western blotting

Thermo SCIENTIFIC invitrogen

Protein transfer technical handbook

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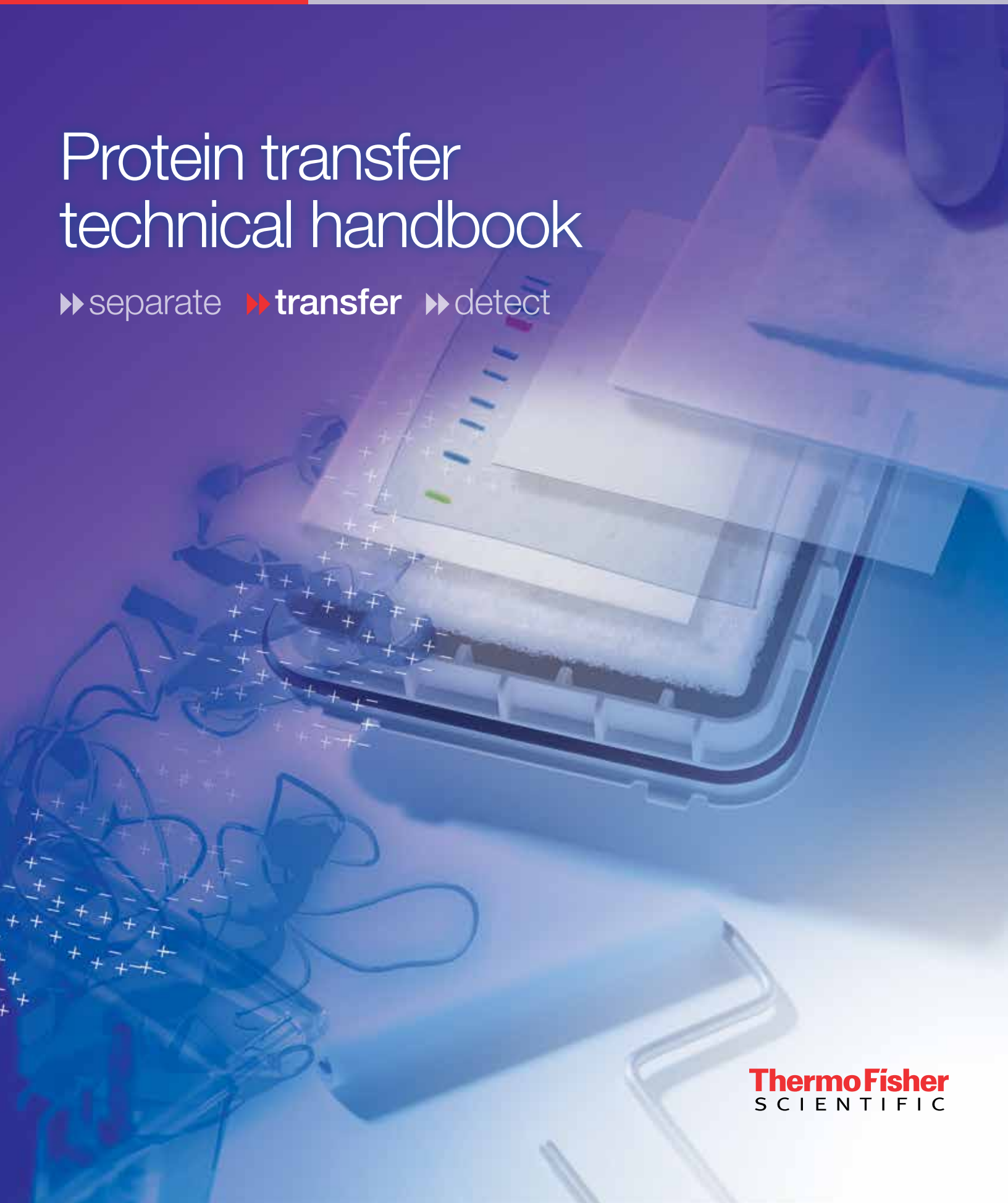


ThermoFisher SCIENTIFIC

Comprehensive protein transfer solutions designed to drive your success

Protein transfer is a vital step in Western blotting involving the transfer of proteins separated in a gel by electrophoresis to a solid support matrix so that specific proteins can be detected using immunodetection techniques. We offer a complete array of products to support rapid and efficient protein transfer for Western blotting. Our portfolio of high quality protein transfer products unites membranes, buffers, stains, molecular weight markers, alongside a comprehensive choice of transfer devices designed to suit your needs and enable better quality Western blot results.

For a complete listing of all available products and more, visit thermofisher.com/western

Contents

Introduction to electrotransfer methods for Western blotting

The transfer of size-separated proteins from a polyacrylamide gel to a membrane support is one of the key steps to subsequent immunodetection of a specific protein via Western blotting. Scientists have used a variety of methods for this transfer step, including diffusion transfer, capillary transfer, heat-accelerated convectional transfer, vacuum blotting and electroblotting (electrotransfer). Among these methods, electroblotting has emerged as the most popular because it is faster and more efficient than the other methods.

Protein transfer from gel to membrane is necessary for two reasons: 1) better handling capability offered by the membrane than the fragile gel during Western blot processing and 2) better target accessibility on the membrane by macromolecules like antibodies. In general, all electrotransfer methods rely on the electrophoretic mobility of proteins to move them out of a gel. The techniques involve placing a protein-containing polyacrylamide gel in direct contact with a piece of nitrocellulose membrane, polyvinylidene difluoride (PVDF) membrane or other suitable protein-binding support. Next, the gel-membrane element is "sandwiched" between two electrodes, which are typically submerged in a conducting solution (transfer buffer) (Figure 1). When an electric field is applied, the proteins move out of the gel and onto the surface of the membrane, where the proteins become tightly attached. The resulting membrane is a copy of the protein pattern that was in the polyacrylamide gel. For a complete workflow, see Figure 2.

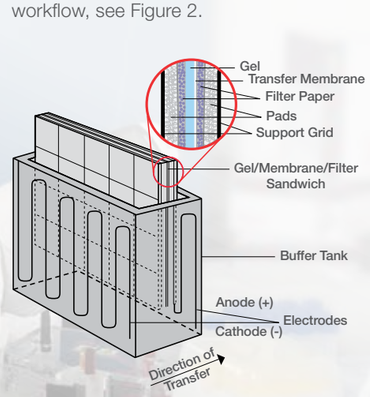


Figure 1. Western blot electrotransfer of proteins from gel to membrane. While this diagram depicts the setup of a typical wet transfer, many of the principles apply to semi-dry and dry methods of protein transfer to membranes.

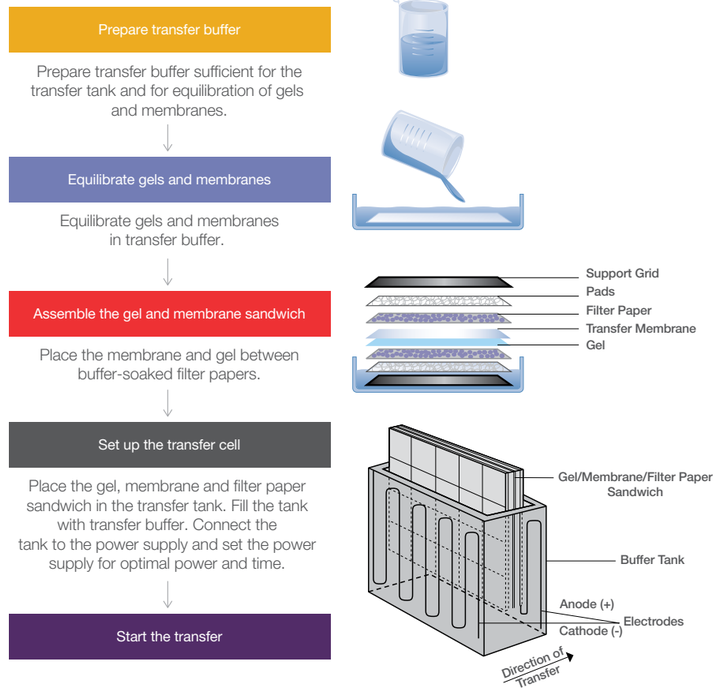


Figure 2. Workflow of the tank electrotransfer of proteins for Western blotting.

There are three ways to electrotransfer proteins from SDS-PAGE or native gels to nitrocellulose or PVDF membranes for the purpose of Western blotting:

- **Wet electroblotting (traditional wet or tank transfer)**
- **Semi-dry electroblotting (semi-dry transfer)**
- **Dry electroblotting (dry transfer)**

This handbook will focus on these three techniques and other considerations to help improve your protein transfer efficiency for better Western blot results.

▸▸Learn more at thermofisher.com/western

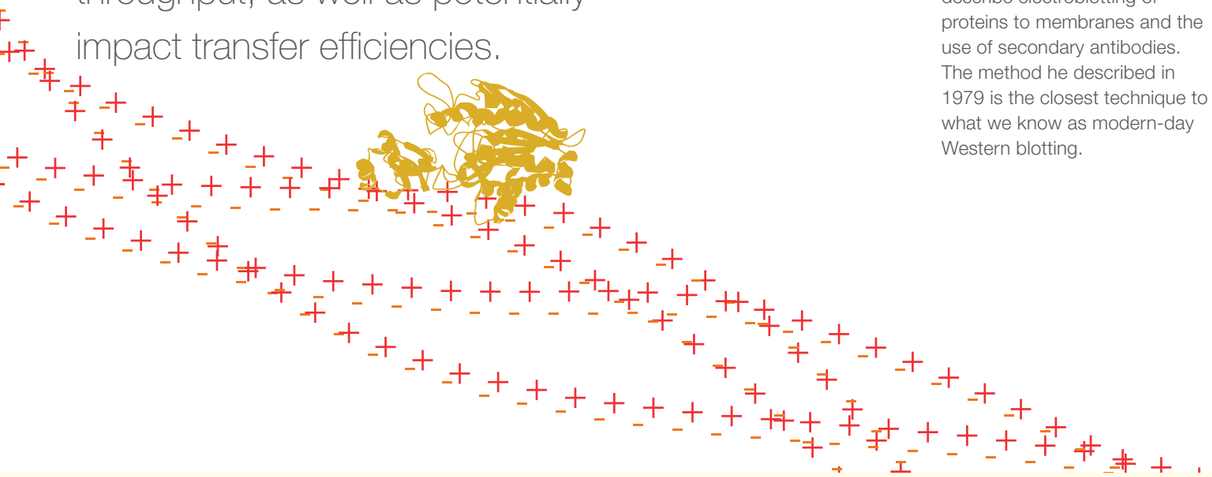
Did you know?

W. Neal Burnette in **Tobert Nowinski's** lab at the Fred Hutchinson Cancer Research Center in Seattle was the first to coin the term "Western blotting." The term honors Edwin Southern, who described blotting of DNA, and is a reference to the West Coast location of Nowinski's lab.

Pre-transfer considerations

Choice of electrotransfer system

When setting up a Western blot experiment, one of the first decisions is the choice of electrotransfer method: wet, semi-dry or dry. This choice will drive what transfer buffers (or lack thereof) will be used and may impact the choice of membrane products because some transfer devices have specific requirements. The choice of transfer device may also drive the gel size, blotting area and throughput, as well as potentially impact transfer efficiencies.



In general, dry and semi-dry transfer methods are faster than wet techniques. If time is a factor, you may want to consider these methods. However, the speed of transfer may affect transfer efficiencies. It is typically perceived that the slower, wet transfer methods tend to offer better transfer efficiencies than the faster semi-dry or dry transfer methods. If the amount of target or antibody quality is an issue, you may want to choose a wet transfer method or consider other signal enhancements (see page 30).

At Thermo Fisher Scientific, we offer transfer systems for the three electrotransfer methods. See Table 1 for a comparison of our systems and read more about the individual transfer methods beginning on page 16.

Table 1. Characteristics of our electrotransfer systems.

	Traditional wet transfer		Rapid semi-dry transfer	Dry transfer
				
	XCell II Blot Module	Mini Blot Module	Pierce Power Blotter	iBlot 2 Gel Transfer Device
Transfer time	60–120 min	60 min	5–10 min	7 min
Power supply	External	External	Internal	Internal

Did you know?

Harry Towbin's group in Basel, Switzerland, was the first to describe electroblotting of proteins to membranes and the use of secondary antibodies. The method he described in 1979 is the closest technique to what we know as modern-day Western blotting.

Building the Western transfer sandwich

After proteins have been separated by electrophoresis, the next step is the assembly of the "transfer sandwich." For wet and semi-dry electrotransfer devices, the transfer sandwich typically consists of a filter paper-gel-membrane-filter paper arrangement, where the filter paper aids in wicking of the transfer buffer. For some commercially available wet and semi-dry transfer devices, pre-assembled transfer stacks are available in which the polyacrylamide gel is inserted.

Dry transfer devices do not require filter paper for wicking transfer buffer. Instead a unique gel matrix transfer stack incorporates buffer, eliminating the need for buffer tanks and wetted filter paper.

Learn more at thermofisher.com/transfer

Choosing the Western blot membrane

The most common immobilization membranes for Western blotting are nitrocellulose, polyvinylidene difluoride (PVDF) and nylon. The following characteristics make these membranes suitable for protein transfer:

- A large surface area to volume ratio
- A high binding capacity
- Extended storage of immobilized proteins
- Easy to use
- Possible optimization for low background, signal and reproducibility

Western blot membranes are typically supplied in either sheets or rolls, and commonly have a thickness of 100µm, with typical pore sizes of 0.1, 0.2 or 0.45µm. Most proteins can be successfully blotted using a 0.45µm pore size membrane, while a 0.1 or 0.2µm pore size membrane is recommended for low molecular weight proteins or peptides (<20kDa). For some transfer instruments, prepackaged membranes and blotting paper "stacks" are available. Table 2 provides a summary of available membranes.

Table 2. Characteristics of our Western blotting membranes.

Membrane type	Applications and uses	Reprobe characteristics	Binding interaction
Nitrocellulose membranes	Western, Southern and Northern blots, amino acid analysis, dot/slot blots	Can be stripped and reprobed	Hydrophobic and electrostatic
PVDF membranes (including Invitrogen™ Invitrolon™ membranes)	Western blots, protein sequencing, amino acid analysis, solid-phase assays	Can be stripped and reprobed	Hydrophobic
Nylon	Southern, Northern and Western blots	Can be stripped and reprobed	Ionic, hydrophobic and electrostatic
iBlot 2 Transfer Stack, Nitrocellulose Membrane	Western blots	Can be stripped and reprobed	Hydrophobic and electrostatic
iBlot 2 Transfer Stack, PVDF Membrane	Western blots	Can be stripped and reprobed	Hydrophobic

Nitrocellulose membranes

High protein-binding affinity, compatibility with a variety of detection methods, and binding affinity of proteins and glycoproteins make nitrocellulose a popular matrix. Protein immobilization on the membrane is thought to occur by hydrophobic interactions. Use of high salt and low methanol concentrations in transfer conditions improves protein immobilization on the membrane, especially with proteins of higher molecular weights.

