Power Blotter 1-Step Transfer Buffer

Catalog Number PB7100 and PB7300

Pub. No. MAN0017056 **Rev.** A.0

Contents

Contents	Cat. No. PB7100	Cat. No. PB7300	Storage
Power Blotter 1-Step Transfer Buffer, 5X concentrate	250 mL	1 L	Store at room temperature.

Product description

Invitrogen™ Power Blotter 1-Step Transfer Buffer is used with the Invitrogen™ Power Blotter to transfer proteins from SDS-PAGE gels to nitrocellulose or PVDF membranes in 5-10 minutes. Refer to the Power Blotter System (PB0012, PB0013) User Guide for more details.

Use 1-Step Transfer Buffer

Note: This procedure is optimized for the following precast gels: Bolt™ Bis-Tris Plus, Novex™ Tris-Glycine, Novex™ Tris-Glycine Plus, NuPAGE™ Bis-Tris, NuPAGE™ Tris-Acetate, and Novex™ Tricine Gels. Gel types not in this list may require further optimization.

Additional materials required

- Power Blotter Pre-Cut Membranes and Filters, Nitrocellulose, Mini Size (Product No. PB7220)
- Power Blotter Pre-Cut Membranes and Filters, PVDF, Mini Size (Product No. PB9220)
- Power Blotter Pre-Cut Membranes and Filters, Nitrocellulose, Regular Size (Product No. PB7320)
- Power Blotter Pre-Cut Membranes and Filters, PVDF, Regular Size (Product No. PB9320)
- Power Blotter Station (Product No. PB0010)
- Power Blotter Cassette or Cassette XL (Product No. PB0002 or PB0003)

Transfer protein from gel to membrane

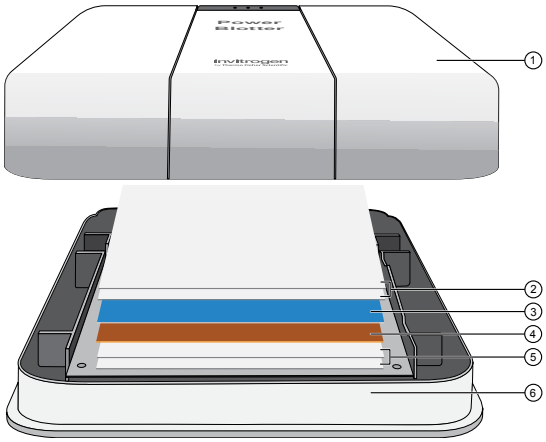
1. Dilute Power Blotter 1-Step Transfer Buffer 5X concentrate to 1X with distilled water (1 part buffer to 4 parts water).
2. Equilibrate filter paper and membrane in pre-diluted 1X Power Blotter 1-Step Transfer Buffer for ≥5 minutes with gentle rocking. For each gel, use 4 sheets of filter paper (each sheet ~0.85 mm thick) and 1 sheet of nitrocellulose or PVDF membrane.

   Note: Use ~50 mL of transfer buffer for 1 mini-size transfer stack and ~100 mL of transfer buffer for 1 regular-size transfer stack.

   Note: PVDF membranes must be pre-wetted with methanol or ethanol before equilibrating in transfer buffer.
3. After electrophoresis, remove the gel(s) from cassette(s) and briefly place in a tray containing deionized water or transfer buffer. This will ensure even wetting, facilitate proper gel placement, and improve contact with the membrane.

4. Using filters and membranes equilibrated in Step 1, assemble the blot directly on the anode plate of the cassette as shown in Figure 1. Eliminate air bubbles between the gel and membrane with a roller or clean pipette.

   Note: When transferring more than one gel, allow for 10 mm spaces between the stacks.



① Cathode
② 2 sheets of pre-wet filter paper (not to exceed 1.8 mm thickness)
③ Pre-run gel
④ Membrane
⑤ 2 sheets of pre-wet filter paper (not to exceed 1.8 mm thickness)
⑥ Anode

5. Using the Power Blotter Station, transfer protein from gel to membrane using continuous amperage (~21 mA/cm^2) for 5-10 minutes (Table 1).

