Affinity® ECL Western Blotting Substrate (pg grade)

Introduction

The Affinity ECL Western Blotting Substrate is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. Affinity ECL Western Blotting Substrate enables the detection of femtogram amounts of antigen and allows for easy detection of HRP using photographic or other imaging methods. 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 Affinity ECL Substrate makes it the ideal substitute for other ECL Substrate without the need for additional optimization of assay conditions.

Important Product Information

• Affinity ECL Substrate requires more dilute antibody concentrations than those used with precipitating colorimetric HRP substrates. To optimize antibody concentrations, perform a systematic dot blot analysis.
• Empirical testing is essential to determine the appropriate blocking reagent for each Western blot system, as crossreactivity of the blocking reagent with antibodies causes nonspecific signal. Blocking buffer also affects system sensitivity.
• Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin, which causes high background signal.
• Use sufficient volumes of wash buffer, blocking buffer, antibody solution and substrate working solution to cover blot and ensure that it never becomes dry. Using large blocking and wash buffer volumes minimizes nonspecific signal.
• For optimal results, use a shaking platform during incubation steps.
• Add Tween™-20 Detergent (final concentration of 0.05-0.1%) to the blocking buffer and all diluted antibody solutions to minimize nonspecific signal.
• Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
• Do not handle membrane with bare hands. Always wear gloves or use clean forceps.
• All equipment must be clean and free of foreign material. Metallic devices (e.g., scissors) must have no visible signs of rust. Rust may cause speckling and high background.
• Exposure to the sun or any other intense light can harm the substrate. For best results keep the substrate working solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the working solution.

Procedure Summary

Note: Antigen and antibody amounts may require optimization.
1. Dilute the primary antibody to 5.0-0.5μg/mL.
2. Dilute the secondary antibody to 1.0-0.067μg/mL.
3. Mix Detection Reagents 1 and 2 at a 1:1 ratio and add it to the blot. Incubate blot for 1-30 minutes.
4. Drain excess reagent. Cover blot with a clear plastic sheet protector or clear plastic wrap.
5. Expose blot to X-ray film.

Detailed Western Blotting Procedure

1. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Reagent for 60 minutes at room temperature (RT) with shaking. If desired, block overnight at 2-8ºC without shaking.
2. Remove the Blocking Reagent and add the primary antibody working dilution. Incubate blot for 1 hour at RT with shaking or overnight at 2-8°C without shaking.
3. Briefly rinse membrane in Wash Buffer two times.
4. Wash membrane by suspending it in Wash Buffer and agitating for ≥ 5 minutes. Replace Wash Buffer at least 4-6 times. Increasing the Wash Buffer volume, the number of washes and wash duration may help minimize background signal.
5. Incubate blot with the HRP-conjugate working dilution for 1 hour at RT with shaking.
6. Repeat Steps 3 and 4 to remove nonbound HRP-conjugate.

Note: Membrane MUST be thoroughly washed after incubation with the HRP-conjugate.

7. Prepare the substrate working solution by mixing equal parts of Detection Reagents 1 and 2. Use 0.125mL Working Solution per cm2 of membrane.

Note: For best results prepare working solution immediately before use. The working solution is stable for 1 hour at RT.

8. Incubate blot with working solution for 1 minute at RT.
9. Remove blot from working solution and place it in a plastic sheet protector or clear plastic wrap. Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and the membrane protector.
10. Place the protected membrane in a film cassette with the protein side facing up. Turn off all lights except those appropriate for X-ray film exposure (e.g., a red safelight).

Note: Film must remain dry during exposure. For optimal results, perform the following precautions:

- Make sure excess substrate is removed from the membrane and the membrane protector.
- Use gloves during the entire film-handling process.
- Never place a blot on developed film, as chemicals on the film may reduce signal.

11. Carefully place X-ray film on top of the membrane. Perform a first-time exposure of 60 seconds. Vary the exposure time to achieve optimal results.
12. Develop film using appropriate developing solution and fixative. If signal is too intense, reduce exposure time or strip and re-probe the blot with decreased antibody concentrations.

Storage

Upon receipt store at 2-8°C.

Cat.#	Product Name	Size
K001	Solution Ⅰ	25/50/250 ml
K002	Solution Ⅱ	25/50/250 ml