

**In This Section**

### Introduction

Protein transfer efficiency in blotting applications is dependent upon multiple factors, including gel percentage, gel thickness, protein size, transfer conditions (e.g., buffer and voltage), and type and quality of membrane. To achieve optimal transfer efficiency, transfer conditions must be adjusted to address these varying factors.

### Choosing the appropriate membrane

| Nitrocellulose | PVDF | Nylon |
|---|---|---|
| Hydrophobic binding | Hydrophobic binding | Hydrophobic & electrostatic binding |
| General purpose membrane | SDS tolerant | Stable if baked |
| Low background | High background | High background |
| Low strength | High strength | High strength |
| Becomes brittle if baked | Suitable for protein sequencing | Least suitable for Western transfer |

### Transfer solutions

Formula for Towbin transfer solution:

**Towbin Transfer Solution**

| 1X Working Solution | Amount for 1X Working Solution |
|---|---|
| 25 mM Tris base | 30.3 g Tris base |
| 192 mM Glycine | 144.1 g Glycine |
| 0.1% SDS | 10.0 g<br>Adjust volume to 8 liters with distilled water<br>Measure, but do not adjust pH; it should be approximately 8.2 to 8.4 |
| 20% Methanol | 2 L Methanol<br>Adjust volume to 10 liters with distilled water |

It may be necessary to lower the concentrations of methanol, SDS or both to obtain the optimal balance of transfer and binding efficiency. The table below outlines the effects that SDS and methanol have on protein transfer.

| SDS | Methanol |
|---|---|
| Improves transfer of proteins >60 kDa | Improves binding efficiency |
| Decreases binding efficiency | Decreases transfer efficiency |
| Not compatible with nylon membranes | Do not soak gel in transfer buffer prior to blotting |
| Include 0.1% - 0.2% in transfer buffer | Include 20% in transfer buffer |

### Tips for increasing transfer efficiency

— Use the lowest concentration gel that will resolve the protein(s) of interest.

— Avoid using gels ≥1 mm thick. Thick gels may require longer blotting times.

— Decrease the concentration of methanol to optimize transfer efficiency of proteins >150 kDa.

— Small proteins tend to transfer more easily than large proteins. Longer transfer times may be used to ensure complete transfer of large proteins (>60 kDa), proteins from native gels, and thicker gels.

— Use two membranes if transferring for an extended period of time (>1 hour). The second membrane will bind any protein that may transfer through the first. This can be verified by membrane staining.

— Use ProSieve® Color Protein Markers (see page 51) to confirm that transfer has occurred to the membrane and not the filter paper.

— Use a chopping motion when removing the well and foot area. Slicing the gel may cause tearing.

— Gently roll out air bubbles between transfer stack layers using a wet glass rod or pipette.

— If the gel sticks to the filter paper or membrane after transfer, soak for 5 - 15 minutes in water then gently peel the filter paper away.

— If proteins are left in the gel after following recommended transfer conditions, increasing the voltage by no more than 5 volts may be helpful.



Section XI: **Blotting Proteins from Polyacrylamide Gels**

Semi-dry Blotting PAGEr® Gold Precast Gels

This protocol is for use with the BioRad® Trans-Blot® Semi-Dry Cell. If using another manufacturer's blotting apparatus, follow manufacturer's instructions for use.

### Tips for Semi-Dry Blotting

— To prevent the stack from drying out, add extra transfer solution (2 ml - 5 ml) to the top layer of filter paper before closing the lid on the blotting apparatus.

— Center the gel on the membrane. Occasionally, the gel will overlap the membrane and stick to the filter paper below. If this occurs, gently break the seal with a scalpel.

### Procedure for semi-dry blotting

**NOTE:** Nitrocellulose or PVDF membranes are recommended for this method and should be wetted in methanol prior to wetting in transfer solution.

1. Electrophorese gel following standard procedure.
2. Carefully trim off stacking gel and bottom foot from the gel.
3. Soak gel for 20 minutes in chilled 1X transfer solution.
4. Cut the filter paper, blot paper and membrane to the size of the gel.

**NOTE:** Transfer membranes should be handled at the edges with gloves worn.

5. Soak membrane, blotting paper and filter paper for 5 - 10 minutes in 1X transfer solution.
6. To make a semi-dry blotting sandwich, stack in the following order:
   - 6a. Mask
   - 6b. Prewet extra thick blot paper
   - 6c. Prewet nitrocellulose membrane
   - 6d. Polyacrylamide gel.
   - 6e. Prewet Whatman® Grade 114 or 54 Filter Paper
   - 6f. Prewet extra thick blot paper
   - 6g. Top platen (stainless steel cathode), and safety cover
7. Turn on the power and transfer at 25 volts (constant) at 400 mA for:

   60 minutes for 10 kDa - 100 kDa proteins

   90 minutes for 100 kDa - 300 kDa proteins

**NOTE:** Optimized conditions will be required for different proteins or different membranes.

Continued on next page.