   Note: A summary of transfer conditions is provided below. See more detailed instructions in the Power Blotter System (PB0012, PB0013) User Guide.

   Table 1 Power supply settings for different gel sizes.

Gel Size	Surface Area (cm^2)	Constant Current (A)	Voltage Limit (V)	Recommended Transfer Time (minutes)		
				Low MW	Mixed MW	High MW
1 mini-sized gel	~60	1.3	25	5	7	10
2 mini-sized gels or 1 midi-sized gel	~120	2.5	25	5	7	10
3 mini-sized gels	~180	3.8	25	5	7	10
4 mini-sized gels or 2 midi-sized gel	~240	5.0	25	5	7	10

   Note: For gels thicker than 1 mm or for homemade gel formulations, add 2 minutes of transfer time.

6. Remove and rinse the membrane with deionized water and proceed to protein detection.

   Note: After transfer: Thoroughly wash the anode and cathode after each use by rinsing or soaking the unassembled cassette under hot water. Remove any residue with a gloved hand. Rinse with deionized water and stand parts in a rack to dry or proceed with additional transfers. Allow 5-10 minutes between repeated transfers to prevent excess instrument heating.

Troubleshooting

Observation	Possible cause	Recommended action
Inefficient transfer.	Salt was deposited on electrodes.	Power Blotter 1-Step Transfer Buffer (1X working solution) is a highly concentrated salt solution. Thoroughly wash the anode and cathode after each use and rinse the unassembled cassette under hot water while removing any sticky salt residue with a gloved hand. Briefly rinse with deionized water and stand in a rack to dry. For more thorough cleaning, immerse the cassette top and bottom in hot water and use a gloved hand or clean sponge to remove salt residue. Rinse with deionized water and stand in a rack to dry. **Note:** Failure to keep cassette top and bottom clean can result in moving parts sticking and lead to poor transfer efficiency.
	Membrane or filter paper was insufficiently equilibrated in Power Blotter 1-Step Transfer Buffer.	Equilibrate membrane and filter paper in Power Blotter 1-Step Transfer Buffer (1X working solution) before transfer. Use a sufficient amount of buffer for the equilibration step.
	Insufficient transfer time.	Increase transfer time of 7-10 minutes to 10-12 minutes.
	PVDF membrane was not pre-wetted with methanol.	Wet PVDF membrane with methanol or ethanol and equilibrate for 10-15 minutes in Power Blotter 1-Step Transfer Buffer (1X working solution) before transfer.
Inconsistent transfer.	Air bubbles were trapped between the gel and membrane.	When assembling sandwich, use a roller or pipette to remove any air bubbles between the gel and the membrane.
Inefficient transfer of high molecular-weight proteins from Tris-glycine gels to nitrocellulose or PVDF membranes.	Inefficient binding of high molecular-weight proteins (<200kDa) to nitrocellulose or PVDF membrane.	Combine ethanol and Power Blotter 1-Step Transfer Buffer (1X working solution) in a 15:85 ratio before equilibrating filter paper and membrane. After electrophoresis and prior to transfer, wash Tris-glycine gel for 10-15 minutes in water.

Related products

Product	Cat. no.
Power Blotter Select Transfer Stacks, Nitrocellulose, Regular Size	PB3310
Power Blotter Select Transfer Stacks, Nitrocellulose, Mini Size	PB3240
Power Blotter Select Transfer Stacks, PVDF, Regular Size	PB5310
Power Blotter Select Transfer Stacks, PVDF, Mini Size	PB5240
Nitrocellulose Membrane, 0.45 µm, 30 cm × 3.5 m roll	88018
PVDF Transfer Membrane, 0.45 µm, 26.5 cm × 3.75 m roll	88518
PageRuler™ Prestained Protein Ladder, 10-170 kDa, 2 × 250 µL	26616
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	34580

Manufacturer: Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA