Introduction

The Thermo Scientific Pierce Fast Semi-Dry Transfer Buffer is for transferring proteins from SDS-PAGE gels to nitrocellulose or PVDF membranes. This transfer buffer is compatible with semi-dry transfer units and accelerates protein transfer to 7-10 minutes compared with the traditional 45-minute transfer. The formulation does not require methanol and is supplied as a 10X concentrate that is simply diluted with deionized water before use.

Procedure for Fast Semi-Dry Transfer of Proteins

Note: This procedure was optimized for the following precast gels: Thermo Scientific Pierce Precise, NuPAGE Bis-Tris, Criterion Tris-HCl and Novex Tris-Glycine Gels. Using gels with other formulations or gels that are thicker than 1 mm might require optimization.

For homemade gels, equilibration (Step 4) is unnecessary; however, transfer might require more time (e.g., 20-45 minutes).

A. Additional Materials Required
- Western blotting filter paper cut to size
- Transfer membrane cut to size

B. Transfer Protein from Gel to Membrane
1. Dilute the Pierce Fast Semi-Dry Transfer Buffer, 10X 1:10 with deionized water and stir before use.
   Note: Use two pieces of thin filter paper (~ 1.25 mm) or one piece of ultra-thick filter paper (~ 2.5 mm) on the top and the bottom of the sandwich. PVDF membranes must be pre-wet with methanol before equilibrating in transfer buffer.
3. After electrophoresis, equilibrate gel in ultrapure water for 5-10 minutes with gentle agitation.
4. Equilibrate gel in diluted Pierce Fast Semi-Dry Buffer for 5-10 minutes with gentle agitation.
5. Assemble blot directly on anode plate of semi-dry transfer unit as described below. Eliminate air bubbles between gel and membrane with roller or clean pipette.

\begin{center}
\begin{tabular}{c}
\hline
\textbf{(-)} & \textbf{Cathode} \\
\hline
\hline
\textbf{Filter paper (2 sheets)} & \textbf{Gel} \\
\hline
\textbf{Membrane} & \textbf{Filter paper (2 sheets)} \\
\hline
\textbf{(+)} & \textbf{Anode} \\
\end{tabular}
\end{center}

6. Using a semi-dry transfer unit, transfer protein from gel to membrane using continuous voltage of 25V for 7-10 minutes.
7. Remove and rinse membrane with ultrapure water. Proceed with protein detection methods.
Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inefficient transfer</td>
<td>Salt build-up on plate electrodes</td>
<td>Wash surface of both electrodes with deionized water after each use and lightly rub electrode surfaces with gloved hand to dislodge any insoluble salts</td>
</tr>
<tr>
<td></td>
<td>Membrane, filter paper or gel insufficiently equilibrated in Pierce Fast Semi-Dry Buffer</td>
<td>Equilibrate membrane, filter paper and gel in Pierce Fast Semi-Dry Buffer before transfer</td>
</tr>
<tr>
<td></td>
<td>Insufficient transfer time</td>
<td>Increase transfer time from 7-10 minutes to 10-15 minutes</td>
</tr>
<tr>
<td></td>
<td>PVDF membrane was not pre-wet with methanol</td>
<td>Wet PVDF membrane with methanol and then equilibrate for 10-15 minutes in Pierce Fast Semi-Dry Buffer before transfer</td>
</tr>
<tr>
<td>Inconsistent transfer</td>
<td>Air bubbles trapped between gel and membrane</td>
<td>When assembling sandwich use a roller or pipette to remove any air bubbles between the gel and the membrane</td>
</tr>
</tbody>
</table>

Related Thermo Scientific Products

- **88600** Western Blotting Filter Paper, 8 cm × 10.5 cm sheet
- **77012** Nitrocellulose Membrane 0.2 µm, 8 cm × 12 cm
- **88013** Nitrocellulose Membrane 0.2 µm, 7.9 cm × 10.5 cm
- **88024** Nitrocellulose Membrane 0.2 µm, 8 cm × 8 cm
- **88018** Nitrocellulose Membrane 0.45 µm, 30 cm × 3.5 m roll
- **77010** Nitrocellulose Membrane 0.45 µm, 8 cm × 12 cm
- **88014** Nitrocellulose Membrane 0.45 µm, 7.9 cm × 10.5 cm
- **88025** Nitrocellulose Membrane 0.45 µm, 8 cm × 8 cm
- **22860** Low-Fluorescence PVDF Transfer Membrane, 0.2 µm, 7 cm × 8.4 cm
- **88518** PVDF Transfer Membrane, 0.45 µm, 26.5 cm × 3.75 m roll
- **88585** PVDF Transfer Membrane, 0.45 µm, 10 cm × 10 cm

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