INSTRUCTIONS

Chemistar ™ High-sig ECL Western Blotting Substrate

Kit Contents

No.	Description
180-501	**Tanon High-sig ECL Western Blotting Substrate**, sufficient reagents for 1000cm^2 of membrane. **Contents:** Luminol Reagent, 50 mL ; Peroxide Solution, 50 mL
180-5001	**Tanon High-sig ECL Western Blotting Substrate**, sufficient reagents for 5000cm^2 of membrane. **Contents:** Luminol Reagent, 250 mL ; Peroxide Solution, 250 mL

Introduction

Tanon High-sig ECL Western Blotting Substrate is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. Tanon High-sig ECL Western Blotting Substrate enables the detection of picogram 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 or Chemiluminescence imaging system to obtain optimal results or stripped of the immunodetection reagents and re-probed. The special formulation of Tanon High-sig ECL Western Blotting Substrate makes it the ideal substitute for Amersham ECL Substrate or Pierce ECL Western Blotting Substrate without the need for additional optimization of assay conditions.

Important Product Information

1. Use the same blotting conditions when switching from Amersham ECL Substrate or Pierce ECL Substrate to Tanon High-sig ECL Western Blotting Substrate.
2. If you are currently using a SuperSignal Chemiluminescent Substrate, switching to Tanon High-sig ECL Western Blotting Substrate requires increasing antigen and antibody concentrations. To determine the appropriate concentrations, perform a systematic dot blot analysis.
3. Tanon High-sig ECL Western Blotting Substrate requires more dilute antibody concentrations than those used with precipitating colorimetric HRP substrates. To optimize antibody c oncentrations, perform a systematic dot blot analysis.
4. Empirical testing is essential to determine the appropriate blocking reagent for each Western blot system, as cross-reactivity of the blocking reagent with antibodies causes nonspecific signal. Blocking buffer also affects system sensitivity.
5. 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.
6. 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.
7. For optimal results, use a shaking platform during incubation steps.
8. Add Tween™-20 Detergent (final concentration of 0.05-0.1%) to the blocking buffer and all diluted antibody solutions to minimize nonspecific signal.
9. Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
10. Do not handle membrane with bare hands. Always wear gloves or use clean forceps.
11. All equipment must be clean and free of foreign material. Metallic devices must have no visible signs of rust. Rust may cause speckling and high background.
12. 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.

NOTE

Tanon High-sig ECL Western Blotting Substrate requires less sample and less primary and secondary antibodies than other reagents such as Amersham ECL and Pierce ECL Substrate.

Note the following recommendations when changing from Pierce ECL Substrate or similar reagents.

1. Storage conditions: store at 2-8℃.
2. Dilute the primary antibody to 1:200-1:5000 from a 1mg/mL stock.
3. Dilute the secondary antibody to 1:2000-1:20000 from a 1mg/mL stock.
4. Mix Detection Reagents 1 and 2 at a 1:1 ratio and add it to the blot (Approximately 0.1mL of working HRP substrate is required per cm^2 membrane area). Incubate blot for 1 minute.
5. Drain excess reagent. Cover blot with a clear plastic sheet protector or clear plastic wrap.
6. Expose blot to X -ray film or Chemiluminescence imaging system.

Troubleshooting

Problem	Possible Cause	Solution
High background	Insufficient washes	Increase wash buffer volumes and wash cycle repetitions.
	Too much HRP in the system	Dilute HRP-conjugate further.
	Poor quality blotting reagents or buffers	Use high grade reagents and Milli-Q water.
	Cross-reactivity between blocking reagent and antibody	Use Tween-20 surfactant in the washing buffer or use different blocking agent.
	Poor quality antibodies	Use high quality affinity-purified antibodies.
	Secondary antibody concentration is too high	Decrease the antibody concentration or reduce x-ray exposure time.
	Insufficient washing or blocking	Increase the concentration or volume of the blocking agent used to compensate for the greater surface area of the membrane. In -addition, incubation times for both the wash and blocking steps may need to be extended.
Weak or no signal	Inefficient protein transfer	Optimize protein transfer. If necessary stain the blot to visualize protein and confirm complete transfer.
	Antigen concentration is too low	Load more antigen on the gel.
	Antibody is inactive	Multiple freeze-thaw or bacterial contamination of antibody solution can change antibody titer or activity. Prepare fresh antibody working solutions.
	Outdated substrate	Prepare fresh working HRP substrate and store properly. Outdated substrate can reduce sensitivity
	Too much HRP exhausted the substrate	Dilute HRP-conjugate further
Speckled background	Aggregate formation in the HRP -conjugate	Filter HRP-conjugate through a 0.2 μm filter before use.
Non-specific bands	Primary antibody concentration too high	Increase primary antibody dilution.
	Secondary antibody concentration too high	Increase secondary antibody dilution.
	Poor antibody specificity	Use SuperSignal Western Blot Enhancer
White bands with a black background	Too much HRP-conjugated antibody	Reduce concentration of secondary, HRP-conjugated antibody.
Uneven blot	Fingerprints, fold marks or forceps imprints on the blot	Avoid touching membrane with bare hands or folding membrane; use gloves and blunt end forceps.
	Air bubbles were trapped during transfer	Using a pipette or a stirring rod, gently roll out any trapped air bubbles while assembling the transfer stack.