Nitrocellulose membranes for Western blotting

We offer a variety of nitrocellulose membranes that fit most of your transfer needs, including transfer stacks, pre-cut and roll formats. A comparison of the various offerings can be found in Table 3.

Features:

- **High quality** — pure, 100% nitrocellulose membranes with high surface area and excellent uniformity
- **Selection** — available in 0.2µm and 0.45µm pore sizes for peptide and protein applications, respectively
- **Convenient** — available as ready-to-use, pre-assembled membrane/filter paper sandwiches, as several sizes of pre-cut sheets or as economically priced rolls for cutting to any dimension
- **High sensitivity** — provides high-affinity protein binding, blocks easily and exhibits very low background in chemiluminescent Western blotting

Learn more at thermofisher.com/membranes

For ordering information refer to page 42.

Table 3. Applications and specifications for our nitrocellulose membranes.

Specs	Pre-cut nitrocellulose						Nitro-cellulose roll	Ready-to-use sandwich		iBlot 2 Transfer Stacks	
Cat. No.	77012	88013	88024	77010	88014	88025	88018	LC2009 LC2000	LC2006 LC2001	IB23002	IB23001
Number of transfers	25	15	15	25	15	15	84 (7.9 × 10.5cm)	16	16	10	10
Quantity	25	15	15	25	15	15	1 roll	16	16	10	10
Pore size	0.2	0.2	0.2	0.45	0.45	0.45	0.45	0.2	0.45	0.2	0.2
Dimensions	8 x 12cm	7.9 x 10.5cm	8 x 8cm	8 x 12cm	7.9 x 10.5cm	8 x 8cm	30cm x 3.5m	8.5 x 13.5cm	8.5 x 13.5cm	8 x 8cm	8.3 x 13cm
Application	Western transfer of proteins <20kDa			Western transfer of proteins >20kDa				Western transfer of proteins <20kDa	Western transfer of proteins >20kDa	For use with iBlot 2 Gel Transfer Device	
Reprobe characteristics	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

PVDF membranes

PVDF membrane is ideal for Western blotting applications as well as for amino acid analysis and protein sequencing of small amounts of proteins (as little as 10pmol). PVDF membranes are highly hydrophobic and must be pre-wetted with methanol or ethanol prior to submersion in transfer buffer. PVDF membranes have a high binding affinity for proteins, with binding likely occurring via dipole and hydrophobic interactions, and offer better retention of adsorbed proteins than other supports. PVDF is also less brittle than nitrocellulose and can be stripped and reprobed without a loss of sensitivity or increased background.

PVDF membranes for Western blotting

We offer a variety of PVDF membranes that fit most of your transfer needs, including transfer stacks, pre-cut and roll formats. A comparison of the various offerings can be found in Table 4.

Features:

- **High quality** — PVDF transfer membranes manufactured especially for protein transfer and Western blot applications and more resistant to discoloration than other commercially available PVDF membranes
- **Durable** — PVDF is compatible with most organic solvents, acids and mild bases; doesn't tear or become brittle like nitrocellulose
- **Selection** — available in 0.2µm and 0.45µm pore sizes; available as ready-to-use, pre-assembled membrane/filter paper sandwiches, as pre-cut sheets or as economically-priced rolls for cutting to any dimension
- **Versatile** — compatible with chemiluminescent, chromogenic and fluorescent Western blot detection

While our 0.2µm PVDF membrane performs well for Western blotting, amino acid analysis and protein sequencing applications, our high-quality 0.45µm PVDF membrane is suited for high sensitivity and low background immunoblotting. For fluorescent blotting applications, choose the Thermo Scientific™ Pierce™ Low-Fluorescence PVDF Transfer Membranes. These membranes are made of high-quality polyvinylidene difluoride and provide high binding capacity for proteins and nucleic acids for Western, Southern and Northern blotting methods.

Blotting paper

Blotting (or filter) paper is an essential component for the transfer sandwich in wet and semi-dry electroblotting methods. The filter paper is first wetted in transfer buffer before building the transfer sandwich. The paper serves to aid wicking transfer buffer through the gel, helping the proteins move out of the gel onto the membrane. Dry electrotransfer conditions do not use filter paper.

Blotting paper should be made of high-quality materials so that it doesn't contribute to possible background issues during the Western blotting detection step. The paper thickness may also be of concern with some transfer systems.

Pierce Western Blotting Filter Papers

Thermo Scientific™ Pierce™ Western Blotting Filter Papers are pre-cut cotton sheets for wet or semi-dry, passive or electrophoretic transfer of proteins from polyacrylamide gels (SDS-PAGE) to PVDF, nitrocellulose or other membranes. Pierce Filter Papers are suitable for use with alcohol or other organic solvents commonly used in protein and nucleic acid blotting applications. Extra-thick paper is available for optimal wicking under certain transfer conditions.

Features:

- **High quality** — clean cotton cellulose fiber paper manufactured with additive-free ultrapure water to minimize sources of background signal and artifacts
- **Easy to use** — pre-cut filter paper sheets in several convenient sizes for use with most mini-gel sizes, tank transfer cassettes and semi-dry blotters
- **Validated** — tested for use with various protein methods, including wet and semi-dry transfer
- **Two thicknesses** — choose standard-thickness paper for traditional procedures; choose extra-thick filter paper for high-capacity blotting or as a replacement for multiple sheets

Learn more at thermofisher.com/filterpaper

For ordering information refer to page 42.

Table 4. Applications and specifications for our PVDF membranes.

Specs	Invitrolon PVDF	0.2µm PVDF	Pre-cut PVDF membrane		PVDF rolls		iBlot 2 Transfer Stacks		Tropifluor PVDF
Cat. No.	**LC2005**	**LC2002**	**88585**	**22860**	**88518**	**88520**	**IB24001**	**IB24002**	**T2234**
Number of transfers	20	20	10	10	111 (8 x 10cm)	111 (8 x 10cm)	10		5
Quantity	20 membrane/ filter paper sandwiches	20 membrane/ filter paper sandwiches	10 pre-cut PVDF sheets	10 pre-cut PVDF sheets	1 roll	1 roll	10 transfer stacks		5 precut sheets
Pore size	0.45µm	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.2µm	0.2µm	0.45µm
Dimensions	8.3 x 7.3cm	8.3 x 7.3cm	10 x 10cm	7 x 8.4cm	26.5cm x 3.75m roll	26.5cm x 3.75m roll	13 x 8.3cm	8 x 8cm	15 x 15cm
Binding capacity	Goat IgG: 294µg/cm^2 BSA: 131µg/cm^2 Insulin: 85µg/cm^2	50–150µg/cm^2 for large, globular proteins >150µg/cm^2 for smaller peptides	Goat IgG: 294µg/cm^2 BSA: 215µg/cm^2 Insulin: 160µg/cm^2	NT†	IgG: 294µg/cm^2 BSA: 215µg/cm^2 Insulin: 160µg/cm^2	Goat IgG: 448µg/cm^2 BSA: 340µg/cm^2 Insulin: 262µg/cm^2	240µg protein/cm^2		125µg protein/cm^2
Application	Optimal Western blot transfers for proteins >10kDa Protein sequencing Amino acid analysis	Western blot transfers Protein sequencing Amino acid analysis Solid-phase assay systems	Western blot transfers Protein sequencing Amino acid analysis	Fluorescence Western blotting	Western blot transfers Protein sequencing Amino acid analysis	Western blot transfers Protein sequencing Amino acid analysis	For use with the iBlot 2 Gel Transfer Device		Western blotting with dioxetane-based detection including Invitrogen™ CSPD™ or CDP-Star™ substrates
Reprobe characteristics	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes

†not tested

Learn more at thermofisher.com/membranes

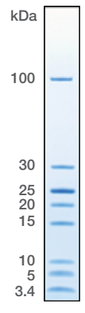
Protein ladder considerations

Typically, protein ladders or standards are included during gel electrophoresis for protein sizing after separation. Protein ladders can have other uses in the context of Western blotting methods, and those uses will drive the choice of the marker. Protein ladders can be used to monitor transfer efficiencies, although only in a qualitative fashion. In this use, pre-stained ladders are the recommended choice. Another use is protein molecular weight estimation (sizing) after Western blot detection steps.
In this scenario, one can choose either markers for near-infrared (NIR) detection or specialty markers that can be detected using chemiluminescence.

Protein ladder selection guide.

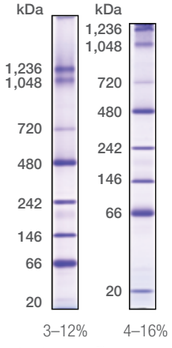
	MW range	Product	No. of proteins	Range
Unstained	Low	PageRuler Unstained Low Range Protein Ladder	8	3.4–100kDa
	Broad	PageRuler Unstained Protein Ladder	14	10–200kDa
	High	NativeMark Unstained Protein Standard	8	20–1,200kDa
Prestained	Low	PageRuler Prestained Protein Ladder	10	10–170kDa
	Broad	PageRuler Plus Prestained Protein Ladder	9	10–250kDa
	High	HiMark Pre-stained Protein Standard	9	30–460kDa
Multicolor prestained	Broad	Spectra Multicolor Broad Range Protein Ladder	10	10–260kDa
	High	Spectra Multicolor High Range Protein Ladder	8	40–300kDa
Other	Western	MagicMark XP Protein Standard	9	20–220kDa
	Specialty	PageRuler Prestained NIR Protein Ladder	10	11–250kDa
		BenchMark Fluorescent Protein Standard	7	11–155kDa
		BenchMark His-tagged Protein Standard	10	10–160kDa
		IEF Marker 3–10	13	3–10 pI

Learn more at thermofisher.com/proteinstandards

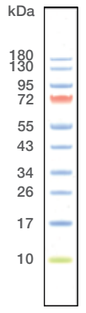


Thermo Scientific™ PageRuler™ Low Range Cat. No. 26632
NuPAGE™ 4–12% Bis-Tris Gel with MES SDS buffer

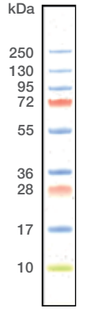
Thermo Scientific™ PageRuler™ Unstained Cat. No. 26614
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



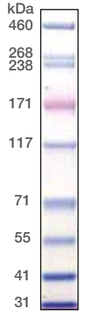
Invitrogen™ NativeMark™ Unstained Cat. No. LC0725
NativePAGE™ Bis-Tris gels stained using Coomassie R-250



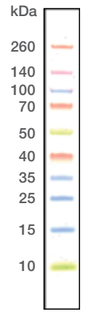
Thermo Scientific™ PageRuler™ Prestained Cat. No. 26616
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



Thermo Scientific™ PageRuler™ Plus Prestained Cat. No. 26619
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

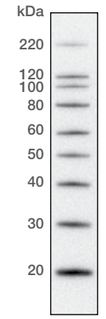


Invitrogen™ HiMark™ Prestained Cat. No. LC5699
NuPAGE™ 3–8% Tris-acetate Gel with SDS buffer

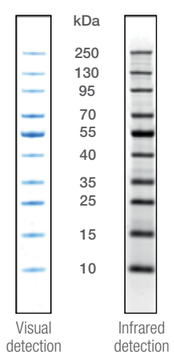


Thermo Scientific™ Spectra™ Multicolor Broad Range Cat. No. 26634
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

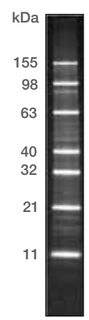
Thermo Scientific™ Spectra™ Multicolor High Range Cat. No. 26625
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



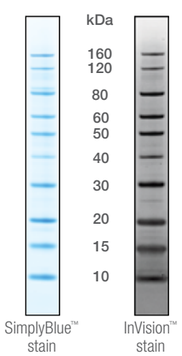
Invitrogen™ MagicMark™ XP Cat. No. LC5602
NuPAGE Bis-Tris gel, blotted to nitrocellulose, detected with Invitrogen™ WesternBreeze™ Chemiluminescent Kit



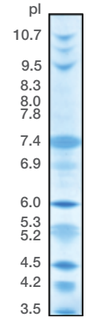
Thermo Scientific™ PageRuler™ Prestained NIR Cat. No. 26635
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



Invitrogen™ BenchMark™ Fluorescent Cat. No. LC5928
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



Invitrogen™ BenchMark™ His-tagged Cat. No. LC5606
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



Invitrogen™ IEF Marker 3-10 Cat. No. 39212-01
Novex™ pH 3–10 IEF Gel

For additional information on protein ladders, refer to the Protein gel electrophoresis technical handbook on thermofisher.com/pagehandbook

For ordering information refer to pages 42-43.

Transfer buffers

Common buffers used for Western blotting are the Towbin buffer system (25mM Tris-HCl pH 8.3, 192mM glycine, 20% (v/v) methanol)[1] and the CAPS buffer system (10mM CAPS pH 10.5, 10% (v/v) methanol). However, the final choice of transfer buffer may depend on the transfer device and will be noted in the device instruction manuals.

In most experiments, SDS should be omitted from the Western transfer buffer because the negative charge imparted to proteins can cause them to pass through the membrane as opposed to binding the membrane (also known as blowout). Typically, residual SDS associated with the proteins in SDS-PAGE gels is sufficient to effectively carry them out of the gel and onto the membrane support. For proteins that tend to precipitate, the addition of low concentrations of SDS (<0.01%) may be necessary. It should be noted that adding SDS to the transfer buffer may require optimization of other transfer parameters (e.g., time, current) to prevent blowout of the proteins through the membrane.

Methanol in the transfer buffer aids in stripping off SDS from proteins in SDS-PAGE gels, thus increasing their ability to bind to support membranes. However, methanol can inactivate enzymes required for downstream analyses and can shrink the gel and membrane, which may increase the transfer time of large molecular weight proteins (>150kDa) with poor solubility in methanol. In the absence of methanol, though, protein gels may swell in low ionic strength buffers, and therefore it is recommended to pre-swell gels for 30 minutes to one hour to prevent band distortion.

To increase speed of transfer with semi-dry methods, high-ionic strength buffers are the choice. These buffers, when combined with a suitable constant high-current power source (1.5 to 5.0A), will decrease protein transfer times to under 10 minutes.

