

Western Blotting is Easy with Thermo Scientific Products

STEP 1: SDS-PAGE

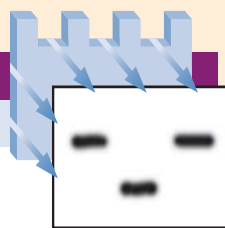
Separate protein sample by electrophoresis.



- Pierce SDS-PAGE Sample Prep Kit (Product # PI-89888)
- Precise™ Protein Gels (many available, see our website)
- Tris-Hepes-SDS Running Buffer (Product # PI-28398)
- Lane Marker Reducing Sample Buffer (5X) (Product # PI-39000)
- Lane Marker Non-Reducing Sample Buffer (5X) (Product # PI-39001)
- Pierce Protein Molecular Weight Markers

STEP 2: Electro-Transfer

Transfer proteins to membrane.

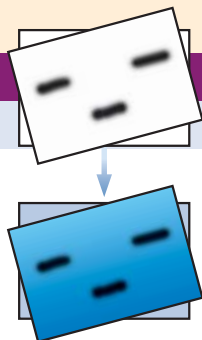


- Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes (Product # PI-24580) and for PVDF Membranes (Product # PI-24585)
- Tris-Glycine Transfer Buffer (Product # PI-28380)
- Pierce Western Blot Signal Enhancer (Product # PI-21050)
- Pierce Antibody Extender NC (Product # PI-32110 and PI-32105)
- Nitrocellulose Membranes, 0.2 µm and 0.45 µm
- PVDF Membrane, 0.45 µm (Product #s PI-88585 and PI-88518)
- Low-fluorescence PVDF Membrane, 0.2 µm (Product # PI-22860)
- Western Blotting Filter Paper (Product # PI-88600)

For detection of proteins that cannot be efficiently transferred to a membrane, Thermo Scientific Pierce In-Gel Detection Technology allows positive identification of proteins directly in a gel (Product #s PI-33500, PI-33505, PI-33510 and PI-33515).

STEP 3: Blocking

Block nonspecific sites.



- Protein-free Blocking Buffer (Product #s PI-37570, PI-37571, PI-37572 and PI-37573)
- StartingBlock™ Blocking Buffers in PBS or TBS with or without 0.05% Tween®-20
- SuperBlock® Blocking Buffers in PBS or TBS with or without 0.05% Tween-20
- Casein in PBS (Product # PI-37528) and in TBS (Product # PI-37532)
- BSA in PBS (Product # PI-37525) and in TBS (Product # PI-37520)

STEP 4: Formulate Wash Buffers

Choose a buffer.

- Phosphate Buffered Saline (PBS, Product #s PI-28372 and PI-28348)
- Tris Buffered Saline (TBS, Product #s PI-28376, PI-28379 and PI-28358)
- Tween-20 (Product # PI-28320)

For convenience and economy, we also offer complete Western blotting kits that include chemiluminescent substrates, enzyme-conjugated antibodies, blocking buffers and standard buffers.

†See patent information on reverse page.

STEP 5: Primary and Secondary Detection Reagents

Incubate the membrane with antibody.

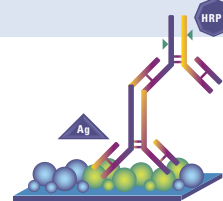
For a complete list, visit the antibody selection guide on our website.

For direct detection methods we offer:

- Monoclonal Antibodies
- Fluorescent Probes and Labeling Kits
- Enzyme Labeling Kits

For indirect detection methods we offer:

- Biotinylation Kits
- Protein A, Protein G and Protein L labeled with fluorescein, rhodamine, HRP, AP or biotin
- Avidin, Streptavidin and NeutrAvidin Biotin-Binding Protein labeled with fluorescein, rhodamine, HRP or AP
- Clean-Blot IP Detection Reagents
- Secondary antibodies labeled with fluorescein, rhodamine, HRP, AP or biotin
- DyLight Secondary Antibody and Streptavidin Conjugates



STEP 6: Enzyme Substrates

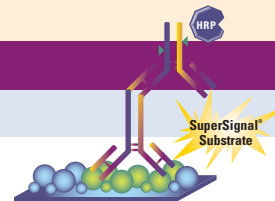
Add the detection reagent.

