

Pierce[®] Fast Western Blot Kit, SuperSignal[®] West Pico Substrate

35060 35061 35065 35066

2172.0

Number	Description
35060	<p>Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Mouse, contains sufficient reagents to perform 20 blots (8 × 10 cm) probed with a mouse primary antibody</p> <p>Kit Contents:</p> <p>Fast Western Antibody Diluent, 400 ml</p> <p>Fast Western 10X Wash Buffer, 200 ml</p> <p>Fast Western Mouse Optimized HRP Reagent, Pico, 20 ml</p> <p>SuperSignal West Pico Luminol/Enhancer Solution, 100 ml</p> <p>SuperSignal West Stable Peroxide Solution, 100 ml</p>
35061	<p>Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Rabbit, contains sufficient reagents to perform 20 blots (8 × 10 cm) probed with a rabbit primary antibody</p> <p>Kit Contents:</p> <p>Fast Western Antibody Diluent, 400 ml</p> <p>Fast Western 10X Wash Buffer, 200 ml</p> <p>Fast Western Rabbit Optimized HRP Reagent, Pico, 20 ml</p> <p>SuperSignal West Pico Luminol/Enhancer Solution, 100 ml</p> <p>SuperSignal West Stable Peroxide Solution, 100 ml</p>
35065	<p>Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Mouse Trial Size, contains sufficient reagents to perform five blots (8 × 10 cm) probed with a mouse primary antibody</p> <p>Kit Contents:</p> <p>Fast Western Antibody Diluent, 100 ml</p> <p>Fast Western 10X Wash Buffer, 50 ml</p> <p>Fast Western Mouse Optimized HRP Reagent, Pico, 5 ml</p> <p>SuperSignal West Pico Luminol/Enhancer Solution, 25 ml</p> <p>SuperSignal West Stable Peroxide Solution, 25 ml</p>
35066	<p>Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Rabbit Trial Size, contains sufficient reagents to perform five blots (8 × 10 cm) probed with a rabbit primary antibody</p> <p>Kit Contents:</p> <p>Fast Western Antibody Diluent, 100 ml</p> <p>Fast Western 10X Wash Buffer, 50 ml</p> <p>Fast Western Rabbit Optimized HRP Reagent, Pico, 5 ml</p> <p>SuperSignal West Pico Luminol/Enhancer Solution, 25 ml</p> <p>SuperSignal West Stable Peroxide Solution, 25 ml</p>

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate contains optimized reagents that shorten the time to perform a typical Western blot from 4 hours to ~55 minutes. The kit provides all the reagents necessary to complete a Western blot that was probed with a mouse or rabbit primary antibody. The protocol requires minimal hands-on time and yields results comparable to classic Western blotting. The SuperSignal West Pico Chemiluminescent Substrate produces a signal that is detected using photographic or other imaging methods. Blots can be repeatedly exposed to film to obtain optimal results or stripped of the primary antibody and immunodetection reagents and then reprobed.

Important Product Information

- The Fast Western Blot Kit reagents are optimized to function together. Use the primary antibody at the concentration typically used in Western blotting procedures with SuperSignal West Pico Substrate (i.e., 0.2-1.0 µg/ml).
- The Pierce Fast Western Blot Kit is optimized for blots that are not pre-blocked. Pre-blocking the membrane can cause a decrease in assay sensitivity.
- Shake the Fast Western Antibody Diluent well before use. The antibody diluent is a saturated solution and settling may occur.
- Use a clean incubation tray for each step of the Fast Western blotting procedure. Trays do not need to be changed between washes.
- For optimal results, use a shaking platform during incubation steps.
- Do not handle membrane with ungloved hands. Always wear gloves or use clean forceps to handle the blot.
- The stability of primary antibodies diluted in the Fast Western Antibody Diluent varies. For best results, prepare the antibody working dilution immediately before use.
- All equipment must be clean and free of foreign material. Metallic devices (e.g., scissors) must have no visible signs of rust. Rust causes speckling and high background.
- The Substrate Working Solution is stable for up to 8 hours at room temperature.
- We offer a variety of protein transfer membranes, primary antibodies and X-ray film. Please consult our web site or catalog for product and ordering information.

Additional Materials Required

- Membrane with transferred 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 a CCD camera
- Rotary platform shaker

Material Preparation

1X Wash Buffer	Mix 1 part of the Fast Western 10X Wash Buffer with 9 parts of water. Example: Mix 10 ml of 10X Fast Western Wash Buffer with 90 ml of water. Prepare at least 60 ml for each 8 × 10 cm blot.
Primary Antibody Solution	Shake the Fast Western Antibody Diluent well before use. Dilute the primary antibody (0.2-1.0 µg/ml) with Antibody Diluent. Use ~0.125 ml of antibody per cm ² of membrane (e.g., 10 ml per 8 × 10 cm blot). The stability of diluted primary antibodies varies depending on the antibody. For best results, prepare working dilution just before use. Example: To prepare 1 µg/ml from a stock concentration of 1 mg/ml, mix 10 µl of the primary antibody with 10 ml of the Fast Western Antibody Diluent.
Optimized HRP Reagent Working Dilution	Mix 1 part of Optimized HRP Reagent with 9 parts of Antibody Diluent. Use 0.125 ml per cm ² of membrane (e.g., 10 ml per 8 × 10 cm blot). For best results, use this solution within 1 hour. Example: Mix 1 ml of the Optimized HRP Reagent with 10 ml of the Antibody Diluent. Note: If using the SNAP i.d. TM System, use from 0.2 to 1 ml of Optimized HRP Reagent and, if needed, adjust to the appropriate volume with the Antibody Diluent.
SuperSignal West Pico Working Solution	Mix SuperSignal West Pico Luminol/Enhancer Solution and SuperSignal West Stable Peroxide Solution at 1:1. Use 0.125 ml Working Solution per cm ² of membrane (e.g., 10 ml per 8 × 10 cm blot). For best results, prepare working solution just before use (Step 8). The working solution is stable for up to 8 hours at room temperature. Example: Mix 5 ml of Luminol/Enhancer Solution with 5 ml of Stable Peroxide Solution.