We offer several ready-to-use buffers for standard wet, semi-dry and fast semi-dry blotting systems (Table 5).

BupH Tris-Glycine Buffer Packs

Each Thermo Scientific™ BupH™ Tris-Glycine Buffer pack yields 500mL of 25mM Tris and 192mM glycine at a pH of approximately 8 when dissolved in 400mL deionized water and 100mL of methanol.

Pierce 10X Tris-Glycine Buffer

Thermo Scientific™ Pierce™ 10X Tris-Glycine Buffer is a space-saving stock solution that is ideal for quickly preparing standard Tris-glycine (pH 8.5) transfer buffer used for Western blotting. Simply dilute with deonized water or 20% methanol.

Bolt Transfer Buffer, 20X

Invitrogen™ Bolt™ Transfer Buffer (20X) is optimized for the transfer of proteins from Invitrogen™ Bolt™ Bis-Tris Plus gels to membranes for Western blotting. When combined with 10% methanol, Bolt Transfer Buffer can be used with the Invitrogen™ Mini Blot Module or XCell™ II Blot Module for wet transfer. It can also be used with Invitrogen™ Bolt™ Antioxidant to enhance transfer of reduced proteins to membranes.

Novex Tris-Glycine Transfer Buffer, 25X

Invitrogen™ Novex™ Tris-Glycine Transfer Buffer (25X) is optimized for Western blot transfer applications using Tris-glycine gels. The buffers are made with high-purity reagents and are strictly quality controlled. The concentrated buffer requires a simple dilution with deionized water before use.

NuPAGE Transfer Buffer, 20X

Invitrogen™ NuPAGE™ Transfer Buffer (20X) is the buffer choice for transfer of proteins from Invitrogen™ NuPAGE™ Novex™ gels to membranes for Western blotting. NuPAGE Transfer Buffer can be used with the Invitrogen™ Mini Blot Module or XCell II Blot Module for wet (tank) transfer. It can also be used with Invitrogen™ NuPAGE™ Antioxidant to enhance transfer of reduced proteins to membranes.

Learn more at thermofisher.com/transferbuffer

Pierce Methanol-Free Transfer Buffer, 10X

Thermo Scientific™ Pierce™ Methanol-Free Transfer Buffer does not require cooling or the addition of methanol. Simply dilute the 10X solution with water and use directly in tank or conventional semi-dry transfer.

Pierce 1-Step Transfer Buffer

Thermo Scientific™ Pierce™ 1-Step Transfer Buffer is a high-ionic strength formulation designed for rapid semi-dry transfer of 10–300kDa proteins from polyacrylamide gels (SDS-PAGE) to nitrocellulose or PVDF membranes using the Thermo Scientific™ Pierce™ Power Blotter. It comes as a ready-to-use 1X solution and contains no alcohol. One 1L bottle is sufficient for 20 mini-sized gels or 10 midi-sized gels.

Table 5. Transfer buffer selection guide.

Format	Dry blend powder	Liquid				Specialty	
Product name	**BupH Tris-Glycine Buffer Packs**	**Pierce 10X Tris-Glycine Buffer**	**Novex 25X Tris-Glycine Transfer Buffer**	**NuPage 20X Transfer Buffer**	**Bolt 20X Transfer Buffer**	**Pierce 10X Methanol-Free Transfer Buffer**	**Pierce 1-Step Transfer Buffer**
Cat. No.	**28380**	**28363**	**LC3675**	**NP0006-1**	**BT00061**	**35040**	**84731**
Concentrate	N/A	10X	25X	20X	20X	10X	1X
Quantity	40 packs	1L	500mL	1L	1L	5L	1L
Number of transfers	40 transfers at 500mL each	20 transfers at 500mL each	25 transfers at 500mL each	40 transfers at 500mL each	40 transfers at 500mL each	100 transfers at 500mL each	20 transfers at 50mL each
Storage	RT†	RT	RT	RT	RT	4°C	RT
Gel compatibility	Tris-glycine gels	Tris-glycine gels	Tris-glycine gels	NuPage Bis-Tris and Tris-Acetate gels	Bolt Bis-Tris Plus Gels	Tris-glycine gels	Tris-glycine, Bis-Tris, Tris-HCl gels
Transfer method compatibility	Wet or traditional semi-dry	Wet or traditional semi-dry	Wet or traditional semi-dry	Wet or traditional semi-dry	Wet or traditional semi-dry	Wet or traditional semi-dry	Rapid semi-dry using Pierce Power Blotter
Preparation	Dissolve in 400mL water and 100mL methanol	Dilute with 20% methanol	Dilute with 20% methanol	Dilute with 20% methanol	Dilute with 10% methanol	Dilute with water; no methanol needed	Use as is; no dilution necessary

† RT = room temperature

Did you know?

Prior to electroblotting, the most popular method for protein transfer was diffusion blotting. A single experiment using diffusion blotting took an average of 1.5 to 2 days. In comparison, a typical dry-transfer electroblotting now averages less than 10 minutes.

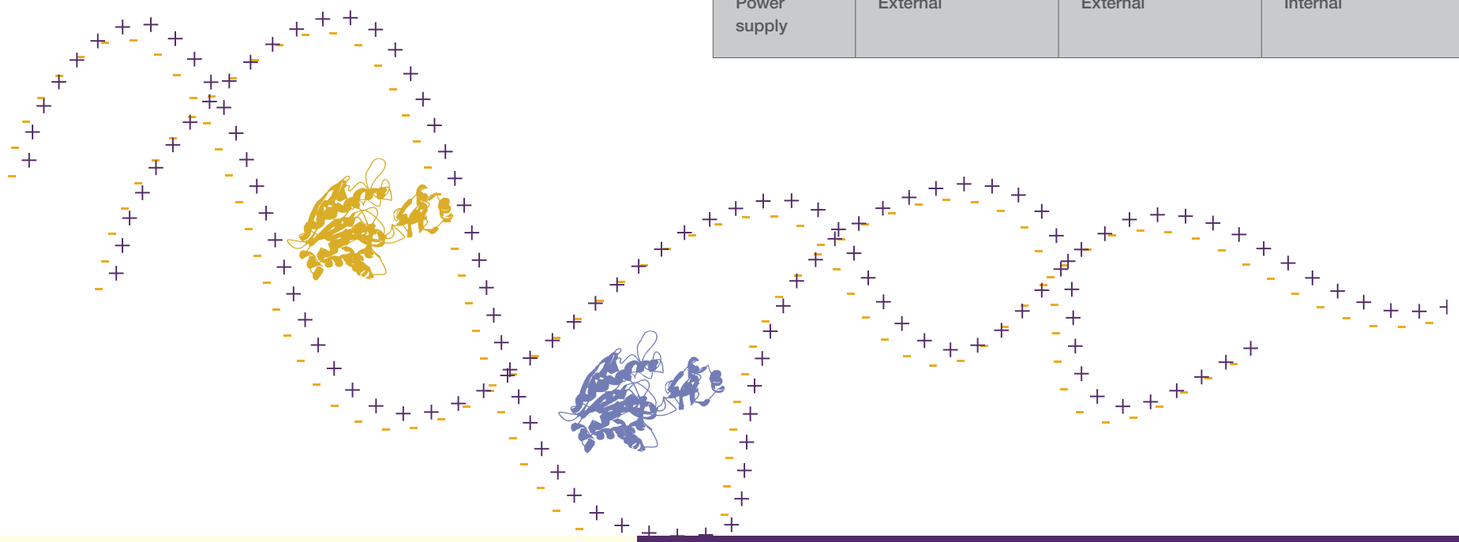
For ordering information refer to page 43.

Electrotransfer systems for Western blotting

All electroblotting methods rely on the electrophoretic mobility of proteins. When an electric field is applied across the transfer sandwich or stack, the proteins move out of the polyacrylamide gel and onto the nitrocellulose or PVDF membrane, creating a copy of the protein separation pattern in the original gel. Wet, semi-dry and dry transfer procedures are commonly used electroblotting methods. The key difference between these systems is the amount of buffer used in the transfer. In traditional wet transfer systems, the membrane-gel sandwich is submerged into a tank that contains transfer buffer. A current passes through the buffer to move proteins from the gel to the membrane. For semi-dry transfer, the membrane-gel sandwich is flanked by filter paper soaked with transfer buffer. Charge is driven through the filter paper to move the proteins from the gel to the membrane. In dry transfer systems, the membrane-gel sandwich is placed between gel matrices that contain ions. These ions move when current is applied, resulting in transfer of the proteins from the separation gel to the membrane.

Characteristics of our electrotransfer systems.

	Traditional wet transfer		Rapid semi-dry transfer	Dry transfer
				
	XCell II Blot Module	Mini Blot Module	Pierce Power Blotter	iBlot 2 Gel Transfer Device
Transfer time	60–120 min	60 min	5–10 min	7 min
Capacity of device	1–2 mini gels	1 mini gel (per module) or 2 mini gels (two modules per tank)	4 mini gels or 2 midi gels	2 mini gels or 1 midi gel
Blotting area	9 x 9cm	9 x 9cm	21 x 22.5cm	8.5 x 13.5cm
Transfer buffer requirement	Yes (1,000mL)	Yes (200–400mL per module)	Yes (50mL per mini gel or 100mL per midi gel)	No
Power supply	External	External	Internal	Internal



Wet electroblotting (tank transfer)

Tank transfer systems for the electrotransfer of proteins to membranes have been used for a long time. In this method, the gel is first equilibrated in transfer buffer. The gel is then placed in the transfer sandwich (filter paper-gel-membrane-filter paper), cushioned by pads and pressed together by a support grid. The supported gel sandwich is placed vertically in a tank between stainless-steel/platinum wire electrodes and filled with a transfer buffer (Figure 3).

Multiple gels may be electrotransferred in the standard field option, which is performed either at constant current (0.1 to 1A) or voltage (5 to 30V) for as little as one hour to overnight. Transfers are typically performed with an ice pack and at 4°C to mitigate the heat produced. A high field option that may bring transfer time down to as little as 30 minutes exists, but it requires the use of high voltage (up to 200V) or high current (up to 1.6A) and a cooling system to dissipate the tremendous heat produced.

For wet transfer, transfer efficiencies are better for lower molecular weight proteins than higher molecular weight proteins, with typical efficiencies of 80–100% for proteins between 14 and 116kDa.[2] The transfer efficiency improves with increased transfer time. However, with increasing time and the use of larger pore-sized membranes (0.45µm), the risk of over-transfer of the proteins through the membrane increases (also known as blowout), especially for lower molecular weight (<30kDa) proteins.

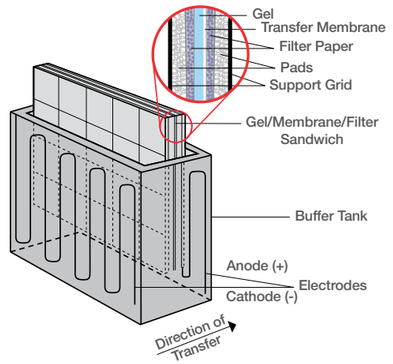
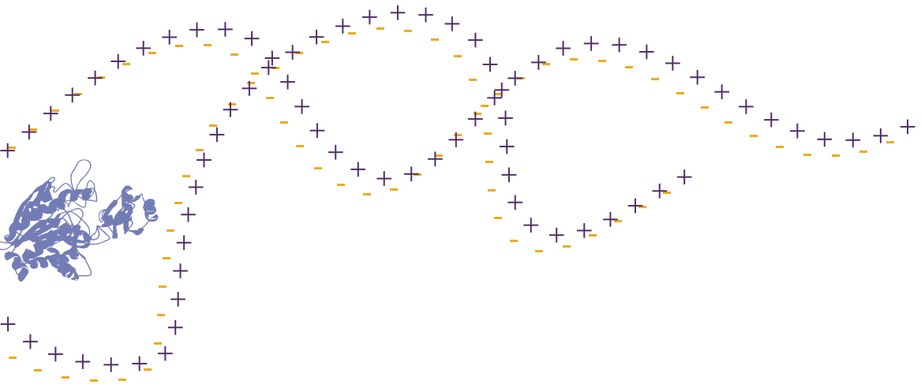


Figure 3. Tank (wet) transfer apparatus for Western blotting with gel-membrane sandwich detail. Schematic showing the assembly of a typical Western blot apparatus with the position of the gel and transfer membrane and direction of protein transfer in relation to the electrode position.

Did you know?

In many cases, ethanol can be substituted for methanol in transfer buffers without impacting transfer efficiency.



Mini Blot Module

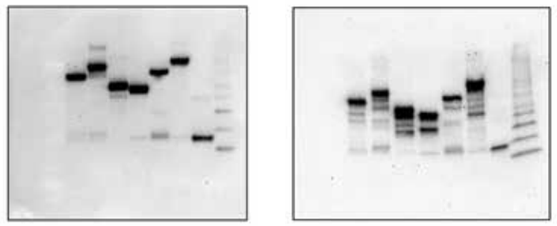
Leak-free, less-buffer wet transfer system

The Invitrogen™ Mini Blot Module is a wet transfer device used exclusively with the Invitrogen™ Mini Gel Tank and is designed to make your Western transfers simple and easy-to-perform. The tank accommodates one Mini Blot Module per chamber, or two blot modules total with the side-by-side layout. The universal connection and molded gasket make the blot module easy-to-use, while the inner core of the blot module allows for use of less methanol-based transfer buffer per wet transfer than other commercially available transfer systems. At the recommended conditions and constant voltage, proteins can be transferred to nitrocellulose or PVDF membranes typically in 30 to 60 minutes.

Features:

- **Universal module design** — allows modules to fit in either chamber of the tank, simplifying the transfer setup
- **Unique gasket seal** — helps prevent buffer leakage to minimize mess during setup of your Western transfer
- **1/2-inch buffer chamber** — requires only half the volume of methanol-based transfer buffer and helps save you money
- **Standard 60-minute transfer protocol** — accelerates your Western workflow so you can get results faster
- **Robust electrodes, sturdy steel plates** — for highly efficient and reliable Western transfers

Learn more at thermofisher.com/wettransfer



A Western blot of a Bolt gel shows clean, sharp protein signals corresponding to only full-length proteins, whereas a Western blot of a Bio-Rad™ TGX™ gel shows multiple low molecular weight degradation products. Protein kinases implicated in cancer (IKKB, HCK, EPHB3, MAPK14, FLT1, and DDR2) were analyzed on **(A)** a Bolt Bis-Tris Plus gel and **(B)** a Bio-Rad TGX Tris-glycine gel. Protein samples were prepared for electrophoresis according to each manufacturer's protocol. The purified kinases (50ng each) as GST fusion proteins, along with MagicMark XP Protein Standard and purified recombinant GST, were loaded in a Bolt 4–12% gel and a Bio-Rad TGX 4–20% gel. The samples were separated and transferred to PVDF membranes using the Mini Blot Module for the Bolt gels or on the Bio-Rad transfer system. Blot detection was performed using an anti-GST antibody and a WesternBreeze Chemiluminescence Detection Kit. The membranes were then imaged using an LAS-1000 system (FujiFilm) with an exposure time of one minute.

