ECL kit (femtogram)

CAT NO.: APL-1023

DESCRIPTION

The ECL Western Blotting Substrate is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. 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 Apolo ECL Substrate makes it the ideal substitute for other ECL Substrate without the need for additional optimization of assay conditions.

CONTENT

	APL-1023
ECL Western Blotting Substrate	100 mL

STORAGE

2-8°C

IMPORTANT PRODUCT INFORMATION

1. 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.
2. 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.
3. 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.
4. 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.
5. For optimal results, use a shaking platform during incubation steps.
6. Add Tween™-20 Detergent (final concentration of 0.05-0.1%) to the blocking buffer and all diluted antibody solutions to minimize nonspecific signal.
7. Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
8. Do not handle membrane with bare hands. Always wear gloves or use clean forceps.
9. 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.

10. 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

1. Dilute the primary antibody to 5.0-0.5 µg/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 1-60 seconds
4. Drain excess reagent. Cover blot with a clear plastic sheet protector or clear plastic wrap
5. Expose blot to X-ray film

NOTE: Antigen and antibody amounts may require optimization.

PRODUCT USE LIMITATION

Research use only.