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9620 Medical Center Drive, Suite 101
Rockville, MD 20850, USA
Web: www.abpbio.com

Product Information

ECL Western Blotting Detection Kit

Catalog Number: P007

Kit Contents

P007A: HRP Substrate Reagent A, 50 ml;

P007B: HRP Substrate Reagent B, 50 ml.

Storage upon receipt:

- 2-8°C
- Protect from light

Product Description

The **ECL Western Blotting Detection Kit** provides high sensitivity in western or dot/slot/spot blotting applications on both PVDF and nitrocellulose transfer membranes, and is compatible with all commonly used buffers and blocking reagents. Blots on PVDF membrane may be reprobed, allowing detection of multiple target proteins on the same blot.

The chemiluminescent detection is based on the catalyzed oxidation of luminol by horseradish peroxidase (HRP). The oxidized luminol emits visible light as it decays to its ground state.

The **ECL Western Blotting Detection Kit** consists of HRP Substrate Reagent A and HRP Substrate Reagent B. Working HRP substrate is prepared by combining equal volumes of Reagent A and Reagent B Solution. The HRP substrate produces a high intensity signal with low background for detection of both high and low abundance proteins.

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.

Antibody Incubations

1. If PVDF membranes were dried after transfer, wet the blots in 100% methanol for 15 seconds. The blot will uniformly change from opaque to semitransparent. **NOTE:** Omit this step if using nitrocellulose membrane.
2. Rinse the blot with water and then place the blot in blocking buffer and incubate for 1 hour with gentle agitation at room temperature.
3. Prepare primary antibody solution by diluting the antibody in wash or blocking buffer.
4. Place the blot in the diluted primary antibody solution and incubate for at least 1 hour with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.
5. Wash the blot with fresh wash buffer a minimum of three times with gentle agitation for 5–10 minutes. Additional or longer washes may further reduce background.
6. Prepare HRP-conjugated secondary antibody solution by diluting the antibody in wash or blocking buffer.
7. Place the blot in the diluted HRP-conjugated secondary antibody solution, and incubate for 1 hour with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.
8. Wash the blot with fresh wash buffer a minimum of three times with gentle agitation for 5–10 minutes. Additional or longer washes may further reduce background.

Chemiluminescent Detection

1. To prepare working HRP substrate, mix equal volumes of Reagent A and Reagent B Solution in a clean container or test tube. Approximately 0.1 mL of working HRP substrate is required per cm^2 membrane area.

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

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

2. Allow the HRP substrate to reach room temperature (~10 minutes).

3. Place the blot protein side up in a clean container, and add the HRP substrate onto the blot.

4. Incubate the blot for 5 minutes at room temperature.

5. Drain the excess substrate.

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 Western HRP 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 HRP substrate can be added to the same blot for consecutive exposures.
NOTE: The working HRP substrate can be stored up to 7 days in the dark at 2–8 °C without any detectable loss of activity.