Specifications	
Mode of Transfer	Wet
Gel Compatibility	NuPAGE Bis-Tris, Tris-acetate, Tris-glycine, Tricine, Bolt Bis-Tris Plus gels
Running Dimension	Vertical
Capacity	≤2 blot modules/mini-gel tank; 1 mini gel/blot module
Gel Size	Mini (8 x 8cm)

Recommended Products

Our **Invitrogen™ PowerEase™ 300W Power Supply, Nitrocellulose Pre-cut Blotting Membranes and Pre-cut PVDF Membranes** are recommended for use with the Mini Blot Module.

XCell II Blot Module

Expands your XCell SureLock and XCell Mini-Cell Systems into Western transfer devices

The Invitrogen™ XCell II™ Blot Module allows you to easily transfer proteins or nucleic acids from mini gels to membranes. It fits neatly into the Invitrogen™ XCell SureLock™ and XCell II™ Mini-Cell System in place of the gel/buffer core assembly. It requires less than 200mL of transfer buffer for Western, Southern and Northern transfers. Tough platinized-titanium and stainless-steel electrodes create a uniform electrical field without clamps or hinged gel holders.

Features:

- **Economical** — requires only 200mL methanol-based transfer buffer and helps save you money
- **Flexible** — fits gel sizes up to 8 x 8cm
- **Robust electrodes, sturdy steel plates** — for highly efficient and reliable Western transfers

Specifications	
Mode of Transfer	Wet
Gel Compatibility	NuPAGE, Bolt Bis-Tris Plus, Novex Mini gels
Running Dimension	Vertical
Capacity	Up to 2 mini gels
Gel Size	Mini (8 x 8cm)

Recommended Products

Our **PowerEase 300W Power Supply or PowerEase 90W Power Supply, Nitrocellulose Pre-cut Blotting Membranes and Pre-cut PVDF Membranes** are recommended for use with the XCell II Blot Module.

For ordering information refer to page 43.

Semi-dry electroblotting (semi-dry transfer)

Semi-dry electroblotting became available as the need for faster results became an issue for researchers. For semi-dry protein transfer, the transfer sandwich is placed horizontally between two plate electrodes in a semi-dry transfer apparatus (Figure 4). The key to improving the speed of transfer with this method is to maximize the current passing through the gel versus around it. To do this, the amount of buffer used in the transfer is limited to that contained in the transfer sandwich. Hence, it is critical that the membrane and filter paper sheets are cut to the gel size without overhang and that the gel and filter paper are thoroughly equilibrated in transfer buffer. Also the use of extra-thick filter paper (approximately 3mm thickness) is helpful in certain semi-dry transfer devices because these sheets can hold more transfer buffer.

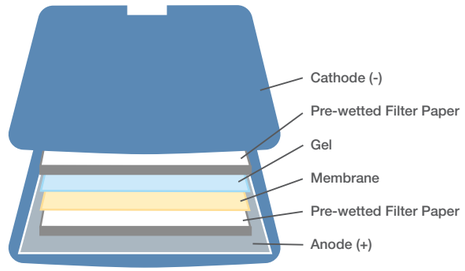


Figure 4. Semi-dry transfer setup with detail of gel-membrane sandwich.

Typically, one to four mini gels may be rapidly electroblotted to membranes. Methanol may be included in the transfer buffer, but other organic solvents, including aromatic hydrocarbons, chlorinated hydrocarbons and acetone, should not be added to avoid damage to the electrode plates. Electrotransfer is performed either at constant current (0.1 up to approximately 0.4A) or voltage (10 to 25V) for 30 to 60 minutes. Fast-blotting, semi-dry techniques use higher ionic strength transfer buffers and a high current power supply to decrease transfer times to under 10 minutes. In rapid methods, amperage is held constant and voltage is limited to a maximum of 25V.

Did you know?

Addition of SDS to the transfer buffer increases the relative current, power and heating during transfer, and may also affect the recognition of some proteins by antibodies.

Pierce Power Blotter

High-performance semi-dry transfers in less than 10 minutes

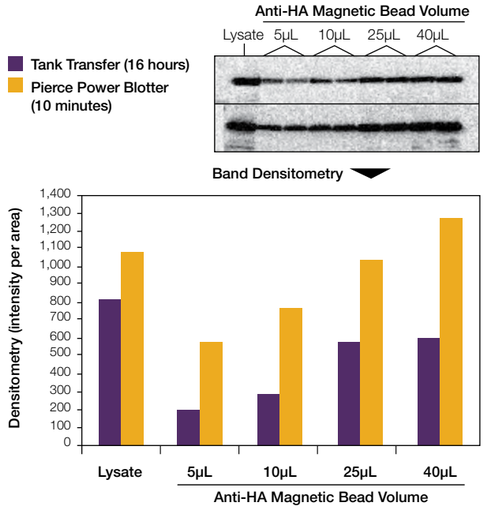
The Thermo Scientific™ Pierce™ Power Blotter is designed specifically for rapid semi-dry transfer of 10-300kDa proteins from polyacrylamide gels to nitrocellulose or PVDF membranes. The Pierce Power Blotter cassette accommodates up to four mini-sized gels or two midi-sized gels per transfer. Either homemade or pre-cast gels can be used. Transfers using the Pierce 1-Step Buffer are complete typically in five to 10 minutes. The instrument is also effective for standard 30- to 60-minute semi-dry transfer protocols using traditional transfer buffers.

The Thermo Scientific™ Pierce™ Power Station component of the Pierce Power Blotter has an easy-to-use color LCD touch-screen interface and preprogrammed transfer methods for different numbers and sizes of gels and ranges of protein molecular weight. The easy-touch programming feature allows custom transfer settings to be quickly created, saved and run. The Pierce Power Blotter is also available as a component of the Thermo Scientific™ Pierce™ Power System.

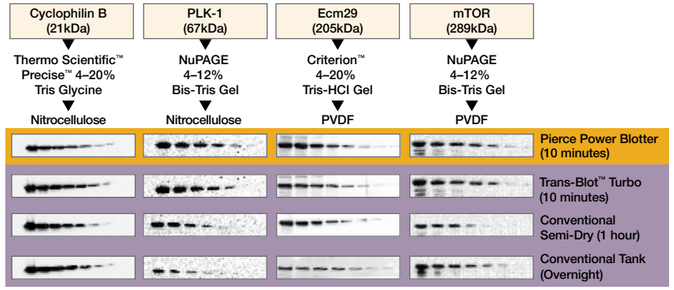
Features:

- **High performance** — achieve high transfer efficiency with a broad range of protein sizes (10–300kDa)
- **Fast** — transfer proteins typically in five to 10 minutes when used with Pierce 1-Step Transfer Buffer
- **High throughput** — simultaneously transfer one to four mini gels or one to two midi-sized gels
- **Integrated power supply** — seamless operation between control unit and cassette enables consistent high efficiency protein transfer
- **Easy-touch programming** — access built-in tutorials and preprogrammed transfer methods or create, save and run customized transfer methods
- **Versatile** — use with Pierce 1-Step Transfer Buffer for rapid blotting programs or Towbin transfer buffer for conventional semi-dry transfer methods

Learn more and see the video at thermofisher.com/powerblotter



Pierce Power Blotter provides highly efficient protein transfer in 10 minutes. GST-PI3K-SH2-HA (37kDa) was expressed in *E. coli* and purified with varying volumes of Thermo Scientific™ Anti-HA Magnetic Beads (Cat. No. 88836). Resulting samples were prepared for SDS-PAGE and electrophoresed. The proteins were then transferred from gel to nitrocellulose membrane using either traditional tank transfer (16 hours) with Towbin transfer buffer (25mM Tris, 192mM glycine, 20% methanol (v/v), pH 8.3) or the Pierce Power Blotter (10 minutes) with Pierce 1-Step Transfer Buffer (Cat. No. 84731). Membranes were probed with anti-HA antibody for one hour, washed five times, probed with goat anti-mouse HRP for 30 minutes, washed five times and incubated in Thermo Scientific™ SuperSignal™ West Femto Chemiluminescent Substrate (Cat. No. 34096). The resulting blots were simultaneously imaged using the Thermo Scientific™ MYECL™ Imager (Cat. No. 62236), and then band densitometry determined using Thermo Scientific™ MYIMAGEANALYSIS™ Software (Cat. No. 62237).



Highly efficient transfer of low, medium and high molecular weight proteins. The Pierce Power Blotter rapidly transfers a wide range of protein sizes from gel to membrane with similar or better efficiency compared to traditional transfer methods (tank and semi-dry) and other commercially available rapid blotting systems. HeLa lysate was serially diluted, prepared for SDS-PAGE and electrophoresed according to the gel suppliers' recommendations. Proteins were then transferred from gel to nitrocellulose or PVDF membrane using the specified methods and devices, following recommended protocols. Finally, blots were probed with respective specific antibodies, detected identically using the Thermo Scientific™ Pierce™ Fast Western Blot Kit (Cat. No. 35075, which uses Thermo Scientific™ SuperSignal™ West Dura Chemiluminescent Substrate), and imaged simultaneously using the MYECL Imager (Cat. No. 62236).

Specifications

Mode of Transfer	Semi-dry
Gel Compatibility	SDS-PAGE gel — either homemade or precast
Running Dimension	Horizontal
Capacity	Up to 4 mini gels or 2 midi gels
Gel Size	Mini (8 x 8cm), Midi (8 x 13cm)

Recommended Products

For transfer times of less than 10 minutes using the Pierce Power Blotter, we recommend **Pierce 1-Step Transfer Buffer**.

Our **Western Blotting Filter Paper** is recommended for building the Pierce Power Blotter transfer sandwich.

For ordering information refer to page 43.

Dry electroblotting (dry transfer)

Dry electroblotting methods use a different transfer sandwich containing innovative components that eliminate use of traditional transfer buffers. A unique gel matrix (transfer stack) that incorporates buffer is used instead of buffer tanks or soaked filter papers (Figure 5). The high ionic density in the gel matrix enables rapid protein transfer. During blotting, the copper anode does not generate oxygen gas as a result of water electrolysis, reducing blot distortion. Conventional protein transfer techniques, including wet and semi-dry, use inert electrodes that generate oxygen. Typically, transfer time is reduced by the shortened distance between electrodes, high field strength and high current.

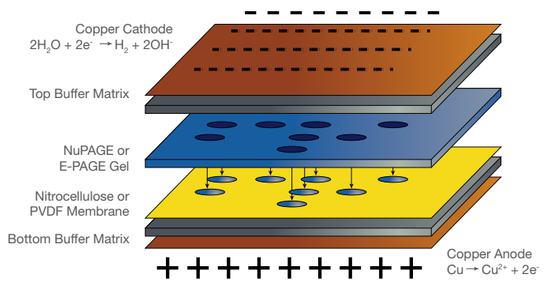
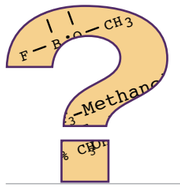
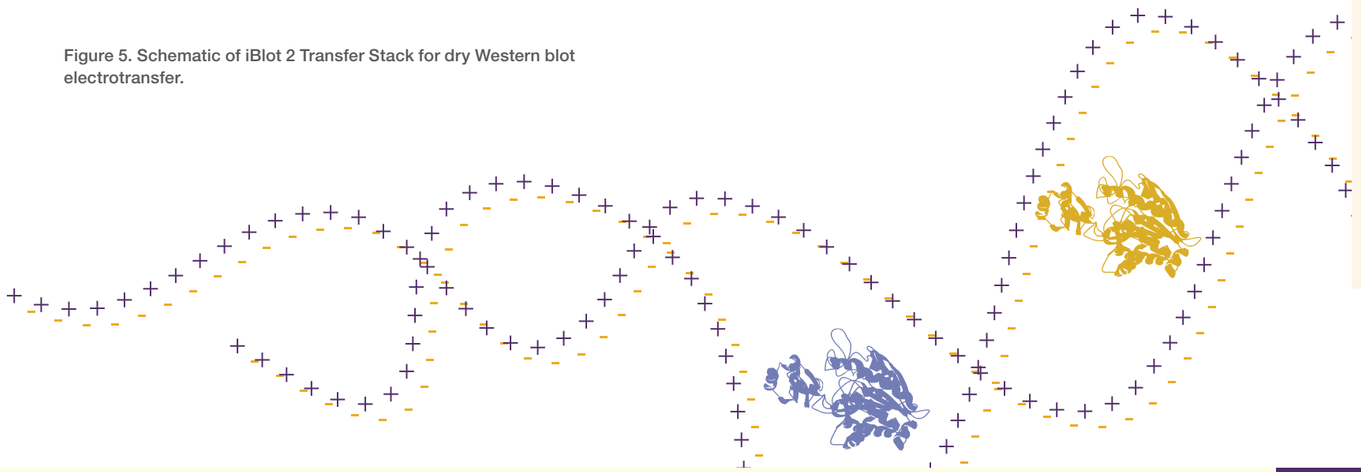


Figure 5. Schematic of iBlot 2 Transfer Stack for dry Western blot electrotransfer.



Did you know?

The use of impure methanol instead of analytical grade methanol can increase transfer buffer conductivity and result in poor protein transfer.



iBlot 2 Gel Transfer Device

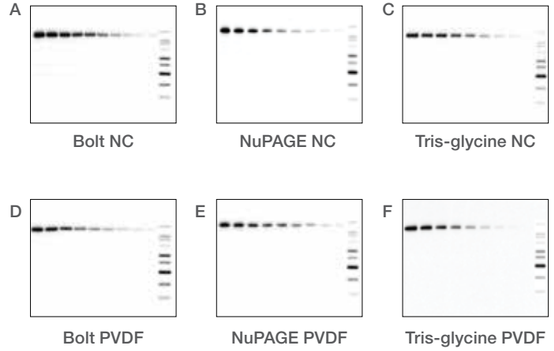
Dry Western transfer in seven minutes, with no transfer buffer needed

The Invitrogen™ iBlot™ 2 Dry Blotting System is for dry electroblotting of proteins from mini-, midi- and E-PAGE gels onto nitrocellulose or PVDF membranes for Western detection. The iBlot 2 system offers exceptional transfer efficiency, convenience and speed, producing crisp and clear bands that remain sharp and straight. Buffer ion reservoirs incorporated into the gel matrix (transfer stacks as opposed to buffer tanks or soaked filter papers) enable rapid protein transfer to either nitrocellulose or PVDF membranes. The shortened distance between electrodes, along with high field strength and current, reduce run times to seven minutes. With the iBlot 2 system, there is no need to prepare buffers, pre-treat your gel or clean up after blotting.