Fast Western Blotting Procedure

1. Remove blot from the transfer apparatus and place in a clean incubation tray.
2. Briefly wash blot in 1X Wash Buffer to remove transfer buffer.
3. Add the Primary Antibody Solution to the blot and incubate for 30 minutes at room temperature (RT) with shaking.
Note: Primary antibody incubation time may be reduced to 10 minutes at RT or increased to an overnight incubation at 4°C. Evaluate each specific antibody/antigen to determine compatibility with incubation time.
4. Remove blot from the primary antibody solution and place it in a clean incubation tray.
5. Add the Optimized HRP Reagent Working Dilution and incubate for 10 minutes at RT with shaking. Higher sensitivity can be obtained by increasing the incubation time to 15 minutes; however, one or more additional washes will be required to reduce background.
6. Remove blot from the HRP solution and place it in a clean incubation tray.
7. Wash membrane by suspending it in approximately 20 ml of Fast Western 1X Wash Buffer and agitating for 5 minutes. Repeat this wash twice.
8. Remove blot and place it in a clean incubation tray. Add the SuperSignal West Pico Working Solution and incubate for 1-5 minutes at RT.
9. Remove blot from SuperSignal West Pico 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 blot and the membrane protector.
10. Expose the blot to film or use your preferred imaging method.

Troubleshooting

Problem	Possible Cause	Solution
High background	Incubation tray is contaminated with HRP	Use a clean incubation tray after every step of the procedure
	Used too much primary antibody	Reduce primary antibody concentration to 0.2-1 µg/ml
	Insufficient washing	Use a minimum of 20 ml of 1X Wash Buffer for each wash
		Use a clean incubation tray to begin the wash steps
		Add an additional wash cycle for a total of four 5 minute washes
Overexposed film	Decrease exposure time or use Thermo Scientific Pierce Background Eliminator (Product No. 21065)	
Omitted the brief pre-wash	Wash membrane in 1X Wash Buffer briefly before beginning the protocol	
Weak signal	Used insufficient quantities of antigen or primary antibody	Strip and re-probe blot using a higher concentration of antibodies
		Load higher concentrations of sample onto the gel
	Used too much primary antibody	Reduce primary antibody concentration to 0.2-1 µg/ml
	Used too much sample	Reduce amount of sample on the gel
Spots within the protein bands	Inefficient protein transfer	Optimize transfer conditions
	Unevenly hydrated membrane	Optimize transfer procedure
	Bubble between X-ray film and membrane	Hydrate membrane according to manufacturer's instructions
Speckling	Over-heating during electrophoresis or transfer	Remove all bubbles before exposing blot to film
		Control temperature during electrophoresis and transfer

Additional Information

Visit our web site for additional information relating to this product including the following:

- Tech Tip #67: Chemiluminescent Western Blotting Technical Guide and Protocols
- Tech Tip #21: Convert to SuperSignal West Pico Substrate from ECL Substrate
- Tech Tip #23: Strip and reprobe Western blots
- Tech Tip #24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip #32: Guide to enzyme substrates for Western blotting
- Tech Tip #43: Protein stability and storage
- Western Blotting Handbook and Troubleshooting Guide

Related Thermo Scientific Products

34079	SuperSignal West Pico Chemiluminescent Substrate, Trial Kit
34077	SuperSignal West Pico Chemiluminescent Substrate, 100 ml
34080	SuperSignal West Pico Chemiluminescent Substrate, 500 ml
34078	SuperSignal West Pico Chemiluminescent Substrate, 1 L
34089	CL-XPosure™ Film, 7 × 9.5 in (18 × 24 cm) sheets, 100/pkg.
34090	CL-XPosure Film, 5 × 7 in (13 × 18 cm) sheets, 100/pkg.
34091	CL-XPosure Film, 8 × 10 in (20 × 25 cm) sheets, 100/pkg.

34097	CL-XPosure Film, 9.5 × 11.8 in (24 × 30 cm) sheets, 100/pkg.
34099	CL-XPosure Film, 14 × 17 in (35 × 40 cm) sheets, 100/pkg.
21059	Restore Western Blot Stripping Buffer, 500 ml
46430	Restore PLUS Western Blot Stripping Buffer, 500 ml
21065	Pierce Background Eliminator Kit, for eliminating background from X-ray film
88018	Nitrocellulose Membrane, 0.45 µm, 33 cm × 3 m, 1 roll
77010	Nitrocellulose Membrane, 0.45 µm, 8 × 12 cm, 25/pkg.
88025	Nitrocellulose Membrane, 0.45 µm, 8 × 8 cm, 15/pkg.
88585	PVDF Transfer Membrane, 0.45 µm, 10 × 10 cm, 10/pkg.
88518	PVDF Transfer Membrane, 0.45 µm, 26.5 cm × 3.75 m, 1 roll
88600	Western Blotting Filter Paper, 10 × 10.5 cm, 100 sheets

SuperSignal Technology is protected by U.S. Patent # 6,432,662

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