WesternBreeze® Chemiluminescent Western Blot Immunodetection Kit

Package Contents

Catalog Numbers
WB7104 Anti-Mouse
WB7106 Anti-Rabbit
WB7108 Anti-Goat

Contents list

Storage Conditions

- Store all solutions at 4°C.
- Once diluted, use the solutions the same day.
- All solutions are proprietary formulations and contain chlorobutanol as a preservative.

Required Materials

List of Materials

Timing

Preparation and immunodetection: ~3 hours

Selection Guide

Western Blotting Kits
Go online to view related products.

Product Description

- WesternBreeze® Chemiluminescent Kits detect proteins that have been immobilized on membranes (nitrocellulose or PVDF) following western transfer or bound directly from solution (dot blots).
- Detection is accomplished with a ready-to-use CDP-Star® chemiluminescent substrate for alkaline phosphatase.
- Protein bands can be captured either by X-ray film or a CDP-Star® compatible imaging system.

Important Guidelines

- Avoid touching the working surface of the membrane, even with gloves.
- Use ultra-filtered water, free from alkaline phosphatase activity. Do not allow the membrane to dry out after adding the chemiluminescent substrate.
- Use a rotary shaker platform rotating at 1 revolution/second for all washing, blocking, and incubating steps.
- Add solutions to the trays slowly, at the membrane edge, to avoid bubbles forming under the membrane. Decant from the same corner of the dish to ensure complete removal of previous solutions.

Online Resources

Visit our product pages for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.

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Print Options

Protocol Outline

This process involves the following phases:

A. Prepare membrane and solutions.
B. Incubate membrane with Primary and then Secondary Antibodies.
C. Add and incubate substrates.
D. Complete detection.

Western Blotting Protocol

See page 2 for quantities and guidelines to prepare the antibodies and solutions.

See page 3 to view a typical immunodetection procedure for use with small membranes (60 cm^2).

Preparing the Membrane

For Westerns or Western dot blots, wash membrane twice for 5 minutes in 20 mL of water to remove gel, transfer buffer, and weakly bound proteins. Then, proceed with immunodetection.
For water washed and dried membranes:

- NC Membranes: Proceed with immunodetection.
- PVDF membranes: Re-wet membrane in methanol followed by two 20-mL water washes for 5 minutes.

Scaling up for Large Membranes

To blot standard size gels (~200 cm^2), scale up the required solution volumes × 3.3. Use a tray that closely matches the dimensions of the membrane for the most efficient use of the solutions.

Limited Product Warranty and Disclaimer Details

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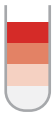
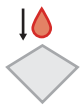
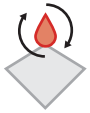
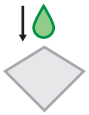
Preparing Solutions for Nitrocellulose and PVDF Membranes

The Blocking Solution is used for blocking and as a Primary Antibody Diluent for NC membranes. For western blots from Novex® or other mini gels, prepare solutions as described in table below for ~60 cm^2 membrane.

Timeline	Steps	Procedure Details			
		For NC Membrane		For PVDF Membrane	
1 	**Prepare Blocking Solution**	Ultra filtered Water	14 mL	Ultra filtered Water	5 mL
		Blocker/Diluent (Part A)	4 mL	Blocker/Diluent (Part A)	2 mL
		Blocker/Diluent (Part B)	2 mL	Blocker/Diluent (Part B)	3 mL
		Total Volume	20 mL	Total Volume	10 mL
		For NC Membrane		For PVDF Membrane	
2 	**Prepare Primary Antibody Diluent**	Dilute your Primary Antibody according to the manufacturers recommendations into 10 mL of NC Blocking Solution (see above). Typically, commercial Primary Antibody preparations are diluted 1:1000 to 1:5000 to a concentration of about 1 to 0.2 µg/mL.		Ultra filtered Water	7 mL
				Blocker/Diluent (Part A)	2 mL
				Blocker/Diluent (Part B)	1 mL
				Total Volume	10 mL
				Dilute your Primary Antibody into this diluent according to manufacturer's recommendations.	
		For NC Membrane		For PVDF Membrane	
3 	**Prepare Antibody Wash**	Ultra-filtered Water	150 mL	Ultra-filtered Water	150 mL
		Antibody Wash Solution (16X)	10 mL	Antibody Wash Solution (16X)	10 mL
		Total Volume	160 mL	Total Volume	160 mL
		For NC Membrane		For PVDF Membrane	
4 	**Prepare Chemiluminescent Substrate**	Chemiluminescent Substrate	2.375 mL	Use the CDP-Star directly from the bottle. DO NOT add the Chemiluminescent Substrate enhancer (Nitro-Block-II enhancer).	
		Chemiluminescent Substrate Enhancer	0.125 mL		
		Total Volume	2.5 mL	Total Volume	2.5 mL

Immunodetecting with the Chemiluminescent Western Blot Kit

The table below describes the procedure for immunodetection using small membranes (60 cm^2).

Timeline	Steps	Procedure Details
Day 1, 1 	**Block membrane**	Use 10 mL of blocking solution in the dish provided. Incubate 30 minutes on a rotary shaker (1 revolution/second). Decant the Blocking Solution.
2 	**Wash membrane with water**	Rinse the membrane with 20 mL of water for 5 minutes, then decant. Repeat once.
3 	**Incubate with Primary Antibody**	Incubate the membrane with 10 mL of Primary Antibody Solution for 1 hour, then decant.
4 	**Wash membrane with Antibody Wash**	Wash membrane for 5 minutes with 20 mL of prepared Antibody Wash, then decant. Repeat 3 times.
5 	**Incubate with Secondary Antibody**	Incubate the membrane in 10 mL of Secondary Antibody Solution for 30 minutes, then decant.
6 	**Wash membrane with Antibody Wash**	Wash the membrane for 5 minutes with 20 mL of Antibody Wash, then decant. Repeat 3 times.
7 	**Wash membrane with water**	Rinse the membrane with 20 mL of water for 2 minutes, then decant. Repeat twice.
8 	**Add substrate**	Place the membrane on sheet of transparency plastic. Do not allow membrane to dry out. Evenly apply 2.5 mL of Chemiluminescent Substrate Solution to the membrane without touching the surface (remember to add Chemiluminescent Substrate Enhancer for NC membranes).
9 	**Incubate with substrate**	Allow reaction to develop for 5 minutes at room temperature.
10 	**Detect**	Blot excess Chemiluminescent Substrate from membrane using filter paper in the kit. Do not allow membrane to dry out. Cover membrane with another piece of transparency plastic. Expose x-ray film. for one second to several minutes, or use another imaging device.