The Invitrogen™ iBlot™ 2 Gel Transfer Device is an integral part of the iBlot 2 Dry Blotting System, which consists of the transfer device and consumable transfer stacks that contain the required buffers and transfer membrane (nitrocellulose or PVDF). The iBlot 2 Device and the transfer stacks are sold separately.

Features:

- **Fast** — complete protein transfer in seven minutes
- **Great results** — high detection sensitivity and even transfer
- **Consistent** — increased blotting reliability and reproducibility
- **Flexible** — mini- and midi-gel size formats and membrane types can be used
- **Easy-to-use** — simple system with built-in tutorials and application notes and with options to create custom methods



Membranes processed on the iBlot 2 Dry Blotting System show consistent transfer across various protein gel chemistries to both nitrocellulose (NC) and PVDF membranes. Total cell extracts from A431 cells were transferred from 4–12% Bolt, 4–12% NuPAGE, and 4–20% Tris-glycine precast gels to NC membranes (A–C), and to PVDF membranes (D–F), using the iBlot 2 Dry Blotting System.

Learn more and see the video at thermofisher.com/iblot2

For ordering information refer to page 43.

Comparison of elapsed time for protein transfer with the iBlot 2 Dry Blotting System to other blotting methods.

	iBlot 2 Dry Blotting System	Conventional semi-dry transfer	Wet transfer
Buffer preparation	0 min	30 min	30 min
Soaking gel in transfer buffer	0 min	20 min	**0 min**
Assembling layers	2 min	10 min	**10 min**
Transfer	7 min	45–90 min	1–3 hrs
Cleanup	0 min	10 min	10 min
Total elapsed time	9 min	1 hr, 55 min– 2 hr, 40 min	1 hr, 50 min– 3 hr, 50 min
Time saved with the iBlot 2 Dry Blotting System	N/A	1 hr, 45 min– 2 hr, 30 min	1 hr, 40 min– 3 hr, 40 min

Specifications

Mode of Transfer	Dry
Gel Compatibility	E-PAGE, NuPAGE Mini/Midi, Novex Midi/Mini, Bolt Bis-Tris Plus gels
Running Dimension	Horizontal
Capacity	Up to 2 mini gels or 1 midi gel
Gel Size	Mini (8 x 8cm), Midi (8 x 13cm)

Recommended Products

We recommend the use of our **iBlot 2 Transfer Stacks** with the iBlot 2 Gel Transfer Device. These are available in nitrocellulose or PVDF, and in regular or mini-gel size.

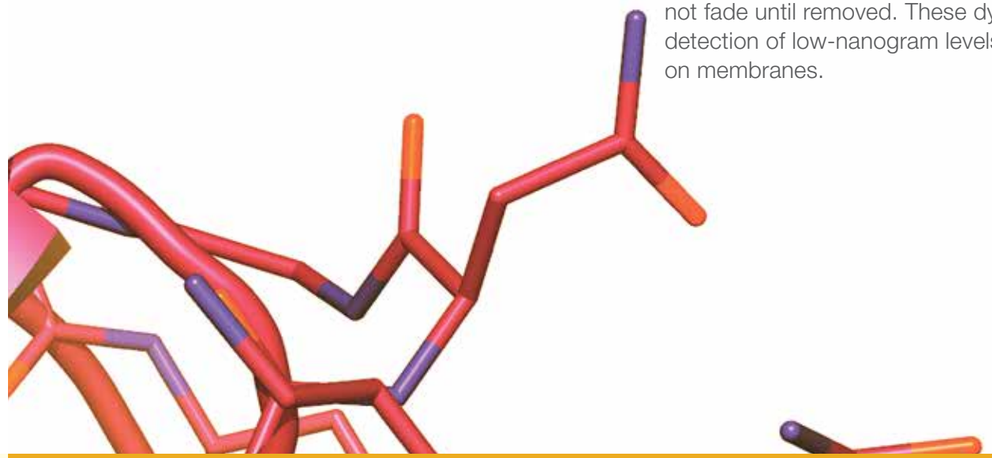
For ordering information refer to page 43.

Did you know?

Although nitrocellulose membranes were originally used to filter out particles, such as bacteria, they are now used primarily to bind macro-molecules in Western and Southern blotting. Macro-molecules such as proteins are thought to bind to the nitrocellulose membranes by hydrophobic interactions.

Post-transfer

Once the protein transfer process is completed, the membrane is removed from the transfer sandwich and is ready for any post-transfer membrane treatment. Before committing valuable time and detection reagents to Western blot processing and detection, it is prudent to assess the efficiency of the SDS-PAGE transfer step by using a reversible membrane stain. Signal enhancers may be used at this stage, before the membrane is incubated with blocking buffer, if there is a need for increased sensitivity.



Monitoring transfer efficiency

Transfer efficiency can vary dramatically among proteins, based upon the ability of a protein to migrate out of the gel and its propensity to bind to the membrane under a particular set of conditions. The efficiency of transfer depends on factors such as the composition of the gel, contact of the gel with the membrane, the position of the electrodes, transfer time, field strength and the presence of detergents, as well as the size and composition of the protein of interest.

Some researchers may stain the gel to confirm that proteins have migrated out of the gel. However, this method is unreliable because it does not necessarily reveal how effectively proteins have transferred to the membrane. A better method to monitor transfer efficiency relies on staining the membrane for total protein with a dye, such as Ponceau S or amido black 10B. Because dyes may interfere with antibody binding and detection, a protein stain that is easily removable is ideal. Ponceau S stain is the most widely used reagent for reversibly staining proteins on a membrane, although it has limited sensitivity, does not photograph well and fades quickly, making documentation difficult. Superior alternatives for staining protein on nitrocellulose or PVDF membranes are available that are easily photographed and do not fade until removed. These dyes also allow the detection of low-nanogram levels of protein on membranes.

Pierce Reversible Protein Stain Kits for Nitrocellulose or PVDF Membranes

A great alternative to Ponceau S

The Thermo Scientific™ Pierce™ Reversible Protein Stain Kits for either Nitrocellulose or PVDF Membranes provide rapid and sensitive alternative to Ponceau S stain for protein detection on nitrocellulose or PVDF membranes after transfer from polyacrylamide gels. The lower limit of detection with this method is 25 to 50ng per band (at least five times more sensitive than traditional Ponceau S staining). The staining protocol is simple, quick and results in turquoise-blue bands that do not fade and are easily photographed for future reference. The stain can be easily reversed in less than 15 minutes. Subsequent Western blot detection is unaffected because the stain does not alter the protein and is completely removed. The treated membrane does not interfere with conventional chemiluminescent or chromogenic detection using horseradish peroxidase and alkaline phosphatase substrates. In addition, the stain is compatible with N-terminal sequence analysis of proteins excised and eluted from the membrane.

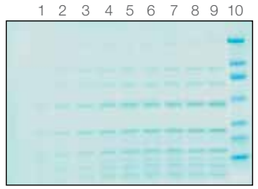
Features:

- **Better than Ponceau S** — more sensitive, easier to document, permanent until reversed (does not fade)
- **Sensitive** — high-avidity, total-protein stain; lower limit of detection equals 25 to 50ng per band
- **Specific** — detects only protein; does not bind or interact with other electrophoresis or sample components
- **Rapid** — stains in less than five minutes (nitrocellulose membranes) or in less than 30 minutes (PVDF membranes); erase and reverse staining in less than 15 minutes
- **Stable** — components are stored at room temperature, to help save refrigerator space and eliminate equilibration steps

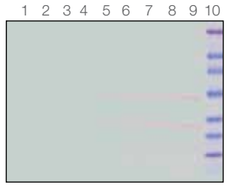
A. Pierce Reversible Stain

B. Ponceau S stain

Pierce Reversible Protein Stain and Ponceau S stain: A comparison of GST lysate staining on nitrocellulose. Increasing amounts of GST lysate protein were applied onto two 4–20% Tris-glycine SDS-polyacrylamide gels and electroblotted. Blot A was treated with Pierce Reversible Stain for 30 seconds and destained according to the protocol. Blot B was stained with 0.1% Ponceau S stain for five minutes and destained. The blot stained with Pierce Reversible Stain demonstrates superior visual detection of bands. GST lysate loading volumes (Lanes 1-3). Lane 1, 5µL; Lane 2, 10µL; Lane 3, 15µL and Lane 4, MW marker, 10µL.



A. Pierce Reversible Stain



B. Ponceau S stain

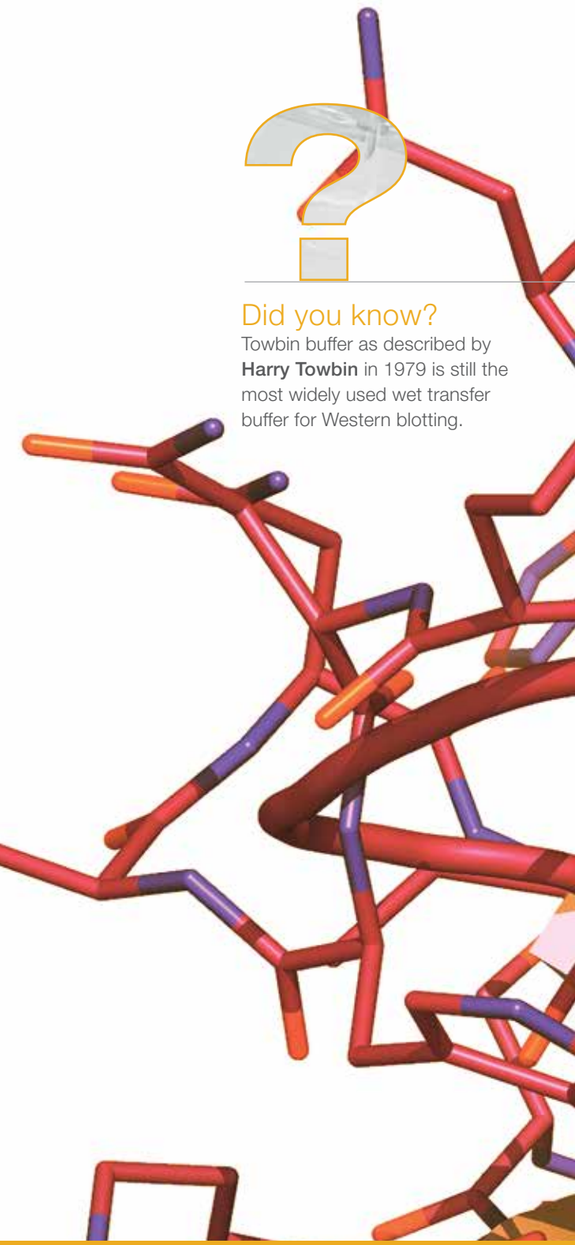
Comparison of Pierce Reversible Protein Stain with Ponceau S stain on PVDF membrane. Pierce Unstained Protein MW Markers, lanes 1–9, were serially diluted, applied to two 4–20% Tris-glycine SDS-polyacrylamide gels and electroblotted to PVDF membrane. Blot A was stained with Pierce Reversible Stain for one minute and destained according to the protocol. Blot B was stained with 0.1% Ponceau S stain in 5% acetic acid for five minutes and destained according to the published protocol. Lane 10, MW Marker.

Comparison of Pierce Reversible Protein Stain with Ponceau S stain.

Pierce Reversible Protein Stain	Ponceau S Stain
• Tight-binding, higher sensitivity, general protein stain	• Weak-binding, low-sensitivity general protein stain
• Detection limit: 25–50ng	• Detection limit: 250ng
• Turquoise blue bands are photographed easily	• Red bands are difficult to photograph
• Turquoise bands do not fade over time, but they can be reversed	• Stained protein bands fade within hours
• Typical staining time: 60 seconds	• Typical staining time: five minutes
• Background eliminated quickly with low pH wash	• No background elimination step

Recommended Products

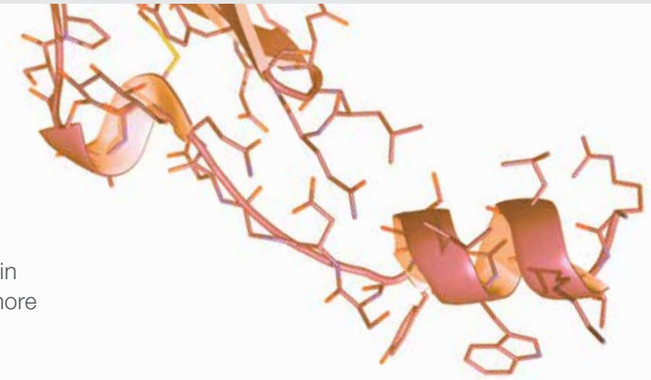
For detection of protein on membranes after Western transfer, we recommend the use of either our **Nitrocellulose or PVDF Membranes**.



Did you know?

Towbin buffer as described by **Harry Towbin** in 1979 is still the most widely used wet transfer buffer for Western blotting.

For ordering information refer to page 43.



Signal enhancements

There are many ways to increase the sensitivity of a Western blot. Some methods are as simple as switching detection substrates or blocking buffers in the immunodetection protocols, while others are more time-consuming, such as optimizing antibody titer.

A number of post-transfer membrane treatments also exist that can improve signal intensity. Some of these methods increase sensitivity and/or decrease background; others conserve the amount of antibody needed downstream while maintaining signal intensity.

SuperSignal Western Blot Enhancer

Increase signal-to-noise ratio and band development for better sensitivity

Thermo Scientific™ SuperSignal™ Western Blot Enhancer contains a membrane treatment reagent and a primary antibody diluent that increases both signal intensity and sensitivity three- to 10-fold compared to a detection performed without it.

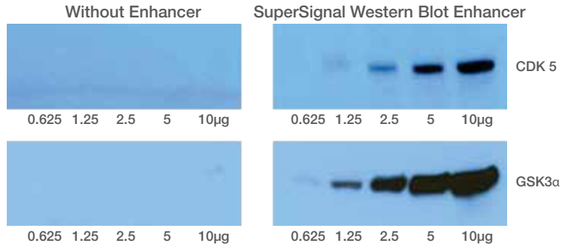
When a protein or antigen is difficult to detect because of low abundance or poor immunoreactivity, use of SuperSignal Western Blot Enhancer can help significantly reduce background and enhance detection of low-abundance and weakly immunoreactive antigens.

