

★ Storage

Store at 2-8 °C for for up to 1 year.

★ Contents

- Product Manual
- Detection Plus Solution A
- Detection Plus Solution B

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

★ Shipping Condition

Ship with ice pack.

★ Introduction

West-Q Chemiluminescent Substrate Kit, Plus is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. West-Q Chemiluminescent Substrate Kit, plus enables 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 to obtain optimal results or stripped of the immunodetection reagents and reprobed.

★ Usage

West-Q Chemiluminescent Substrate Kit, Plus is a high-sensitivity substrate that is more sensitive than most chemiluminescent products. For optimal performance of West-Q Chemiluminescent Substrate Kit, Plus antibody must be more dilute than those used with other substrates. If you have been using other chemiluminescent substrate, dilute both primary and secondary antibodies at least 5-fold more. Recommended dilution ranges are listed below.

- Primary Antibody Dilution Range from a 1 mg/ml stock
1:500-1:5,000 or 0.1-1 ug/ml
- Secondary Antibody Dilution Range from a 1 mg/ml stock
1:20,000-1:100,000 or 2-10 ng/ml

★ Procedure

Note: Antigen and antibody amounts may require optimization. Recommended antibody dilutions must be used to obtain positive results. For recommended dilution ranges please see usage section.

1. Prepare the Substrate Working Solution by mixing equal parts of the West-Q Chemiluminescent Substrate Kit, Plus Solution A and B. Use 0.125 ml Working Solution per cm² of membrane. The Working Solution is stable for 24 hours at room temperature.

Note: For best results prepare Working Solution immediately before use.

	Final Working Solution	Advantage
Lowest Sensitivity	0.04 - 0.06 ml/cm ²	Reduce background
Standard Sensitivity	0.06 - 0.08 ml/cm ²	Cost less than any other ECL
Pico Sensitivity	0.1 - 0.125 ml/cm ²	Conserve costly antibodies

Note: All the results can be varied by experimental conditions. For best result, optimize final working solution volume, exposure time, and antibody concentration.

2. Incubate membrane with Working Solution for 1-5 minutes at RT.
3. Remove membrane 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 membrane and the protector.
4. 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.
5. 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. Enhanced or pre-flashed film is not necessary. Caution: Any movement between the film and membrane can cause artifacts on the film.
6. Develop film using appropriate developing solution and fixative. If signal is too intense, reduce exposure time or strip and reprobe the membrane with decreased antibody concentrations.

★ Tips

- Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each Western blot system. Determining the proper blocking buffer can help increase sensitivity and prevent non-specific signal caused by cross-reactivity between the antibody and the blocking reagent.
- 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 membrane and ensure that it never becomes dry. Using large blocking and wash buffer volumes minimizes nonspecific signal.
- Add Tween-20 (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.
- 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.

★ Related Products

Product Name	Cat No
Tween 20, Molecular Biology Grade	T9100
West-EZier Super Blocking Buffer	W3700
West-Ez Blocking Buffer, 3% BSA-IgG Free	W3710
West-Ez Stripping Buffer	S2100