Storage

Store at 2-8°C, Stable for up to 1 year.

Contents

Product Manual
- West-Ezier Rapid Blocking Biffer,   100ml
- West-EZier Antibody Dilution Buffer,   100ml
- West-EZier Washing Buffer (10X),   100ml
- West-EZier 2nd anti-Rabbit IgG-HRP,   100ul
- West-Q Pico Dura ECL Solution.   20ml

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-Ezier Rapid Western Kit contains optimized reagents which allows one-hour rapid Western protocol to replace the classical three-step Western blot process, which takes at least four hours after the gel transfer. West-EZier Rapid Western Kit provides optimized reagents for blocking, antibody dilution and washing. The West-EZier Rapid Western Kit requires minimal hands-on time and provides high signal with low background. West-EZier time and provides high signal with low background. West-EZier Rapid Western protocol is rapid and efficient method for developing Western blot without the need of purchase expensive instrument and consumables which required by other rapid Western blotting systems.

Materials Required

- Membrane with transfered protein.
- Primary Antibody: Choose a mouse or rabbit antibody that is specific to the target protein(s).
- X-ray film, film cassette, developing and fixing reagents for film processing, or an imaging instrument such as CCD camera.
- Rotary platform shaker.

Tips

- Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenou biotin, which causes high background signal.
- Use sufficient volumes of wash buffer, blcking buffer, antibody solution and substrate working solution to cover membrane and ensure that it never becomes dry. Using large blocking and wash buffer voluems minimizes 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.

Procedure

Preparation Step

1. Dilute 10X West-EZier Washing buffer with deionized water to make 1X Washing buffer.
2. Dilute the primary antibody in a West-EZier Antibody Dilution Buffer. The dilution factor should be determined empirically for each antibody.
3. Mix the Enhancer Solution and the Substrate Solution before use.

Blotting and Detection Step

1. After transfer, briefly wash nitrocellulose membrane or PVDF membrane in West-EZier Washing Buffer (1X) to remove transfer buffer. If using a PVDF mem-brane that is dried up, re-wet in 100% methanol and rinse in distilled water for 5 minutes.
2. Place your blot into an incubation tray and add enough West-EZier Blocking Buffer to cover the blot completely and allow to flow in buffer. Block for 5 minutes at room temperature with gentle agitation.
3. Discard blocking buffer
  Note: Do not wash in this step
4. Add enough of diluted primary antibody solution to cover the blot completely and incubate for 10 minutes at room temperauture with gentle agitation.
  Note: Do not wash in this step
5. Add 10ul of preoptimized West-EZier 2nd anti-Rabbit IgG-HRP solution and incubate 20 minutes at room temperature with gentle agitation.
6. Remove the blot and place it in a clean incubation tray.
7. Briefly rinse the membrane twice with the wah buffer.
8. Wash the membrane in > 4ml/cm² of wah buffer for 5 minutes at room temper-ature with gentle agitation. Repeat this wah at least 3 times.
9. Remove the blot and place it in a clean tray. Add the West-Q Pico Dura ECL Solution and place it in a plastic sheet protector or clean 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.
11. Expose the blot to film or use your perferred imaging method.