6. Use of prestained protein markers visible to the naked eye and fluorography-autoradiography exposure labels helps determine the exact position and molecular weight of bands on the film.
7. NaN_3 inhibits HRP activity. Avoid NaN_3 when recycling secondary antibody; if necessary, its concentration should not exceed 0.01%.