INSTRUCTIONS

Pierce® Fast Western Blot Kit, ECL Substrate

35050 35055

2133.1

Number	Description
35050	**Pierce Fast Western Blot Kit, ECL Substrate,** contains sufficient reagents to perform 25 Western blots (8 × 10 cm) probed with mouse or rabbit primary antibodies **Kit Contents:** **Fast Western Antibody Diluent,** 500 ml **Fast Western 10X Wash Buffer,** 250 ml **Fast Western Optimized HRP Reagent,** 25 ml **Pierce ECL Detection Reagent 1,** 125 ml **Pierce ECL Detection Reagent 2,** 125 ml
35055	**Pierce Fast Western Blot Kit, Trial Size,** contains sufficient reagents to perform five Western blots (8 × 10 cm) probed with mouse or rabbit primary antibodies **Kit Contents:** **Fast Western Antibody Diluent,** 100 ml **Fast Western 10X Wash Buffer,** 50 ml **Fast Western Optimized HRP Reagent,** 5 ml **Pierce ECL Detection Reagent 1,** 25 ml **Pierce ECL Detection Reagent 2,** 25 ml

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

Introduction

The Thermo Scientific Pierce Fast Western Blot Kit, ECL 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 Pierce ECL Substrate produces a chemiluminescent signal, which 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 reprobed.

Important Product Information

- The Fast Western Blotting Kit reagents are optimized to function together. Use the primary antibody at the concentration typically used in Western blotting procedures with ECL substrate.
- The Pierce Fast Western Blotting 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.
- 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 1 hour at room temperature.
- We offer a variety of protein transfer membranes, primary antibodies, and Autoradiography Film. Please consult the Thermo Fisher Scientific Pierce Products website or catalog for product and ordering information.

Additional Materials Required

- Membrane with transferred protein. Use any suitable protocol to separate proteins by electrophoresis and transfer them to a nitrocellulose or PVDF membrane.
- Primary Antibody: Choose a mouse or rabbit antibody that is specific to the target protein(s). The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane.
- Autoradiography film, film cassette, developing and fixing reagents for processing autoradiography film or an imaging instrument such as a CCD Camera.
- Rotary platform shaker for agitation of membrane during incubations.

Material Preparation

Fast Western 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 Working Dilution	Shake the Fast Western Antibody Diluent well before use. Dilute the primary antibody with diluent to a concentration ranging from 0.2 to10 µg/ml. 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 immediately before use. Example: To prepare 1 µg/ml from a solution with a starting concentration of 1 mg/ml, mix 10 µl of the primary antibody with 10 ml of the Fast Western Antibody Diluent.
Fast Western Optimized HRP Reagent Working Dilution	Mix 1 part of Fast Western Optimized HRP Reagent with 9 parts of Fast Western 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 from 0.2 to 1 ml of Optimized HRP Reagent and, if needed, adjust to the appropriate volume with the Antibody Diluent.
Detection Reagent Working Solution	Mix Detection Reagents 1 and 2 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 immediately before use (Step 9). The working solution is stable for up to 1 hour at room temperature. Example: Mix 5 ml of Detection Reagent 1 with 5 ml of Detection Reagent 2.

Procedure for Fast Western Blotting

1. Remove blot from the transfer apparatus and place in a clean incubation tray.
2. Briefly wash blot in Fast Western 1X Wash Buffer to remove transfer buffer.
3. Add the Primary Antibody Working Dilution 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. Remove blot from the primary antibody solution and place it in a clean incubation tray.
5. Add the Fast Western 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 Detection Reagent Working Solution and incubate for 1-5 minutes at RT.
9. Remove blot from Detection Reagent 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
	Insufficient washing	Use a minimum of 20 ml of Fast Western 1X Wash Buffer for each wash
		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
	Inefficient protein transfer	Optimize transfer conditions
Spots within the protein bands	Inefficient protein transfer	Optimize transfer procedure
	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 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 #43: Protein stability and storage
- Western Blotting Handbook and Troubleshooting Guide

Related Thermo Scientific Products

32106	**Pierce ECL Western Blotting Substrate,** 500 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
88600	**Western Blotting Filter Paper, 10 × 10.5 cm,** 100 sheets

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