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Super ECL Western Blotting Detection Kit

Table 1. Kit Components and Storage

Cat. No.	Contents	Amount	Storage	Stability
FP300	Detection Reagent A Detection Reagent B	50 mL 50 mL	2-8 °C	The product is stable for 12 months when stored as directed.
FP301	Detection Reagent A Detection Reagent B	250 mL 250 mL		

Product Description

The **Super ECL Western Blotting Detection Kit** is a highly sensitive, luminol-based enhanced chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. The **Super ECL Western Blotting Detection Kit** enables low picogram or high femtogram detection of antigen by oxidizing luminol in the presence of HRP and peroxide. This reaction produces a prolonged chemiluminescence that can be visualized on X-ray film or other imaging system. Blots can be repeatedly exposed to X-ray film to obtain optimal results or stripped of the immunodetection reagents and re-probed. The special formulation of Super ECL Substrate makes it an ideal substitute for Amersham ECL Prime Substrate without the need for additional optimization of assay conditions.

The **Super ECL Western Blotting Detection Kit** consists of Luminol/Enhancer solution and Peroxide solution. The working ECL substrate solution is prepared by combining equal volumes of both solutions. The ECL substrate produces a high intensity signal for detection of high to low abundant proteins.

Table 2. Antibody dilution ranges to use with Super ECL Western Blotting Detection Kit

Primary Antibody Dilution Range from 1 mg/ml stock	Secondary Antibody Dilution Range from 1 mg/ml stock
1:1,000-1:30,000	1:50,000-1:200,000

Notes

- Using the same blotting conditions when switching from Amersham ECL Prime Substrate to Super ECL Substrate.
- Use a sufficient volume of all solutions to ensure membrane never becomes dry.
- For optimal results, use a shaking or rocking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers, as it inhibits HRP.
- Always wear gloves or use clean, plastic forceps.
- For best results prepare the substrate working solution freshly.

Western Blotting Protocol

Protein Transfer

1. Resolve the protein mixture on a 1-D or 2-D polyacrylamide gel.
2. Immerse the gel in an appropriate transfer buffer and allow it to equilibrate for 10–15 minutes.
3. **If working with a PVDF membrane:** Wet the membrane in 100% methanol for 15 seconds, or until the membrane appearance changes uniformly from opaque to semitransparent.

   If working with a nitrocellulose membrane: Proceed to step 4. Nitrocellulose membranes do not require prewetting.
4. Equilibrate the membrane for at least 5 minutes in the transfer buffer.
5. Soak filter paper in the transfer buffer for at least 30 seconds.
6. Assemble the transfer stack, and transfer proteins according to blotting apparatus manufacturer's instructions.
7. Remove the blot from the transfer system and briefly rinse the membrane in Milli-Q water to remove gel debris. Proceed with immunodetection protocol below. If required, the PVDF membrane blot may be air dried and stored refrigerated for several months.

Blocking

1. Incubate in suitable blocking solution for 1 hour at room temperature on an orbital shaker. Alternatively, membranes may be left in the blocking solution overnight in a refrigerator at 2-8 ^{o}C.
2. Briefly rinse the membrane with two changes of wash buffer. Note: wash buffer for all protocols: PBST or TBST.

Antibody Incubations

1. Prepare primary antibody solution by diluting the antibody in PBST or TBST.
2. Place the blot in the diluted primary antibody solution and incubate for at least 1 hour at room temperature with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.
3. Briefly rinse the membrane with two changes of wash buffer.
4. Wash the blot 4 to 6 times in wash buffer for 5 minutes each at room temperature on an orbital shaker. Additional or longer washes may further reduce background.
5. Prepare HRP-conjugated secondary antibody solution by diluting the antibody in PBST or TBST.
6. Place the blot in the diluted HRP-conjugated secondary antibody solution, and incubate for 1 hour at room temperature with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.
7. Briefly rinse the membrane with two changes of wash buffer.
8. Wash the blot 4 to 6 times in wash buffer for 5 minutes each at room temperature on an orbital shaker. Additional or longer washes may further reduce background.

Chemiluminescent Detection

1. Allow the detection solutions to equilibrate to room temperature for 20 minutes.
2. To prepare working ECL substrate, mix equal volumes of Solution A and Solution B in a clean container or test tube. Approximately 0.1 mL of working ECL substrate is required per cm^2 membrane area.

   The volumes of working ECL substrate needed for some common membrane sizes are indicated below:

Blot Size	Working ECL Substrate Required
7 × 8.5 cm	6 mL (3 mL Solution A + 3 mL Solution B)
10 × 10 cm	10 mL (5 mL Solution A + 5 mL Solution B)
8.5 × 13.5 cm	12 mL (6 mL Solution A + 6 mL Solution B)

3. Drain the excess wash buffer from the washed membrane and place it protein side up in a clean container. Add detection reagent onto the blot and make sure it completely covers the membrane.
4. Incubate the blot for 2 minutes at room temperature.
5. Drain off the excess substrate by holding the membrane edge gently against a tissue.
6. Cover the blot with a clean plastic wrap or sheet protector and remove any air bubbles. Ensure that the surface of the plastic wrap or sheet protector is dry and unwrinkled.
7. Expose the blot to a suitable X-ray film for an appropriate duration. Because of the high sensitivity of the ECL Substrate, a shorter exposure time may be required. The recommended initial exposure time is 30 seconds. The chemiluminescent signal on the blot will last at least two hours. If necessary, fresh ECL substrate can be added to the same blot for consecutive exposures.