- Materials and Reagents
  - Mask
  - A piece of GelBond® Film (see page 61), or similar polyester film, the size of the bottom anode, with a rectangular hole the size of the gel cut out of the center. The purpose of the mask is to focus current through the gel stack.
  - Whatman® Grade 114 or 54 Filter Paper
  - Extra thick blot paper
  - Transfer membrane
  - Scalpel or razor blade
  - Scissors
  - Glass rod or pipette
  - 1XTowbin transfer solution at 4°C
  - Shallow tray for soaking membranes and filter paper

Section XI: **Blotting Proteins from Polyacrylamide Gels**

Tank Blotting PAGEr® Gold Precast Gels

For hydrophobic proteins or proteins >100 kDa, tank blotting is preferable to semi-dry blotting because prolonged transfers are possible without gel drying. For more detailed information and protocols concerning tank blotting, consult the blotting apparatus manufacturer's instructions.

### Procedure for tank blotting

**NOTE:** Transfer is performed at 4°C.

**NOTE:** Nitrocellulose or PVDF membranes are recommended for this method and should be wetted in methanol prior to wetting in transfer solution.

1. Electrophorese gel following standard procedures.
2. Carefully trim off stacking gel and bottom foot from the gel.
3. Soak gel for 20 minutes in chilled 1X Transfer solution.
4. Cut the filter paper, blotting paper and membrane to the size of the gel.

**NOTE:** Transfer membranes should be handled at the edges with gloves worn.

5. Soak fiber pads, nitrocellulose membrane and blotting paper for 2 minutes in 1X Transfer solution.
6. To make a tank blotting sandwich, stack in the following order:
   - 6a. Cathode unit
   - 6b. 1 prewet fiber pad
   - 6c. 1 sheet prewet extra thick blot paper
   - 6d. 1 sheet prewet Whatman® 114 or 54 Blotting Paper
   - 6e. Polyacrylamide gel
   - 6f. Prewet nitrocellulose membrane
   - 6g. 1 sheet prewet extra thick blot paper
   - 6h. 1 prewet fiber pad
   - 6i. Anode unit
7. Place in tank with nitrocellulose membrane closest to anode (+).
8. Cover with chilled 1X Transfer solution.
9. Turn on the power and transfer by running at 100 volts (constant) at 400 mA for:

   90 minutes for 10 kDa–100 kDa proteins

   120 minutes for 100 kDa–300 kDa proteins

**NOTE:** Optimized conditions will be required for different proteins or different membranes.

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## Materials

- Whatman® Grade 114 or 54 Filter Paper
- Extra thick blot paper
- Transfer membrane
- Fiber or foam pad
- Scissors
- Glass rod or pipette
- Scalpel or razor blade
- 1X Towbin transfer solution at 4°C
- Shallow tray for soaking membranes and filter paper

### Monitoring protein transfer

Protein transfer can be monitored by staining the gel following transfer (see page 166), and/or staining the membrane. Membrane staining should only be performed when duplicate samples have been run on a gel and the membrane can be cut in half, or when a second membrane has been used. Stains commonly used for this purpose include SYPRO® Ruby Protein Blot Stain, (see page 55), India ink stain or colloidal gold stain. Described below is the use of the GE Healthcare AuroDye® Forte Kit which is a colloidal gold stain.

## Materials and Reagents

- Previously blotted membrane
- Orbital shaker set to 37°C
- Orbital or rocking platform shaker
- Glass dish
- Thermal seal pouch and sealing unit
- Distilled water
- 1X PBS, 0.3% Tween® 20

### Staining membranes with the GE Healthcare AuroDye® Forte Kit

This method is compatible with PVDF and nitrocellulose membranes. For more detailed information and protocols, consult the instructions provided with the kit.

1. Place the membrane in a clean glass dish.
2. Add 1X PBS, 0.3% Tween 20.
3. Shake slowly on a shaker for 30 minutes at 37°C.
4. Remove solution and replace with fresh 1X PBS, 0.3% Tween® 20.
5. Shake slowly on a shaker for 5 minutes at room temperature.
6. Repeat steps 4 –5 two more times.
7. Briefly rinse the membrane in distilled water.
8. Place the membrane in a thermal seal pouch with 15 ml - 20 ml AuroDye® Forte Kit for 2 - 4 hours at room temperature.
9. After fully developed, rinse briefly with distilled water and air dry.



### References

Ausubel, F.M., *et al., Current Protocols in Molecular Biology,* Wiley & Sons, 1995.
Sambrook, J., *et al., Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

## Notes