Electroblot proteins by wet or semi-dry transfer

TR0073.1

Introduction

Typical Western blotting experiments involve polyacrylamide gel electrophoresis (PAGE) of protein samples followed by transfer of the size-separated proteins from gel to nitrocellulose or PVDF membrane. Bands or spots of specific proteins are then visualized on the membrane surface when probed with antibodies and detected with enzyme-substrate reporter systems.

Three different methods can be used for protein transfer: passive-diffusion blotting, vacuum blotting, and electroblotting. Among these methods, electroblotting is the most popular because it is both faster and more efficient than the others. Electrophoretic transfer also is more quantitative. Proteins are transferred by an electric current passed through the gel.

Wet (tank) transfer and semi-dry transfer have been developed to electrophoretically blot proteins and nucleic acids from gels to membranes. Transfer efficiency in these two variants of electroblotting is dependant upon gel type, membrane type, transfer buffer composition, equilibration time, size of protein(s), transfer temperature, number of gels, and volume of buffer.

The purpose of this Tech Tip is to provide very simple, generalized protocols for wet and semi-dry transfer of proteins from standard mini gels (approx. 10cm × 10cm) using typical commercial transfer devices and power supplies. When possible consult and follow the instructions for the specific equipment you own. Relevant Thermo Scientific™ Pierce™ products are mentioned in each protocol, and a fuller list of these products occurs at the end this document.

Protein blotting using a wet (tank) transfer apparatus

1. Separate the proteins in the sample by gel electrophoresis (e.g., reducing, denaturing SDS-PAGE).
2. Prepare the transfer buffer. Tris-glycine buffer with methanol (25mM Tris, 192mM glycine, pH 8.3, 20% methanol) is the most common choice for wet transfer. Weigh and prepare sufficient Tris and glycine for 500mL; dissolve these components in less than 400mL of water, adjust the pH with concentrated HCl, and bring the volume up to 400mL with water; finally, add 100mL of methanol. Use and store the transfer buffer at 4°C.
3. Dismantle the mini-gel cassette and place the gel in a tray containing 25-50mL of transfer buffer for 10-15 minutes with gentle agitation.
4. Wet two sheets of filter paper, the transfer membrane and the transfer-cassette pads with transfer buffer by submerging them briefly. If using a PVDF membrane, submerge membrane in 100% methanol for about 30 seconds to pre-wet it before placing it in transfer buffer.
5. Construct the gel "sandwich" in the transfer cassette (Figure 1). The gel must be on the cathode (red electrode) side relative to the membrane; the membrane must be on the anode (black electrode) side relative to the gel. When assembling the gel sandwich, avoid air bubbles between any of the layers.
6. Load cassette into wet/tank transfer apparatus. Set power supply at 30V or 100mA and perform an overnight transfer keeping the buffer temperature at 4°C. Alternatively, transfer may be completed in 60-90 minute at 100V or 350mA; however, greater care must be taken to keep the buffer cool (e.g., by performing the procedure in a cold room or using an ice pack). Small molecular weight proteins tend to pass through the membrane and may require shorter transfer times. Consult manufacturer's instructions for recommendations tailored to your transfer unit.
7. Remove the membrane from the transfer unit and rinse it briefly with ultrapure water.
8. Optional: If desired, confirm the efficiency of protein transfer by staining the membrane using a reversible stain that is compatible with subsequent Western blotting, such as Pierce Reversible Stain Kits for Nitrocellulose or PVDF Membranes (Part No. 24580 and 24585, respectively). Alternatively, stain the gel to confirm that most of the original protein has been removed.
9. Proceed with Western blotting steps, beginning with incubation of the membrane in blocking buffer.

   Note: Treatment procedures with certain signal-enhancement reagents (e.g., Part No. 32110, 21050, 46640) must be performed before blocking.

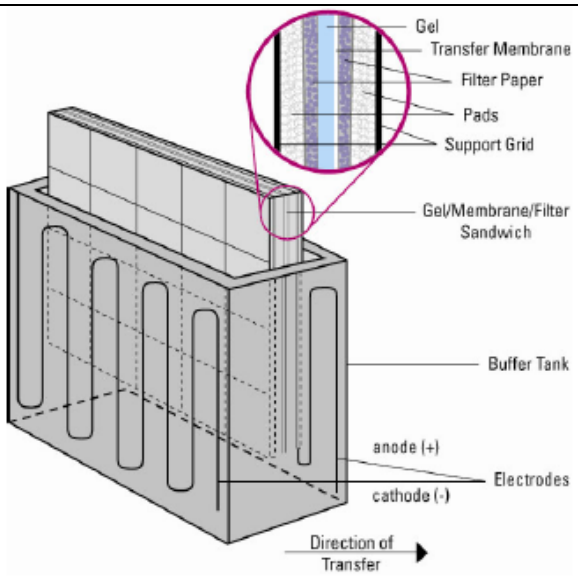
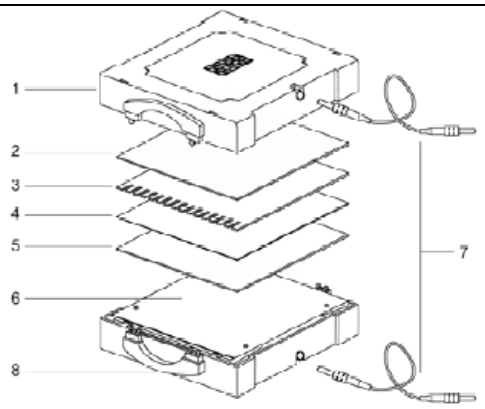


