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

For ordering information refer to page 43.

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.

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

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