Using The Multi-Replica Blotting Kit for Protein Analysis with a Semi-dry Electrophoretic Transfer Apparatus

GENERAL CONSIDERATIONS

Since it was first introduced in the early eighties, semi-dry electrophoretic transfer (semi-dry transfer) of proteins from polyacrylamide gels has become a method of choice for many investigators. Convenience of use and high reproducibility of experiments are among the most attractive features. However, since the strength of the electric field during semi-dry transfer is many fold higher than the field generated in wet transfer set-ups, this method of protein transfer to traditional membrane substrates (nitrocellulose and PVDF membrane) requires a fair amount of optimization (1-3). Use of Multi-Replica Blotting Kit membranes’ (“multi-replica membranes”) with semi-dry transfer adds an additional layer of complexity. These membranes are about 10 fold thinner than traditional membranes and as a result, if exposed to a high electric field for an extended time some of the membranes may be depleted of proteins (FIGURE 2A, B). It is therefore not surprising that it is hard to generate a universal protocol for uniform transfer from gel and binding to Multi-Replica membranes. Variables that will influence the uniformity of the transfer are size of the protein, percentage of gel used, strength of the electric field and the time of the transfer.

One parameter we didn’t evaluate was the effect of the equipment used to transfer proteins from the gel to the membranes. Note that all experiments were done with a 5-membrane stack: at this time we do not recommend the use of a 10-membrane stack for semi-dry transfer.

FIGURE 1. Total protein staining on the Multi-Replica membranes. Samples were eluted in parallel from a 10% gel (left two lanes) and a 4-20% gel (right two lanes) to a single stack of 5 Multi-Replica membranes. Protein sizes are indicated on membrane #1. Lane A had 20 \( \mu \text{g} \) of total protein lysate from Jurkat cells and lane B had 40 \( \mu \text{g} \) of the same sample. The membranes were stained with FastBlue Protein Stain (Chemicon). In the case of both set of samples total variability between membranes was less then 15% in total protein binding.
FIGURE 2. Detection of individual proteins on multiblot membranes by immunoassay. Fifteen µg of total protein lysate from Jurkat cells (lymphatic cell origin; lane 1) and HN12 cells (keratinocyte cell origin; lane 2) were separated in duplicate by PAGE on a 10% gel. Half the gel was transferred with Mini Trans-Blot Transfer Cell (BioRad) and the other half in a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad) onto a 5-membrane stack. Membranes from both transfer types were paired according to membrane number and incubated together in primary antibodies against the proteins indicated. After immunodetection, membranes were stained with FastBlue Protein Stain (Chemicon) to assure that comparable amount of total protein was present on the paired membranes.

PROTOCOL: For transfer of proteins using a Trans-Blot SD Semi Dry Electrophoretic Transfer Cell (BioRad) from 10 and 4-20% gels to a 5-membrane stack

1. PAGE separation of the proteins
Proteins can be separated on number of commercially available precast gels in addition to homemade ones. If the gel used is not 10% or 4-20%, the following transfer protocol may need to be additionally modified. In some cases, running a 10% gel (rather than 4-20%) may give better results after immunodetection.

2. Preparing for the transfer
   • Prepare 200 ml of 1x transfer buffer by mixing 20 ml of buffer stock provided in the kit with 140 ml of ddH2O and 40 ml of 100% methanol. Mix well. (NOTE! this is 10-fold dilution of the stock solution provided with the kit. Wet transfer requires the use of a different dilution of the Transfer Buffer). Divide between two flat trays.
   • Cut 4 pieces of Whatman filter paper for every gel to be transferred. The pieces have to be slightly larger than the gel used.
   • Find a glass or plastic rod of appropriate length to be used in the process of bubble removal.

