VECTOR LABORATORIES

part of **Maravai LifeSciences**

USER GUIDE

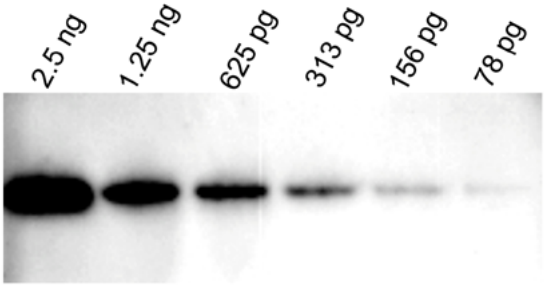
WestVision™ Peroxidase (HRP) Polymer

for Western Blot Detection

Cat. No.: WB-1000 Anti-Rabbit IgG
WB-2000 Anti-Mouse IgG

Storage: 2-8 °C

Unit Size: 0.8 ml



Human recovered plasma with Rabbit Anti-Transferrin and detected with WestVision Horse Anti-Rabbit IgG. Developed with DuoLuX Chemiluminescent Substrate. Membrane blocked with 1x Casein.

DESCRIPTION

WestVision Peroxidase Polymer antibody conjugates are intended to be used in western blot applications to detect primary antibodies made in mouse (WB-2000) or rabbit (WB-1000) on nitrocellulose or PVDF membranes.

Reagents Not Provided

- Wash Buffer: Phosphate buffered saline with Tween® 20; 10 mM Na_2HPO_4, pH 7.5, 150 mM NaCl, and 0.1% Tween 20 (PBST).
- Blocking Solutions: We recommend WestVision Block and Diluent (SP-7000).
- Primary Antibody: Dilute primary antibody in appropriate blocking solution or PBST according to manufacturer's recommendation.
- Peroxidase Substrate: Chromogenic or chemiluminescent

WestVision Reagent Preparation

- WestVision Working Solution. Dilute the WestVision reagent in PBST or the appropriate blocking solution. The diluent must not contain sodium azide. For chromogenic substrates dilute the WestVision reagent to 1:500 – 1:2500. For chemiluminescent substrates dilute the WestVision reagent to 1:5,000 – 1:200,000.

DETECTION PROTOCOL

Recommended volumes are based on the development of 100 cm^2 membranes. Volumes may be proportionally adjusted for blots of different sizes.

1. Remove the blot from the transfer apparatus and block the membrane in 10 ml blocking solution for 30-60 minutes at room temperature with gentle agitation.
2. Incubate the membrane in 10 ml of primary antibody solution for 30-60 minutes at room temperature or at 4 °C overnight with gentle agitation (or for a time established to be optimal for the concentration of primary antibody used).
3. Wash the membrane 3x 5 minutes each in 10 ml PBST with gentle agitation.
4. Incubate the membrane for 45 minutes at room temperature with 10 ml WestVision working solution with gentle agitation.
5. Wash the membrane 3x 5 minutes each in 10 ml PBST with gentle agitation.
6. Prepare substrate working solution according to the substrate kit instructions. Following are protocols for signal development using either a chromogenic or chemiluminescent substrate.

Chromogenic signal development with Vector® TMB substrate (SK-4400):

7. Equilibrate membrane for 2 minutes in PBS in a clean vessel.
8. Incubate membrane in the substrate working solution at room temperature with gentle agitation for 5 minutes or until suitable staining develops. Briefly rinse the membrane in PBS and air-dry.

Chemiluminescent signal development using DuoLuX® Substrate (SK-6604):

7. Equilibrate membrane for 2 minutes in PBS in a clean vessel.
8. Remove excess buffer by holding the membrane vertically and touching the edge of the membrane to absorbent paper.
9. Place membrane target-side-up on plastic wrap on a level surface.
10. Pipette 5 ml of DuoLuX Substrate working solution onto the membrane surface.
11. Incubate for 5 minutes under subdued light. (If high background is present, rinse the membrane in PBS for a few seconds and remove excess as in Step 8.)
12. Place the membrane between two pieces of plastic wrap or a clear sheet protector. Acquire image with the gel imager or expose the membrane to x-ray film for the appropriate time.

Detailed product listings, specifications, protocols and additional information is available on our website: **vectorlabs.com**

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