Chemiluminescent Substrates:

- Pierce ECL Substrate (Product #s PI-32106, PI-32209 and PI-32109)
- SuperSignal West Pico Chemiluminescent Substrate (Product #s PI-34077 and PI-34080); also available in an economical 1-L package (Product # PI-34078)
- SuperSignal West Femto Maximum Sensitivity Substrate (Product #s PI-34096 and PI-34095)
- SuperSignal West Dura Extended Duration Substrate (Product #s PI-34076 and PI-A34075)
- Lumi-Phos™ WB Substrate for Alkaline Phosphatase (Product # PI-34150)

Colorimetric Substrates:

- Pierce Chloronaphthol (Product # PI-34012)
- TMB-Blotting (Product # PI-34018)
- NBT/BCIP (Product # PI-34042)
- Metal Enhanced DAB (Product # PI-34065)



STEP 7: Film

Expose the membrane to X-ray film.

- CL-Xposure Film 5 x 7" sheets (Product #s PI-34090 and PI-34092); 8 x 10" sheets (Product #s PI-34091 and PI-34093); 9.5 x 11.8" sheets (Product # PI-34097); 18 x 24 cm sheets (Product # PI-34089) and 35 x 43 cm sheets (Product # PI-34099)
- Pierce Background Eliminator Kit (Product # PI-21065)

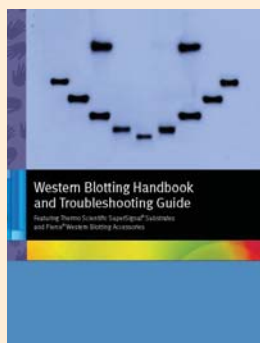
STEP 8: Stripping Buffer

Reprobe the blot if necessary.

- Restore Western Blot Stripping Buffer (Product # PI-21059)
- Restore PLUS Western Blot Stripping Buffer (Product #s PI-46428 and PI-46430) for High-Affinity Antibodies

Western Blotting is Easy with Thermo Scientific Products

Free Western Blotting Handbook and Troubleshooting Guide



This recently updated 73-page handbook contains full-length and abbreviated Western blotting protocols, tips on antibody and blocking buffer optimization, a chemiluminescent substrate selection guide, a procedure for stripping and reprobing Western blots, tips on clearer film development, and other information that will be useful to experts and first-timers alike (BN0128081).

Request your free copy today!

Membranes

Product #	Description	Pkg. Size
PI-88013	Nitrocellulose Membrane, 0.2 µm 7.9 cm x 10.5 cm	15/pkg.
PI-88018	Nitrocellulose Membrane, 0.45 µm 30 cm x 3.5 m	1 roll
PI-88014	Nitrocellulose Membrane, 0.45 µm 7.9 cm x 10.5 cm	15/pkg.
PI-88024	Nitrocellulose Membrane, 0.2 µm 8 cm x 8 cm	15/pkg.
PI-77012	Nitrocellulose Membrane, 0.2 µm 8 cm x 12 cm	25/pkg.
PI-88025	Nitrocellulose Membrane, 0.45 µm 8 cm x 8 cm	15/pkg.
PI-77010	Nitrocellulose Membrane, 0.45 µm 8 cm x 12 cm	25/pkg.
PI-22860	Low-Fluorescence PVDF Transfer Membrane, 0.2 µm 7 cm x 8.4 cm	10/pkg.
PI-88585	PVDF Transfer Membrane, 0.45 µm 10 cm x 10 cm	10 sheets
PI-88518	PVDF Transfer Membrane, 0.45 µm 26.5 cm x 3.75 m	1 roll
PI-88600	Western Blotting Filter Paper	100 sheets

Blocking Buffers

PI-37538	StartingBlock (PBS) Blocking Buffer A protein-based blocker formulation in phosphate-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.	1 L
PI-37542	StartingBlock (TBS) Blocking Buffer A protein-based blocker formulation in Tris-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.	1 L
EN-37539	StartingBlock T20 (PBS) Blocking Buffer A protein-based blocker formulation in phosphate-buffered saline at pH 7.5 with 0.05% Tween-20 Detergent and Kathon® Antimicrobial Agent.	1 L
EN-37543	StartingBlock T20 (TBS) Blocking Buffer A protein-based blocker formulation in Tris-buffered saline at pH 7.5 with 0.05% Tween-20 Detergent and Kathon Antimicrobial Agent.	1 L
PI-37515	SuperBlock (PBS) Blocking Buffer	1 L
PI-37516	SuperBlock T20 (PBS) Blocking Buffer (Contains 0.05% Tween-20 Detergent)	1 L
PI-37535	SuperBlock (TBS) Blocking Buffer	1 L
PI-37545	SuperBlock (TBS) Blocking Buffer Dry Blend	5 pouches
PI-37536	SuperBlock T20 (TBS) Blocking Buffer (Contains 0.05% Tween-20 Detergent)	1 L