3. Setting up the transfer
   • Expose the Anode Plate by removing the Safety Lid and Cathode Plate Assembly.
   • Place the 5-membrane stack in one of the trays with the 1X transfer buffer. Remove any bubbles trapped between layers with a gloved finger. NOTE! There is no need for extensive soaking of membranes in transfer buffer.
• Stack two pieces of filter paper and immerse them in buffer until completely wet. Keep them in a vertical position for couple of seconds to drain excess buffer and then place them on the transfer platform. Roll the glass or plastic rod over the stack to remove any bubbles trapped between them.
• Place the membrane stack on top of the filter paper stack. The membrane numbered ‘5’ should be in contact with the filter paper. Roll the glass or plastic rod over the stack to remove any bubbles trapped between them. Remove any excess buffer by blotting the sides of the transfer stack with a paper towel.
• Open the gel casting unit and expose one side of the gel. Cover the gel with 1 piece of DRY filter paper and press gently. Remove from the plate by gently pealing the paper from the top of the gel. Place the gel with its filter paper backing inside the unused tray with transfer buffer and remove any bubbles trapped between the gel and the paper. This can be easily done with a gloved finger. NOTE! The gel should not be equilibrated for more than five minutes in the transfer buffer!
• Place the gel with the paper backing gel-side-down on top of the transfer stack. The membrane numbered ‘1’ should be in contact with the gel. Roll the glass or plastic rod over the stack to remove any bubbles trapped between them. Remove any excess buffer by blotting the sides of the transfer stack with a paper towel.
• Wet 1 piece of filter paper in the transfer buffer and put it on top of the transfer stack. Roll a glass or plastic rod over the stack to remove any bubbles trapped between them. Remove any excess buffer by blotting the sides of the transfer stack with paper towel.
• Repeat all the steps for every additional gel to be transferred. NOTE! The Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad) anode platform can accommodate up to four mini gels (placed side-by-side) at one time!
• Place the Cathode Assembly and Safety Lid back in place and plug the unit into the power supply.

4. Transferring proteins in BioRad Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell
• Run on ‘constant current’ setting. Determine strength of the field and length of the transfer from TABLE 1.

<table>
<thead>
<tr>
<th>GEL PERCENTAGE</th>
<th>NUMBER OF GELS TRANSFERRED</th>
<th>CURRENT DELIVERED(mA)</th>
<th>TIME REQUIRED(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1</td>
<td>100</td>
<td>30-45</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>200</td>
<td>30-45</td>
</tr>
<tr>
<td>10%</td>
<td>3</td>
<td>300</td>
<td>30-45</td>
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<tr>
<td>10%</td>
<td>4</td>
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<td>60</td>
</tr>
<tr>
<td>10%</td>
<td>100</td>
<td>100</td>
<td>30-45</td>
</tr>
<tr>
<td>10%</td>
<td>200</td>
<td>150</td>
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</tr>
<tr>
<td>10%</td>
<td>300</td>
<td>60</td>
<td>30-45</td>
</tr>
<tr>
<td>10%</td>
<td>400</td>
<td>60</td>
<td>30-45</td>
</tr>
<tr>
<td>GEL PERCENTAGE</td>
<td>NUMBER OF GELS</td>
<td>CURRENT DELIVERED(mA)</td>
<td>TIME REQUIRED(min)</td>
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<tr>
<td>----------------</td>
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</tr>
<tr>
<td>4-20%</td>
<td>4</td>
<td>200</td>
<td>60</td>
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</tbody>
</table>

**TABLE 1.** Recommended conditions for the transfer of mini gels in Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell

- After transfer is complete, carefully open the Safety Lid and remove Cathode Assembly.
- Carefully move transfer stack(s) to the flat surface. Make sure that membrane stack and gel stay in close contact. Without removing paper backing from the gel, introduce needle marks at every well.
- Peal off gel with paper backing so as to expose the membrane stack. Use a 23 – 25 g needle to make marks through the prestained protein standard bands. Push the needle through the stack.
- Remove the membrane stack from the paper below and cut the tabs from the frame.
- Place the membrane stack in TBS buffer (50 mM TRIS pH 8.0, 150 mM NaCl) and wash for 5 minutes.
- Place individual membranes on clean filter paper.
- Label individual membranes by using the needle marks as a guide.

**REFERENCES**