It minimizes the routine problem of overexposing blots, thereby reducing the need to experiment with multiple exposure times to acquire the “perfect blot.” This kit works with both PVDF and nitrocellulose membranes and is compatible with fluorescence, chromogenic and chemiluminescent detection.

Features:

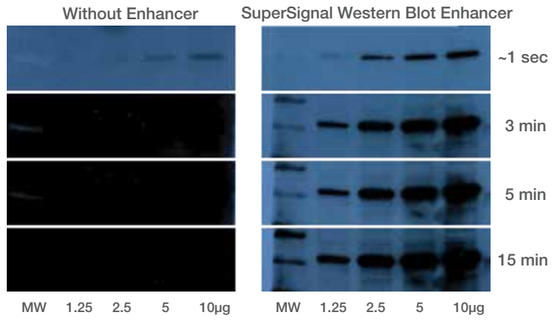
- **Increase sensitivity** — achieve three- to 10-fold increase in signal intensity and sensitivity
- **Improve specificity** — helps improve signal-to-noise ratio for poor quality and low affinity antibodies
- **Reduce background** — helps improve clarity for cleaner Western blots
- **Membrane compatibility** — provides effective signal enhancement with PVDF and nitrocellulose membranes
- **Substrate compatibility** — validated for use with chromogenic, chemiluminescent and fluorescent detection methods

Learn more at thermofisher.com/supersignalenhancer



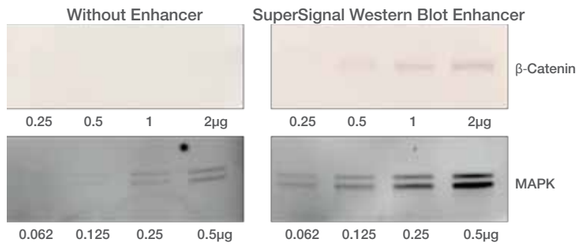
SuperSignal Western Blot Enhancer improves the lower detection limit for chemiluminescent substrates. Cell lysates were separated by electrophoresis, transferred to nitrocellulose and Western blotting was performed using the conventional method (left) or using the SuperSignal Western Blotting Enhancer protocol (right).

	Top panel	Bottom Panel
Blocking buffer	5% milk in Tris-Buffered Saline with 0.05% Tween 20	BLOTTO in TBS
Primary antibody	Mouse anti-CDK 5 (LabVision Cat. No. MS-1059-PABX) at 1µg/mL	Rabbit anti-GSK3α (Cell Signaling Technology Cat. No. 9338) at 1µg/mL
Secondary antibody	Horseradish peroxidase conjugated goat anti-mouse IgG (Cat. No. 31430) at 0.1µg/mL	Horseradish peroxidase-conjugated goat anti-rabbit IgG (Cat. No. 31460) at 0.1µg/mL
Substrate	Pierce ECL Substrate	SuperSignal West Pico Chemiluminescent Substrate
Detection	X-ray with 1-min exposure time	X-ray with 10-min exposure time



SuperSignal Western Blot Enhancer reduces background to enable detection of low-abundance targets. K562 cell lysate was loaded into Tris-glycine SDS-PAGE gels at 1.25, 2.5, 5 and 10µg per lane. After electrophoresis, the proteins were transferred to nitrocellulose membranes (Cat. No. 88013).

Blocking buffer	Thermo Scientific™ SuperBlock™ Blocking Buffer (TBS) (Cat. No. 37535)
Primary antibody	Mouse anti-ERK 1 (Cat. No. MA1-13041) primary antibody at 1µg/mL
Secondary antibody	Horseradish peroxidase-conjugated goat anti-mouse IgG (Cat. No. 31430) at 0.08µg/mL
Substrate	Pierce ECL Substrate (Cat. No. 32209)
Detection	X-ray film



SuperSignal Western Blot Enhancer is compatible with chromogenic and fluorescent detection methods. HeLa cell lysate was separated by electrophoresis, transferred to nitrocellulose (top) or Low-Fluorescence PVDF Membrane (Cat. No. 22860) (bottom) and Western blotting was performed using the conventional method (left) or using the SuperSignal Western Blot Enhancer protocol (right).

	Top Panel	Bottom Panel
Blocking buffer	SuperBlock Buffer in TBS (Cat. No. 37535)	Blocker BLOTTO in TBS (Cat. No. 37530)
Primary antibody	Rabbit anti-β-Catenin (LabVision Part No. PAb RB-1491-PABX) at 0.2µg/mL	Rabbit anti-MAP Kinase at 1µg/mL
Secondary antibody	Alkaline phosphatase-conjugated goat anti-rabbit IgG (Cat. No. 31340) at 0.04µg/mL	DyLight™ 488-conjugated and goat anti-rabbit IgG (Cat. No. 35552) at 0.1µg/mL
Substrate	Pierce 1-Step NBT/BCIP Substrate (Cat. No. 34042)	
Detection	Visual	Typhoon™ 9410

Recommended Products

Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate and **Thermo Scientific™ Pierce™ ECL Substrate** are recommended for use with the SuperSignal Western Blot Enhancer. For chromogenic detection, we recommend **Thermo Scientific™ 1-Step NBT/BCIP Substrate**.

Thermo Scientific™ Goat anti-mouse or Goat anti-rabbit HRP-conjugated secondary antibodies are recommended for your Western blot detection.

For ordering information refer to page 43.

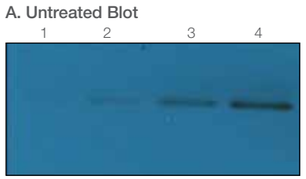
Pierce Western Blot Signal Enhancer

It's like having an intensifying screen in a bottle

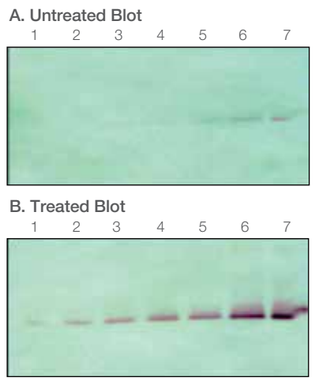
Thermo Scientific™ Pierce™ Western Blot Signal Enhancer is a two-reagent system for conditioning protein blots after transfer to greatly enhance the effectiveness of primary antibodies and intensify the final detection signal in Western blot experiments. The Pierce Western Blot Signal Enhancer membrane treatment procedure is very simple, takes only 15 minutes and can be added to nearly any existing Western blotting protocol. The result is an increase in the intensity of target protein bands on the Western blot or detection of target proteins at levels that were previously not possible. The product is effective for signal intensification with both chemiluminescent and chromogenic substrates, especially with nitrocellulose membranes.

Features:

- **Increases protein detection** — most protein targets show a three- to 10-fold increase in signal intensity, improving detection of low amounts of protein with the same substrate and method
- **Improves antibody binding** — the membrane-treatment reagent exposes and conditions target proteins so that specific antibodies can bind more effectively
- **Works for nearly any protein** — signal enhancement has been demonstrated with targets such as IL-6, p53, NFκB, BRCA1 and EGF
- **Effective with any substrate** — enhances both chemiluminescent and colorimetric detection for Western blots
- **Compatible with any membrane** — enhances signal on nitrocellulose and PVDF membrane, regardless of pore size (enhancement is less pronounced with PVDF)
- **Fast** — 15-minute protocol optimized for a combination of simplicity, speed and signal enhancement for most proteins
- **Convenient** — Ready-to-use reagents, stable at room temperature



Enhanced chemiluminescent detection of identical serial dilutions of IL-6. Panel A: before and Panel B: after treatment with Pierce Western Blot Signal Enhancer. Lane 1: 250pg, Lane 2: 500pg, Lane 3: 1,000pg and Lane 4: 2,000pg.



Enhanced chromogenic detection of identical serial dilutions of IL-6. Panel A: before and Panel B: after treatment with Pierce Western Blot Signal Enhancer. Lane 1: 100pg, Lane 2: 200pg, Lane 3: 300pg, Lane 4: 400pg, Lane 5: 500pg, Lane 6: 1,000pg and Lane 7: 5,000pg.

Comparison of our Western blot enhancers.

Product	Benefit	Mode of action	Use when…
SuperSignal Western Blot Enhancer	Increases sensitivity through enhanced signal and decreased background	Increases antigen specificity by decreasing weak antibody-antigen interactions	More sensitivity is needed and when primary antibody is "dirty" yielding nonspecific bands
Pierce Western Blot Signal Enhancer	Enhances sensitivity	Unraveling targets for increased epitope accessibility	More sensitivity is needed

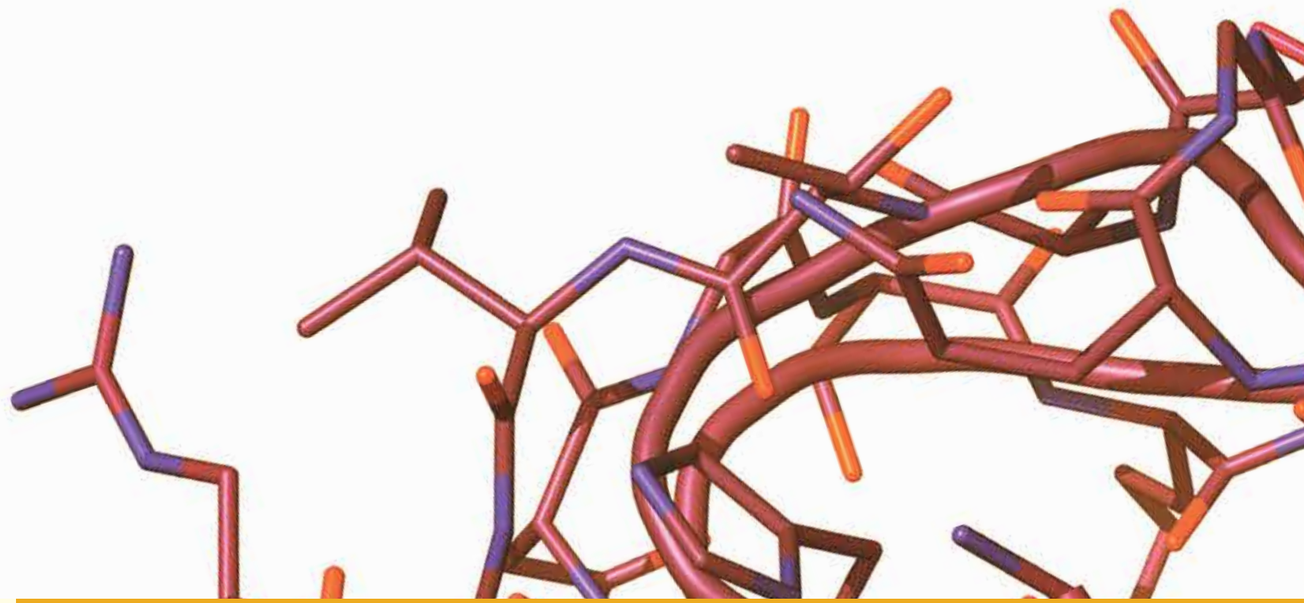
Recommended Products

We recommend the use of **Pierce ECL Substrate** for chemiluminescent Western blot detection or **1-Step NBT/BCIP** for chromogenic Western blot detection of membranes treated with Pierce Western Blot Signal Enhancer.

Thermo Scientific™ CL-Xposure™ Film or MyECL Imager are recommended for the capture of chemiluminescent signals from your Western blots.

Did you know?

The addition of up to 20% methanol or ethanol to a transfer buffer improves small molecular weight protein transfer.



For ordering information refer to page 43.

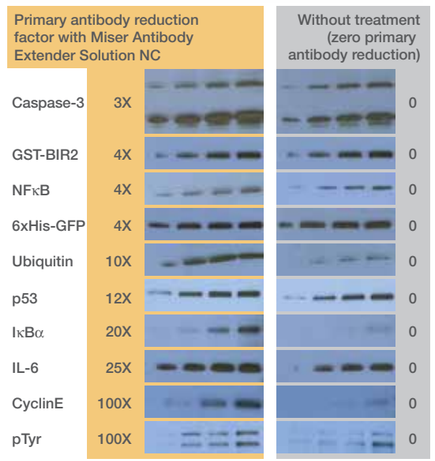
Miser Antibody Extender Solution NC

Get the most out of your primary antibody

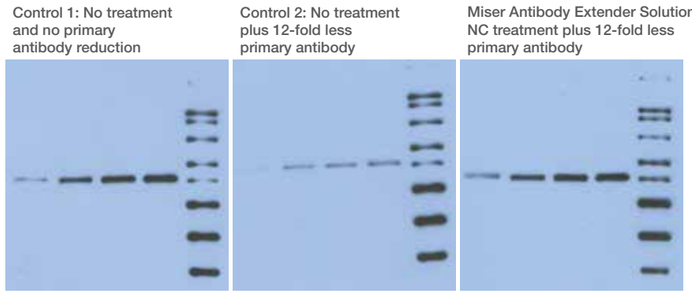
Thermo Scientific™ Miser™ Antibody Extender Solution NC is a post-transfer, nitrocellulose membrane-treatment reagent that enables Western blot detection with much less primary antibody. By treating nitrocellulose membranes for a few minutes in this reagent immediately after transfer and before probing, you can use more dilute primary antibody solutions while obtaining the same level of detection. This conserves use of expensive and rare antibodies, allowing for more experiments with the same amount of antibody. The reagent is so effective that our scientists have observed antibody cost reductions of 10, 25 and even 100 times, depending on the antigen and primary antibody directed against that antigen.

Features:

- **Achieve equivalent signal** — use at least three times less primary antibody than normal and retain comparable or better signal detectability
- **Any detection mode or enzyme** — the method works with both colorimetric and chemiluminescent detection modes, and with horseradish peroxidase (HRP) and alkaline phosphatase (AP) systems
- **Easy to perform** — with a 10-minute protocol, helps save money on your primary antibody; primary antibody use reductions of up to 100-fold have been observed



Use three to 100 times less primary antibody after membrane treatment with Miser Antibody Extender Solution NC. Example Western blot experiments with ten different proteins, demonstrating that equivalent or better detection is possible with at least three times more dilute primary antibody when the Miser Antibody Extender Solution is used.



Treatment with Miser Antibody Extender Solution NC enables p53 detection with 12 times less primary antibody. Aliquots of a nuclear extract from A431 cells were loaded (1, 2, 3 and 4µg per lane) and separated on three identical mini gels (4–20% Tris-glycine) and then transferred to nitrocellulose membranes. Control blots 1 and 2 (left and center) were blocked and then probed with an optimized (Control 1) or 12-fold greater dilution (Control 2) of anti-p53 antibody. The third blot (right) was treated with Miser Antibody Extender Solution NC, then blocked and probed with the 12-fold greater dilution of anti-p53 antibody. Final detection in all cases was done with goat anti-mouse-HRP secondary antibody and chemiluminescent substrate. Comparable sensitivity was observed between Control 1 and the treated blot.