Figure 1. Diagram of a typical wet (tank) transfer assembly. Most wet/tank transfer systems require preparing the sandwich within a plastic cassette. A typical method for assembling the sandwich is as follows: Lay the opened cassette flat on the bench and layer onto one side of the cassette cover each pre-wetted component: (1) pad, (2) paper, (3) gel, (4) membrane, (5) paper, (6) pad. Air bubbles can be removed after addition of each layer by adding a puddle of buffer and using a serological pipet as a roller to push air bubbles outward from the center. Finally, close the cassette (i.e., fold over and latch the opposite cover).

Protein blotting using a semi-dry transfer apparatus

1. Separate the proteins in the sample by gel electrophoresis (e.g., reducing, denaturing SDS-PAGE).
2. For rapid transfer with a semi-dry blotter and capable power supply (e.g., Pierce G2 Fast Blotter, Part No. 62288), use Pierce 1-Step Transfer Buffer (Part No. 84731). Otherwise, use Tris-glycine transfer buffer with 20% methanol (see Step 2 of the Wet Transfer protocol). Use and store the transfer buffer at 4°C.
3. Construct a gel "sandwich" (Figure 2), using extra-thick (~3mm) filter paper (or several sheets of regular filter paper). The membrane must be on the anode (bottom or black electrode) side relative to the gel.
4. Close the semi-dry transfer apparatus and connect the electrodes to an appropriate power supply.
5. If using the Pierce 1-Step Transfer Buffer, apply a continuous voltage of 25V for 5-10 minutes to complete the transfer. If using traditional Tris-glycine methanol buffer, apply a continuous voltage of 25V for 15-60 minutes.
6. Remove the membrane from the transfer unit and rinse it briefly with ultrapure water.
7. Optional: If desired, confirm the efficiency of protein transfer by staining the membrane using a reversible stain that is compatible with subsequent Western blotting, such as Pierce Reversible Stain Kits for Nitrocellulose or PVDF Membranes (Part No. 24580 and 24585, respectively). Alternatively, stain the gel to confirm that most of the original protein has been removed.
8. Proceed with Western blotting steps, beginning with incubation of the membrane in blocking buffer.

   Note: Treatment procedures with certain signal-enhancement reagents (e.g., Part No. 32110, 21050, 46640) must be performed before blocking.



1. Upper lid, spring-loaded cathode plate electrode
2. Filter paper (two sheets)
3. Gel
4. Membrane
5. Filter paper (two sheets)
6. Spring-loaded anode plate electrode
7. Electrical cable
8. Lower base
9. Gel-support frame

Figure 2. Diagram of a typical semi-dry transfer assembly. This is an example of a non-powered blotter whose electrical cables must be connected to a power supply. Newer devices, such as the Pierce G2 Fast Blotter (Part No. 62288), have integrated cassette and power control units that provide convenience and optimal performance for rapid transfer protocols.

Related Thermo Scientific Products

62288	Pierce G2 Fast Blotter, powered semi-dry transfer device
84731	Pierce 1-Step Transfer Buffer, 1L
28363	Pierce Tris-Glycine Buffer, 10X
28380	BupH™ Tris-Glycine Transfer Buffer Packs, 40 packs, each makes 500mL
35045	Pierce Western Blot Transfer Buffer, Methanol-free, 10X
84783	Western Blotting Filter Paper, 7cm x 8.4cm (several sizes available)
37570	Pierce Protein-Free (TBS) Blocking Buffer, 1L
37539	StartingBlock™ T20 (PBS) Blocking Buffer, 1L
37516	SuperBlock™ T20 (PBS) Blocking Buffer, 1L
37525	Blocker™ BSA in PBS (10X), 1L
77010	Nitrocellulose Membrane, 0.45µm, 8 × 12cm, 25 sheets/pkg. (several sizes available)
88585	PVDF Transfer Membrane, 0.45µm, 10 × 10cm, 10 sheets/pkg. (several sizes available)
32110	Pierce Antibody Extender Solution NC, 500mL
21050	Pierce Western Blot Signal Enhancer, 10-blot kit
46640	SuperSignal Western Blot Enhancer, 25-blot kit

Current versions of product instructions are available at *www.thermoscientific.com/pierce*.. For a faxed copy, call 800-874-3723 or your local distributor.