INSTRUCTIONS

Pierce 1-Step Transfer Buffer

84731 84742 2460.2

Number	Description
84731	**Pierce 1-Step Transfer Buffer,** 1L
84742	**Pierce 1-Step Transfer Buffer,** 200mL

Storage: Upon receipt store at room temperature. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Pierce™ 1-Step Transfer Buffer is used with the Thermo Scientific Pierce G2 Fast Blotter to transfer proteins from SDS-PAGE gels to nitrocellulose or PVDF membranes in 5-10 minutes.

Procedure

Note: This procedure is optimized for the following precast gels: Thermo Scientific Precise Protein, Precise™ Tris-Glycine, NuPAGE™ Bis-Tris, Criterion™ Tris-HCl and Mini-PROTEAN™ TGX Gels. Gel types not in this list may require further optimization.

A. Additional Materials Required

- Western blotting filter paper (~0.83mm thick) cut-to-size
- Transfer membrane cut-to-size
- Pierce G2 Fast Blotter Control Unit and Cassette

B. Transfer Protein from Gel to Membrane

1. Equilibrate filter paper and membrane in undiluted 1-Step Transfer Buffer for 10 minutes with gentle rocking. For each gel, use four sheets of ~0.83mm thick Western Blotting filter paper and one sheet of nitrocellulose or PVDF membrane cut to same size.

   Note: Use ~50mL of transfer buffer for one mini-gel and ~100mL for one midi-gel size filter paper and membrane.

   Note: PVDF membranes must be pre-wetted with methanol or ethanol before equilibrating in transfer buffer.

2. After electrophoresis, remove gel 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.

   Note: Do not wash the gel in water after electrophoresis.

   Assemble blot directly on anode plate of the Cassette as described in Figure 1. Eliminate air bubbles between gel and membrane with a roller or clean pipette.

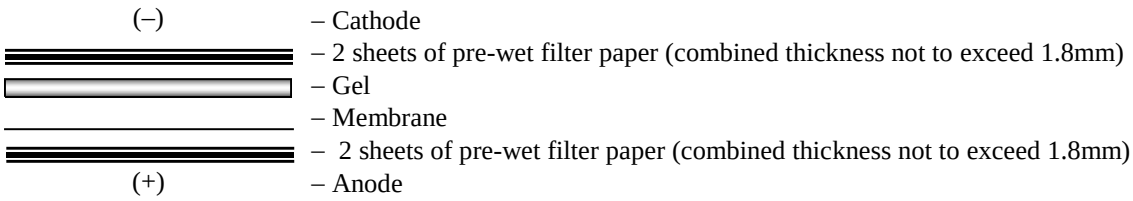

Figure 1. Side view of transfer sandwich.

3. Using the Pierce G2 Fast Blotter Control Unit and Cassette, transfer protein from gel to membrane using continuous amperage (~21mA/cm^2) for 5-10 minutes (Table 1).

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 gels	~240	5.0	25	5	7	10

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

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

Troubleshooting

Problem	Possible Cause	Solution
Inefficient transfer	Salt deposited on electrodes	Pierce 1-Step Transfer Buffer 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 Pierce 1-Step Transfer Buffer	Equilibrate membrane and filter paper in Pierce 1-Step Transfer Buffer before transfer. Use sufficient amount of buffer for the equilibration step
	Insufficient transfer time	Increase transfer time from 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 Pierce 1-Step Transfer Buffer before transfer
Inconsistent transfer	Air bubbles trapped between 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 low molecular-weight proteins to PVDF	Inefficient binding of some low molecular-weight proteins (< 25kDa) to PVDF membrane	Combine ethanol and Pierce 1-Step Transfer Buffer in a 15:85 ratio before equilibrating filter paper and membrane

Related Thermo Scientific Products

84783	**Western Blotting Filter Paper, 7cm × 8.4cm, 0.83mm thickness**
84784	**Western Blotting Filter Paper, 8cm × 13.5cm, 0.83mm thickness**
88600	**Western Blotting Filter Paper, 8cm × 10.5cm, 0.83mm thickness**
84747	**Western Blot Roller**
88018	**Nitrocellulose Membrane, 0.45µm,** 30cm × 3.5m roll
88518	**PVDF Transfer Membrane, 0.45µm,** 26.5cm × 3.75m roll
62236	**MYECL™ Imager**
62237	**MYImageAnalysis™ Software**
26616	**PageRuler™ Prestained Protein Ladder, 10-170kDa,** 2 × 250µL
32106	**Pierce ECL Western Blotting Substrate**
34080	**SuperSignal™ West Pico Chemiluminescent Substrate**

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