INSTRUCTIONS

Pierce® Fast Western Blot Kit, SuperSignal® West Femto Substrate

35080 35081 35085 35086

2174.0

Number	Description
35080	**Pierce Fast Western Blot Kit, SuperSignal West Femto Substrate, Mouse,** contains sufficient reagents to perform 10 blots (8 × 10 cm) probed with a mouse primary antibody **Kit Contents:** **Fast Western Antibody Diluent**, 200 ml **Fast Western 10X Wash Buffer**, 100 ml **Fast Western Mouse Optimized HRP Reagent**, **Femto**, 10 ml **SuperSignal West Femto Luminol/Enhancer Solution**, 50 ml **SuperSignal West Stable Peroxide Solution**, 50 ml
35081	**Pierce Fast Western Blot Kit, SuperSignal West Femto Substrate, Rabbit,** contains sufficient reagents to perform 10 Western blots (8 × 10 cm) probed with a rabbit primary antibody **Kit Contents:** **Fast Western Antibody Diluent**, 200 ml **Fast Western 10X Wash Buffer**, 100 ml **Fast Western Rabbit Optimized HRP Reagent**, **Femto**, 10 ml **SuperSignal West Femto Luminol/Enhancer Solution**, 50 ml **SuperSignal West Stable Peroxide Solution**, 50 ml
35085	**Pierce Fast Western Blot Kit, SuperSignal West Femto Substrate, Mouse Trial Size**, contains sufficient reagents to perform two blots (8 × 10 cm) probed with a mouse primary antibody **Kit Contents:** **Fast Western Antibody Diluent**, 40 ml **Fast Western 10X Wash Buffer**, 20 ml **Fast Western Mouse Optimized HRP Reagent, Femto**, 2 ml **SuperSignal West Femto Luminol/Enhancer Solution**, 10 ml **SuperSignal West Stable Peroxide Solution**, 10 ml
35086	**Pierce Fast Western Blot Kit, SuperSignal West Femto Substrate, Rabbit Trial Size**, contains sufficient reagents to perform two blots (8 × 10 cm) probed with a rabbit primary antibody **Kit Contents:** **Fast Western Antibody Diluent**, 40 ml **Fast Western 10X Wash Buffer**, 20 ml **Fast Western Rabbit Optimized HRP Reagent, Femto**, 2 ml **SuperSignal West Femto Luminol/Enhancer Solution**, 10 ml **SuperSignal West Stable Peroxide Solution**, 10 ml

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

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Introduction

The Thermo Scientific Pierce Fast Western Blot Kit, SuperSignal West Femto Substrate contains optimized reagents that shorten the time to perform a typical Western blot from 4 hours to ~60 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. This kit includes SuperSignal West Femto Chemiluminescent Substrate, a maximum sensitivity HRP substrate that is detected using photographic or other imaging methods. This substrate produces a signal that is stable for several hours, enabling repeated film exposure to obtain optimal results.

Important Product Information

- The Pierce 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 Femto Substrate (i.e., 0.01-0.2 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 blotting procedure. Trays do not need to be changed between washes, but it is critical to use a clean or new tray when beginning the wash steps.
- 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: 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 primary antibody (0.01-0.2 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.™ System, use 0.2-1 ml of Optimized HRP Reagent and adjust to the appropriate volume with the Antibody Diluent. Reagent volume and incubation time might require optimization.
SuperSignal West Femto Working Solution	Mix SuperSignal West Femto 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 4ºC. Evaluate each specific antibody/antigen to determine compatibility with incubation time.
4. Discard the primary antibody solution from the tray, or place blot in a new 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.

   Note: To reuse the tray, remove the HRP solution and completely fill the tray containing the blot with ultrapure water and decant. Repeat this wash twice.
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.

   Note: To further reduce background, a rapid water rinse may be used after each wash with Fast Western 1X Wash Buffer by completely filling the tray with ultrapure water and decanting.
8. Remove blot and place it in a clean incubation tray. Add the SuperSignal West Femto Working Solution and incubate for 5 minutes at RT.
9. Remove blot from SuperSignal West Femto 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
		If reusing the tray, rinse tray and blot with ultrapure water three times before washing with Fast Western 1X Wash Buffer
	Used too much primary antibody	Reduce primary antibody concentration; use the antibody at 0.01-0.2 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	Antigen or primary antibody amounts were not optimal	Strip and re-probe blot using different primary antibody concentration; use the primary antibody at 0.01-0.2 µg/ml
		Optimize the amount of sample applied to the gel
	Inefficient protein transfer	Optimize transfer conditions
Spots within the protein bands	Inefficient protein transfer	Optimize transfer conditions
	Unevenly hydrated membrane	Hydrate membrane according to manufacturer's instructions
	Bubble between X-ray film and membrane	Remove all bubbles before exposing blot to film
Speckling	Over-heating during electrophoresis or transfer	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 #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

34094 **SuperSignal West Femto Chemiluminescent Substrate,** Trial Kit

34095 **SuperSignal West Femto Chemiluminescent Substrate,** 100 ml

34096 **SuperSignal West Femto Chemiluminescent Substrate,** 200 ml

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
88605	**Western Blotting Filter Paper, Extra Thick, 7 cm × 8.4 cm,** 50 sheets
88610	**Western Blotting Filter Paper, Extra Thick, 8.5 cm × 9 cm,** 50 sheets
88615	**Western Blotting Filter Paper, Extra Thick, 8 cm × 13.5 cm,** 50 sheets
88620	**Western Blotting Filter Paper, Extra Thick, 20 cm × 20 cm,** 50 sheets
88600	**Western Blotting Filter Paper, 10 × 10.5 cm,** 100 sheets
35035	**Pierce Fast Semi-Dry Transfer Buffer, 10X, methanol-free,** 500 ml
88217	**Pierce Fast Semi-Dry Blotter**

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

SNAP i.d. is a trademark of Millipore Corporation.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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