Secondary Antibodies and Detection Reagents

Product #	Description	Pkg. Size
PI-31430	Goat Anti-Mouse IgG (H+L) Peroxidase Conjugated	2 ml
PI-31460	Goat Anti-Rabbit IgG (H+L) Peroxidase Conjugated	2 ml
PI-32430	Stabilized Goat Anti-Mouse IgG (H+L) Peroxidase Conjugated, 10 µg/ml	2 ml
PI-32460	Stabilized Goat Anti-Rabbit IgG (H+L) Peroxidase Conjugated, 10 µg/ml	2 ml
PI-21127	Streptavidin, Horseradish Peroxidase Conjugated	5 mg
PI-21130	High Sensitivity Streptavidin-HRP	0.5 ml
PI-21230	Clean-Blot IP Detection Reagent (HRP)	2.5 ml
PI-21232	Clean-Blot IP Detection Kit (HRP)	Kit
PI-21233	Clean-Blot IP Detection Reagent (AP)	2.5 ml

DyLight Conjugates: Package size for these items is 1 mg at 1 mg/ml.

Description	DyLight 488 Dye	DyLight 549 Dye	DyLight 649 Dye	DyLight 680 Dye	DyLight 800 Dye
Goat Anti-Mouse IgG (H+L)	PI-35502	PI-35507	PI-35515	PI-35518	PI-35521
Goat Anti-Rabbit IgG (H+L)	PI-35552	PI-35557	PI-35565	PI-35568	PI-35571
Streptavidin	PI-21832	PI-21837	PI-21845	PI-21848	PI-21851
NeutrAvidin Protein	PI-22832	PI-22837	PI-22845	PI-22848	PI-22853

Substrates

PI-32106	Pierce ECL Western Blotting Substrate	500 ml kit
PI-32209	Pierce ECL Western Blotting Substrate	250 ml kit
PI-32109	Pierce ECL Western Blotting Substrate	50 ml kit
PI-34080	SuperSignal West Pico Chemiluminescent Substrate	500 ml
PI-34078	SuperSignal West Pico Chemiluminescent Substrate	1 L
PI-A34075	SuperSignal West Dura Extended Duration Substrate	100 ml
PI-34095	SuperSignal West Femto Maximum Sensitivity Substrate	100 ml
PI-34150	Lumi-Phos WB Chemiluminescent Substrate	100 ml

Film and Stripping Buffers

PI-34090	CL-XPosure Film, 5 x 7 in (13 x 18 cm)	100/pkg.
PI-34092	CL-XPosure Film, 5 x 7 in (13 x 18 cm)	25/pkg.
PI-34089	CL-XPosure Film, 7 x 9.5 in (18 x 24 cm)	100/pkg.
PI-34091	CL-XPosure Film, 8 x 10 in (20 x 25 cm)	100/pkg.
PI-34093	CL-XPosure Film, 8 x 10 in (20 x 25 cm)	50/pkg.
PI-34097	CL-XPosure Film, 9.5 x 11.8 in (24 x 30 cm)	100/pkg.
PI-34099	CL-XPosure Film, 14 x 17 in (35 x 43 cm)	100/pkg.
PI-21065	Pierce Background Eliminator Kit Sufficient reagents to prepare 3L working solution.	Kit
PI-21059	Restore Western Blot Stripping Buffer Sufficient for stripping 25 (8 cm x 10 cm) blots.	500 ml
PI-21062	Restore Western Blot Stripping Buffer Sufficient for stripping one (8 cm x 10 cm) blot.	30 ml
PI-46428	Restore PLUS Western Blot Stripping Buffer Sufficient reagent to strip one to two (8 cm x 10 cm) blots.	30 ml
PI-46430	Restore PLUS Western Blot Stripping Buffer Sufficient reagent to strip 25 (8 cm x 10 cm) blots.	500 ml

Thermo Scientific SuperSignal Technology is protected by U.S. patent # 6,432,662.

Thermo Scientific Pierce Direct Detection of Biomolecules Technology is protected by U.S. patent # 7,112,411.

U.S. patents pending on Thermo Scientific Pierce Western Blot Signal Enhancer Technology.

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Alexa Fluor® is a trademark of Invitrogen Corporation.

Tween® is a trademark of ICI Americas Inc.

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