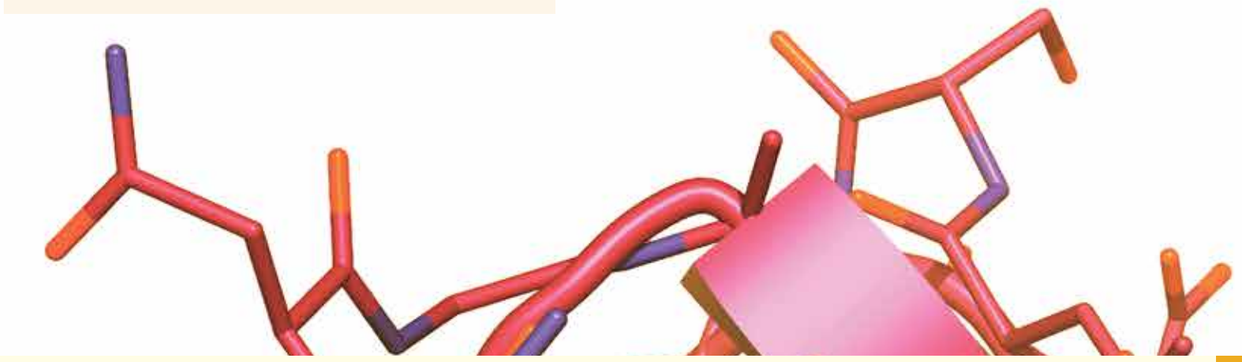
Recommended Products

For molecular weight estimation of target proteins in Westerns, we recommend using **MagicMark XP Western Protein Standard**.

For capturing your chemiluminescent detection results, we recommend **CL-XPosure Film or the MyECL Imager**.

Did you know?

The addition of 0.05% SDS to the transfer buffer helps with large molecular weight protein transfer.



For ordering information refer to page 43.

Protocol quick reference

Pierce Electrophoretic Blotting Quick Start Guide

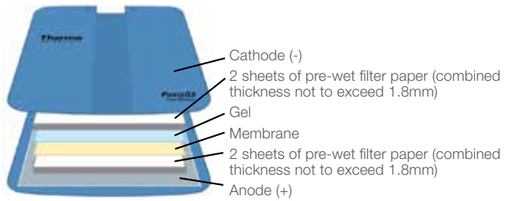
Thermo Scientific™ Pierce™ Power Blotter (Cat. No. 22834), Thermo Scientific™ Pierce™ Power System (Cat. No. 22830), or Thermo Scientific™ Pierce™ G2 Fast Blotter (Cat. No. 62291 upgraded with Cat. No. 62287), and Thermo Scientific™ Pierce™ Power Blot Cassette (Cat. No. 22835)

IMPORTANT Review and implement guidelines for proper set-up before installation. For detailed instructions refer to the manual at *thermoscientific.com/pierce-power-system.*

1. Using the supplied power cord, connect the Pierce Power Station to an electrical outlet.
2. Press the Power Button (located at the rear) to turn ON the Pierce Power Station.

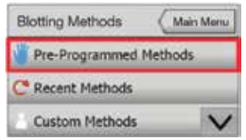
   IMPORTANT: For first use, refer to **"Using the Pierce Power Station for First Time"** section. If you currently use a Pierce Power Station for staining and are adding blotting functionality, refer to **"Using the Pierce Power Blot Cassette for First Time"** section. Upgraded Pierce G2 Fast Blotter/Stainer Control Units do not require any special activation in order to recognize the Power Stain Cassette or the Power Blot Cassette.
3. For each gel, use four sheets of ~0.83mm thick Western blotting filter paper and one sheet of nitrocellulose or PVDF membrane cut to the same size.
4. Equilibrate filter paper and membrane in Pierce 1-Step Transfer Buffer for at least 5 minutes. Use sufficient buffer to cover filter paper and membrane [~50mL per mini-sized (7cm x 8.4cm) sandwich and ~100mL per midi-sized (8cm x 13.5cm) sandwich].

   Note: PVDF membrane must be wetted with methanol or ethanol before equilibration in Pierce 1-Step Transfer Buffer.
5. After electrophoresis, remove gel(s) from cassette(s) and briefly place into tray containing deionized water or transfer buffer. This will ensure even wetting, facilitate proper gel placement and improve gel contact with membrane.
6. Assemble and center sandwich(es) on the anode (see figure below) to ensure even pressure. When transferring two gels allow for 10mm spaces between sandwiches. Use a blot roller to remove any trapped air bubbles.

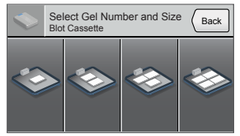


7. Lock top of the cassette (cathode) into place and slide the assembled cassette into the Pierce Power Station.
8. Select **Begin Blotting**.

9. Select **Pre-Programmed Methods** in the Main Menu.

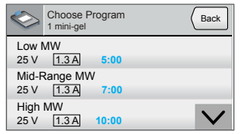


10. Select the number of gels and gel size (mini or midi) for transfer:
    a. **1 mini-gel** b. **2 mini-gels or 1 midi-gel**
    c. **3 mini-gels** d. **4 mini-gels or 2 midi-gels**



11. Select the appropriate program to run:
    a. **Low MW (< 25kDa)**
    b. **Mixed Range MW (25-150kDa)**
    c. **High MW (>150kDa)**
    d. **Std Semi-Dry**
    e. **1.5mm thick gels or if unknown**

    Note: For fast-blotting programs (program a, b, c and e) use Pierce 1-Step Transfer Buffer. Transfer time may be increased to 12 minutes for high molecular-weight proteins (> 200kDa). Do not use the Std Semi-Dry transfer program (program d) with Pierce 1-Step Transfer Buffer.



12. Select the **Start** button to begin transfer.

13. Pierce 1-Step Transfer Buffer is a highly concentrated salt solution. **Thoroughly** wash the anode and cathode after each use by rinsing the unassembled cassette under hot water while removing any salt residue with a gloved hand. Rinse with deionized water and stand parts in a rack to dry. For more thorough cleaning, immerse the unassembled cassette top (cathode) and bottom (anode) in hot water and use a gloved hand or clean sponge to remove salt residue. Rinse with deionized water and stand parts in a rack to dry.

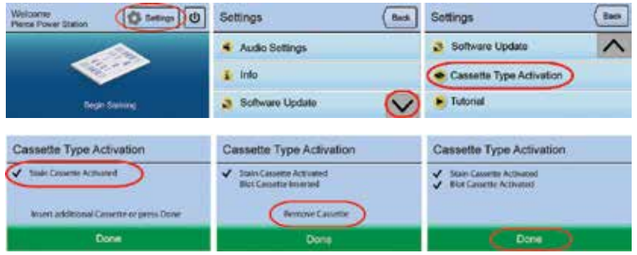
Note: Failure to keep cassette top and bottom clean can result in sticking of movable parts and lead to poor transfer efficiency.

Using the Pierce Power Station for the First Time

When turning on the Pierce Power Station for the first time, you will see the "Cassette Type Activation" screen. Follow the directions on the screen and insert the Power Blot Cassette into the Power Station to activate the Blot Cassette and blotting software. Remove the Power Blot Cassette and insert another cassette type to activate other cassette and software application (e.g., staining), or select "Done".

Using the Pierce Power Blot Cassette for the First Time

First you must activate the blotting software. To do so, press the "Settings" button on the Welcome screen. Press the down arrow to view more menu options, and select the "Cassette Type Activation" button. Previously activated cassette types will appear on the screen with a check mark to the left. To activate the Blot Cassette and blotting software, insert the Power Blot Cassette into the Power Station and remove when instructed. Press "Done" or activate an additional cassette type in the same manner. For detailed instructions refer to the manual at *www.thermoscientific.com/pierce-power-system.* The Pierce Power Station will recognize G2 Fast Blotter Cassettes as Power Blot Cassettes.



Related Thermo Scientific™ Products

88018	Nitrocellulose Membrane, 0.45µm, 30cm X 3.5m roll
88518	PVDF Transfer Membrane, 0.45µm, 25cm X 3.75m roll
84731	Pierce 1-Step Transfer Buffer
84783	Pierce Western Blotting Filter Paper, 7 x 8.4cm, 100 sheets (0.83mm thick)
84784	Pierce Western Blotting Filter Paper, 8 x 13.5cm, 100 sheets (0.83mm thick)
22840	Pierce™ Mini Gel Power Staining Kit
22830	Pierce Power System
22838	Pierce Power Station
22834	Pierce Power Blotter
22835	Pierce Power Blot Cassette
22833	Pierce Power Stainer
22836	Pierce Power Stain Cassette
84747	Western Blot Roller
22841	Midi Gel Incubation Trays, 10 ea
22843	Mini Gel Incubation Trays, 10 ea

WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates. When continuously processing multiple samples allow the cassette to cool for 30 minutes or use multiple cassettes to avoid excessive cassette heating.

WARNING Stainer use outside of the workflows described in this manual may put the operator at risk of dangerous exposure to electrical shock. Do not use this instrument for any purposes or in any configurations not described in this manual.

WARNING The Pierce Power Station can contain dangerous electricity and is not designed to be opened by the user. Disconnect all power to the Pierce Power Station before maintenance by a qualified technician.

WARNING Do not overfill the cassette with liquid. Excess liquid can overflow into the base unit and possibly cause electric shock. Follow the appropriate instructions for reagent amounts and empty any remaining liquid in the cassette upon run completion.

NOTE A grounded circuit capable of delivering the appropriate current and voltage is required for installation. Electrical requirements can be located on the rear panel of the blotter base. The blotter electrical system will adjust to the proper voltage for the respective country. Connect the blotter power cord to the rear left side panel of the device and plug into a grounded power outlet.

ENVIRONMENTALLY FRIENDLY DISPOSAL

According to EU directive 2002/96/EC on electric and electronic equipment and its implementation into national law, all electric equipments must be separately collected and environmentally friendly recycled. Alternative disposal: If the owner of the electric equipments does not return it to the manufacturer, he is responsible for proper disposal at a designated collection point that prepares the device for recycling according to national recycling laws and regulations. This does not include accessories and tools without electric or electronic components.

Blotter Electrical Parameter	Rating
Supply Voltage (VAC)	100–240
Frequency (Hz)	50/60
Maximum Power Rating (W)	168
Fuse (Power Center)	T3AL, 250V, 3A

iBlot 2 Dry Blotting System

Catalog Number IB21001
Publication Number MAN0009113 **Rev.** A.0

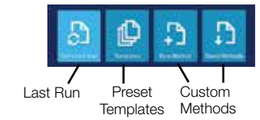
Instructions for using the iBlot 2 Gel Transfer Device to perform dry blotting of proteins from mini or midi gels with iBlot 2 Transfer Stacks are described below. For detailed instructions refer to the manual at thermofisher.com/iblot2.

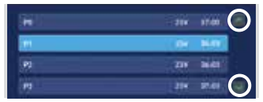
General Guidelines

- **Read "Unpacking Instructions" in the manual for setup instructions when using the iBlot 2 Gel Transfer Device for the first time.**
- Use the Blotting Roller to remove any bubbles between layers of the stack.
- Do not trim the membrane or iBlot 2 Transfer Stacks to fit your gel size.
- Use the iBlot 2 Transfer Stacks, Regular for transferring E-PAGE, 1 midi (1mm thick), or 2 mini gels (1.0 or 1.5mm thick).
- Use iBlot 2 Transfer Stacks, Mini for transferring 1 mini gel (1.0 or 1.5mm thick).
- Method **P0 for 7 minutes** is recommended for transferring most proteins (30–150kDa) with **nitrocellulose** and **PVDF** stacks.
- Based on the initial results, you can increase or decrease the transfer time to optimize results.
  - A Run Time of 5–6 minutes may be necessary for transferring proteins of interest <30kDa.
  - A Run Time of 8–10 minutes may be necessary for transferring proteins of interest >150kDa.
- (Optional) Equilibration of the gel in 20% ethanol for 5–10 minutes prior to transfer may increase overall protein transfer efficiency.

Selecting a Method

1. Press the power switch to turn ON the iBlot 2 Gel Transfer Device. The internal fan should begin running, and the digital display should show the available action icons for selection.
2. Use the up/down arrows to scroll through the list of available methods. Touch the appropriate icon to select a method.

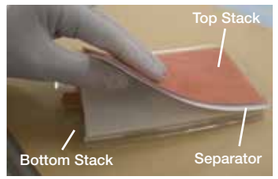




The iBlot 2 Gel Transfer Device is preprogrammed with the six preset templates listed below:

Method	Voltage	Default Run Time	Recommended Run Time Limit
P0	20V for 1 min 23V for 4 min 25V for remainder	7 min	13 min
P1	25V	6 min	10 min
P2	23V	6 min	11 min
P3	20V	7 min	13 min
P4	15V	7 min	16 min
P5	10V	7 min	25 min

Using the iBlot 2 Gel Transfer Device



1. Unseal the Transfer Stack. Set the Top Stack to one side and discard the white separator.*
   Keep the Bottom Stack in the plastic tray.

2 Place the Bottom Stack (in the tray) on the blotting surface. Align electrical contacts on the tray with the corresponding electrical contacts on the blotting surface of the iBlot 2 Gel Transfer Device.

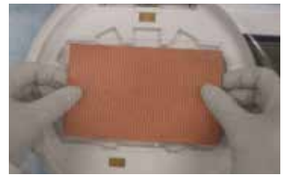
3 Wet the pre-run gel(s) and place on the transfer membrane of the Bottom Stack.

* Make sure the membrane is not stuck to the separator before proceeding to the next step.

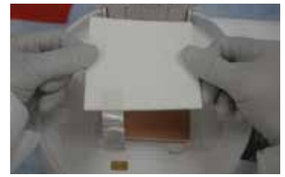
For Research Use Only. Not for use in diagnostic procedures.

Using the iBlot 2 Gel Transfer Device, continued

4. Place a pre-soaked (in deionized water) iBlot Filter Paper on the gel and remove air bubbles using the Blotting Roller.



5. Place the Top Stack over the pre-soaked filter paper. Remove air bubbles using the Blotting Roller.



6. Place the Absorbent Pad on top of the Top Stack such that the electrical contacts are aligned with the corresponding electrical contacts on the blotting surface of the iBlot 2 Gel Transfer Device.
7. Close the lid of the device and proceed to Performing Transfer, below.

