

User Tips for Multi-Replica Blotting for Proteins

Updated March 1, 2003

Selecting a protein gel	<i>10% gels allow the transfer of the greatest size range of proteins (at least 30 to 120 kDa). If the gel percentage is higher then the transfer time should be extended. Gradient gels (4-20%) may also be used although our experience with these is limited.</i>
Loading the protein gel	<i>The membrane dimensions have been set so that they can be used with both the Novex and Bio-Rad submarine transfer devices. The Novex device requires use of a smaller mini-gel so Bio-Rad users will often 'lose' a lane as the membranes are a little small. For this reason, use the first and/or last lanes for markers, not for sample intended for immunoanalysis. The membrane dimensions (not including the frame) are 8cm wide by 7 cm long. More of the marker will need to be used than with conventional blots. We recommend 3-5 fold more (e.g., if the normal recommendation is to load 5 μl, we suggest loading between 15 and 25 μl of the marker).</i>
Equilibrating the gel before transfer	<i>It is possible to 'over-equilibrate' the gel. Do NOT soak the gel for more than 15 minutes before transfer. In fact, it is not necessary to equilibrate the gel at all.</i>
Transfer buffer pH	<i>The 1X Transfer Buffer is pH 8.3. As in other Western blotting protocols, proteins with a pI around the pH of the transfer buffer will not move well out of the gel.</i>
Optimizing transfer conditions	<i>Proteins of between 30 and 120 kDa transfer very well under the conditions recommended in the protocol. Even outside this range, begin with standard conditions: there's often no need to optimize. For example EGFR (175 kDa) has been repeatedly transferred using routine voltage and transfer time. As with conventional Western blotting, extending the transfer time (not increasing voltage) might help. Conversely, with very small proteins, reduce transfer time. If the gel percentage is higher than 10% the transfer time will also need to be extended.</i>
Checking transfer efficiency	<i>It is recommended to use the Protein Transfer Standard (PTS) provided with product available from 20/20 GeneSystems. The PTS is a pre-labeled protein that is run on the gel and will show as a blue band in about the center of each membrane if transfer is successful: no staining is required. More details are in the product instruction manual. Total protein staining with Ponceau S will not work with these membranes.</i>
Visualizing the protein markers	<i>NOTE: The marker will be visible on the stack of membranes after transfer. It is usually NOT visible on individual membranes. Circle bands with a ball point pen and/or poke through bands with a 23-25 g needle to visualize on individual blots.</i>

Storing membranes after transfer	<i>Membranes can be stored for at least 6 months at room temperature and longer if refrigerated. After transfer, allow the membrane to air-dry then store it in a plastic bag in a cool dark place. This storage of membranes for later use is helpful if it's not desirable to probe all 5 or 10 membranes from a transfer at the same time. It can also provide a useful way to archive samples for later analysis when other antibodies are available.</i>
Blocking the membrane	<i>It is not necessary to block the membranes; in fact, leaving out the blocking step may result in better signal. If the preference is to block, use casein or BSA at 0.5%. Do not use skim milk. Whether the membranes are blocked or not, it is important that they be thoroughly wetted with TBST prior to immunoanalysis.</i>
Semi-dry transfer and the Multi-Replica Blotting Kit	<i>We have developed a protocol for use with the Bio-Rad semi-dry transfer device and will be pleased to forward this to you. You can also find it on our website as a 'Application Note'.</i>
Immuno-analysis: antibody amounts	<i>Antibody concentrations may need to be adjusted. For many antibodies, the best results are obtained using a 2 to 5-fold higher concentration of the primary antibody than is used with conventional Western blotting. Exposure times (chemiluminescent) should also be adjusted to be 2-5 fold longer.</i>
Immuno-analysis: carrier protein	<i>Although membranes don't need to be blocked, a carrier protein is recommended. The best carrier found to date is Detector Block (KPL). 0.5 % solutions of BSA, casein, or various PVAs (poly vinyl alcohols) have also worked well. Of these we have had the best success with BSA. Skim milk is NOT recommended.</i>
Immuno-analysis: detection	<i>Both chemiluminescent and colorimetric detection are compatible with the Multi-Replica Blotting Kit membranes. Best is to choose a system optimal for the type of detection performed. For chemiluminescent detection, horse radish peroxidase (HRP)-based detection is suggested. A good choice is Amersham's ECL Plus detection reagent. For colorimetric detection, alkaline phosphatase (AP)-bases systems will normally give the best results. DAB reagent tends to leave brown staining pattern on the membrane; membranes after AP staining are clean. Fluorescence-based detection using the dyes Cy3, Cy5 and FITC can also be used although the signals may be weak.</i>