Performing Transfer

Perform blotting within 15 minutes of assembling the stacks with the gel.

1. Select the desired method and make sure the parameters are correct.
   Touch **Start Run**, or if desired, use the **Start Last Run** icon.

2. Once transfer begins, the elapsed time is displayed on the screen.

3. The end of transfer is indicated by beeping sounds, and a message on the digital display. Touch **Done**.

Disassembling the iBlot 2 Transfer Stack

1. Turn off the iBlot 2 Gel Transfer Device.
2. Open the lid of the iBlot 2 Gel Transfer Device.
3. Discard the Absorbent Pad and Top Stack.
4. Carefully remove and discard the gel and filter paper. Remove the transfer membrane from the stack and proceed with the blocking procedure or stain the membrane.
5. Discard the Bottom Stack.
6. At this point, the iBlot 2 Gel Transfer Device is ready for another run (no cooling period is required). If you are not using the device, turn off the power switch.

Downloading Upgrades

To download iBlot 2 Gel Transfer Device firmware upgrades, go to thermofisher.com/iblot2. Follow instructions on the page to download the upgrades.

Troubleshooting

Protein bands weak or absent from the membrane

Possible cause	Solution(s)
Small proteins transferring through membrane (“blowout”)	Use lower membrane pore size (0.2µm). Decrease transfer time. Wash gel in water to remove residual SDS. Increase methanol concentration 20–40% in transfer buffer.
Large proteins not transferring well onto the membrane	Use PVDF or nylon membrane. Decrease methanol concentration in transfer buffer. Soak gel in 0.05% SDS solution for five minutes. Increase voltage and transfer time.
Transfer stack assembled in the wrong orientation	Ensure that the stack is assembled in the correct orientation.
Short circuit in the transfer	For semi-dry methods, be sure there are no pools of liquids. Tilt unit after setting up the transfer sandwich to remove excess transfer buffer. Make sure there are no overhangs of filter paper outside of the transfer sandwich. For iBlot 2 device, check that the stack is fully aligned to the end of the device.
pH of transfer buffer is incorrect	Verify that pH is correct. pH of transfer buffer should be correct if accurately following recipe.
Too much methanol in transfer buffer	Methanol removes residual SDS from proteins. Decrease accordingly for large molecular weight protein transfer and increase for small molecular weight protein transfer.
SDS concentration is too low/high	SDS coats the proteins to allow transfer; adjust wash appropriately. Increase SDS for large molecular weight protein transfer and remove SDS for small molecular weight protein transfer.

Incomplete transfer

Possible cause	Solution(s)
Insufficient/no current	Check current during run; also check power/electrode connections for dry/semi-dry transfer. Check hardware and make sure the anode and cathode plates are clean and free of salt deposits. Check transfer buffer formulation or dilution.
Large protein transfer	Incubate with SDS (nitrocellulose membranes), transfer longer or increase voltage.
Transfer from a 1.5mm gel	Increase transfer time.
Transfer time too short	Increase transfer time.

Spots/spaces on membrane after transfer

Possible cause	Solution(s)
Air bubbles between gel and membrane	Use roller to ensure that there are no air pockets.
Under- or overcompression of the transfer stack	Check that you are using the number of filter paper sheets and sponges recommended by the manufacturer.
Proteins not binding to membrane	When using PVDF membrane, make sure it is fully activated by pre-wetting in methanol for 5 to 10 seconds.

Recommended references for Western blotting

Cited references

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2. Tovey ER, Baldo BA (1987) Comparison of semi-dry and conventional tank-buffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes. *Electrophoresis* 8:384–387.

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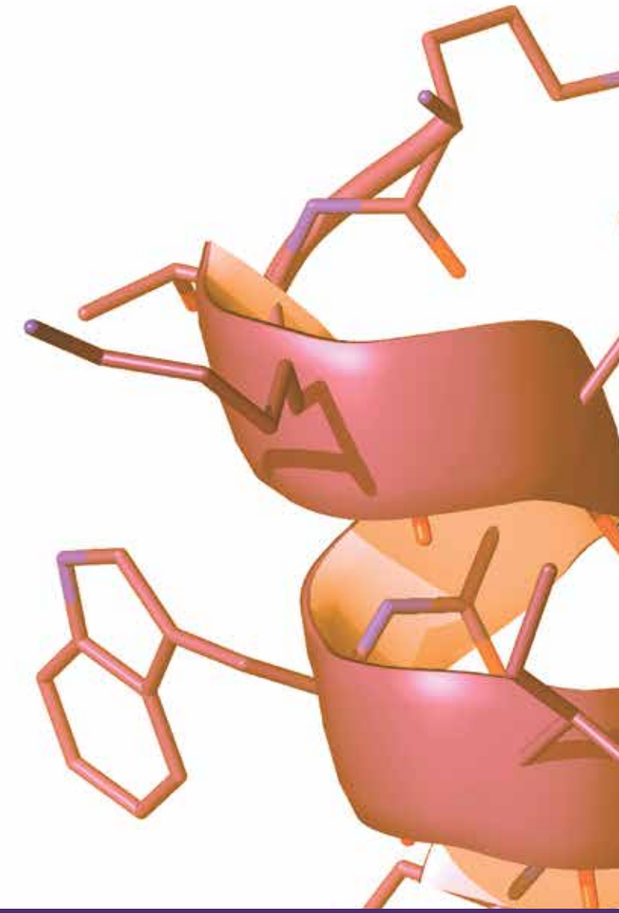

Ordering information

Pre-transfer

Product	Quantity	Cat. No.
Membranes		
Ready-to-use sandwiches		
Nitrocellulose/Filter Paper Sandwich, 0.2µm pore size, 8.5 x 13.5cm	16/pack	LC2009
Nitrocellulose/Filter Paper Sandwich, 0.45µm pore size, 8.5 x 13.5cm	16/pack	LC2006
iBlot 2 Transfer Stacks, Nitrocellulose, Mini	10 stacks	IB23002
iBlot 2 Transfer Stacks, Nitrocellulose, Regular	10 stacks	IB23001
iBlot 2 Transfer Stacks, PVDF, Mini	10 stacks	IB24002
iBlot 2 Transfer Stacks, PVDF, Regular	10 stacks	IB24001
Pre-cut nitrocellulose membranes		
Nitrocellulose Pre-Cut Blotting Membranes, 0.2µm pore size	20/pack	LC2000
Nitrocellulose Pre-Cut Blotting Membranes, 0.45µm pore size	20/pack	LC2001
Nitrocellulose Membrane, 0.2µm, 8 x 12cm	25 sheets	77012
Nitrocellulose Membrane, 0.2µm, 7.9 x 10.5cm	15 sheets	88013
Nitrocellulose Membrane, 0.2µm, 8 x 8cm	15 sheets	88024
Nitrocellulose Membrane, 0.45µm, 8 x 12cm	25 sheets	77010
Nitrocellulose Membrane, 0.45µm, 7.9 x 10.5cm	15 sheets	88014
Nitrocellulose Membrane, 0.45µm, 8 x 8cm	15 sheets	88025
Nitrocellulose rolls		
Nitrocellulose Membrane, 0.45µm, 30cm x 3.5m	1 roll	88018
Pre-cut PVDF membranes		
Invitrolon PVDF/Filter Paper Sandwich, 0.45µm pore size, 8.3 x 7.3cm (for mini gels)	20/pack	LC2005
Invitrolon PVDF/Filter Paper Sandwich, 0.45µm pore size, 8.5 x 13.5cm	16/pack	LC2007
Low-Fluorescence PVDF Transfer Membrane, 0.2µm, 7 x 8.4cm	10 sheets	22860
PVDF Pre-cut Blotting Membranes, 0.2µm pore size	20/pack	LC2002
PVDF Transfer Membrane, 0.45µm, 10 x 10cm	10 sheets	88585

Pre-transfer

Product	Quantity	Cat. No.
Tropifluor PVDF Membrane, 0.45µm pore size, 15 X 15cm	5 sheets	T2234
PVDF rolls		
PVDF Transfer Membrane, 0.2µm, 26.5cm x 3.75m	1 roll	88520
PVDF Transfer Membrane, 0.45µm, 26.5cm x 3.75m	1 roll	88518
Filter paper for blotting		
Western Blotting Filter Paper, 8 x 10.5cm	100 sheets	88600
Western Blotting Filter Paper, 7 x 8.4cm	100 sheets	84783
Western Blotting Filter Paper, 8 x 13.5cm	100 sheets	84784
Western Blotting Filter Paper, Extra Thick, 7 x 8.4cm	50 sheets	88605
Western Blotting Filter Paper, Extra Thick, 8.5 x 9cm	50 sheets	88610
Western Blotting Filter Paper, Extra Thick, 8 x 13.5cm	50 sheets	88615
Western Blotting Filter Paper, Extra Thick, 20 x 20cm	50 sheets	88620
Blotting Filter Papers, 2.5mm thickness, 8.6 x 13.5cm	50 each	LC2008
Blotting Filter Papers, 2.5mm thickness, 7.5 x 8.4cm	50 sheets	LC2010
Protein ladders		
PageRuler Plus Prestained Protein Ladder, 10 to 250kDa SDS-PAGE	2 x 250µL	26619
PageRuler Plus Prestained Protein Ladder, 10 to 250kDa SDS-PAGE	10 x 250µL	26620
PageRuler Prestained Protein Ladder, 10 to 180kDa SDS-PAGE	2 x 250µL	26616
PageRuler Prestained Protein Ladder, 10 to 180kDa SDS-PAGE	10 x 250µL	26617
HiMark Pre-stained Protein Standard, 30 to 460kDa, SDS-PAGE	250µL	LC5699
Spectra Multicolor Broad Range Protein Ladder, SDS-PAGE	10 x 250µL	26623
Spectra Multicolor Broad Range Protein Ladder, SDS-PAGE	2 x 250µL	26634
Spectra Multicolor High Range Protein Ladder, SDS-PAGE	2 x 250µL	26625

Pre-transfer

Product	Quantity	Cat. No.
PageRuler Unstained Protein Ladder, SDS-PAGE	2 x 250µL	26614
PageRuler Unstained Low Range Protein Ladder, 3.4 to 100kDa, SDS-PAGE	2 x 250µL	26632
NativeMark Unstained Protein Standard, 20 to 1,200kDa, NativePAGE	5 x 50µL	LC0725
MagicMark XP Western Protein Standard, 20 to 220kDa, SDS-PAGE	250µL	LC5602
MagicMark XP Western Protein Standard, 20 to 220kDa, SDS-PAGE	50µL	LC5603
BenchMark Fluorescent Protein Standard, 11 to 155kDa, SDS-PAGE	125µL	LC5928
PageRuler Prestained NIR Protein Ladder, 11 to 250kDa, SDS-PAGE	2 x 250µL	26635
BenchMark His-tagged Protein Standard, 10 to 160kDa, SDS-PAGE	125µL	LC5606
IEF Marker 3–10, 3 to 10pI	500µL	39212-01
Transfer buffers		
Bolt Transfer Buffer (20X)	125mL	BT0006
Bolt Transfer Buffer (20X)	1L	BT00061
BupH Tris-Glycine Buffer Packs	40 packs	28380
Novex Tris-Glycine Transfer Buffer (25X)	500mL	LC3675
NuPAGE Transfer Buffer (20X)	125mL	NP0006
NuPAGE Transfer Buffer (20X)	1L	NP0006-1
Pierce 1-Step Transfer Buffer	1L	84731
Pierce 1-Step Transfer Buffer	5 x 1L	84731X5
Pierce 1-Step Transfer Buffer	200mL	84742
Pierce 10X Tris-Glycine Buffer	1L	28363
Pierce 10X Western Blot Transfer Buffer, Methanol-free	5L	35040
Accessories		
Blotting Roller	1 unit	LC2100
Western Blot Roller	1 unit	84747

Transfer

Product	Quantity	Cat. No.
Wet transfer systems		
Mini Blot Module	1 unit	B1000
Mini Gel Tank + Blot Module Set	1 unit	NW2000
XCell II Blot Module	1 unit	EI9051
XCell SureLock Mini-Cell and XCell II Blot Module	1 unit	EI0002
PowerEase 300W Power Supply	1 unit	PS0300
PowerEase 90W Power Supply	1 unit	PS0090
Bolt Western Pack A (Nitrocellulose)	1 kit	B1000A
Bolt Western Pack B (PVDF)	1 kit	B1000B
Semi-dry transfer systems		
Pierce Power Blotter	1 unit	22834
Pierce Power Stainer	1 unit	22833
Pierce Power Station	1 unit	22838
Pierce Power System	1 unit	22830
Dry transfer systems		
iBlot 2 Gel Transfer Device	1 device	IB21001
iBlot 2 Electrode Replacement Kit	1 kit	IB28001

Post-transfer

Product	Quantity	Cat. No.
Reversible protein stain kits		
Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes	1.5L	24580
Pierce Reversible Protein Stain Kit for PVDF Membranes	1.75L	24585
Signal enhancement		
Miser Antibody Extender Solution NC	500mL	32110
Pierce Western Blot Signal Enhancer	500mL	21050
SuperSignal Western Blot Enhancer	500mL	46640
SuperSignal Western Blot Enhancer	50mL	46641

Protein transfer word find challenge

To participate in the word find challenge, go to thermofisher.com/transferwordfind

1 H H C P O I D B O Y R
(7)

2 R C O U E L L M A
(1)

G W E I T H
(3)

3 B I T O N W
(5)

4 C D N T T V I U O I C Y
(4)

5 O N T A M E H L
(14) (2)

6 F E D L I
(13)

T T R N E H S G
(12)

7 L R L U O E L T E C S O N I
(11) (6)

8 E E V R E L B S I R
(15)

A S I T N
(10)

9 L E T O A G V
(8)

10 W E R P O
(9)

L T T O E R B

Final word

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Clues

1. Proteins bind to the PVDF membrane via _____ interactions.
2. Name one of the critical factors that governs the selection of transfer membrane pore size.
3. The scientist who first described electroblotting.
4. Impurities in the methanol of your transfer buffer may affect what?
5. The addition of _____ can improve the transfer of small proteins and retention of proteins binding to the membrane.
6. Along with the short distance between electrodes, the increase of which factor helps reduce the blotting time in the dry blotting workflow?
7. What membrane would you use for low background detection of small molecular weight proteins?
8. What is the recommended method to ensure protein was transferred after Western blotting to your membrane?
9. What factor along with transfer time is most commonly altered in dry blotting to improve the transfer efficiency of proteins?
10. Which Thermo Scientific blotting device will allow you to perform high-throughput transfer of four mini gels in less than 10 minutes?

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