INSTRUCTIONS

Nitrocellulose Transfer Membranes

0391.5

Number	Description
77010	**Nitrocellulose Membrane, 0.45µm, 8 × 12cm,** 25 each
77012	**Nitrocellulose Membrane, 0.20µm, 8 × 12cm,** 25 each
88013	**Nitrocellulose Membrane, 0.20µm, 7.9 × 10.5cm,** 15 each
88014	**Nitrocellulose Membrane, 0.45µm, 7.9 × 10.5cm,** 15 each
88015	**Nitrocellulose Membrane, 0.45µm, 7.9 × 10.5cm,** 5 each
88018	**Nitrocellulose Membrane, 0.45µm, 30cm × 3.5m,** 1 roll, ≥ 84 sheets when cut to 7.9 × 10.5cm, or ≥ 52 sheets when cut to 11.5 × 12.5cm
88024	**Nitrocellulose Membrane, 0.20µm, 8 × 8cm,** 15 each
88025	**Nitrocellulose Membrane, 0.45µm, 8 × 8cm,** 15 each

Storage: Store membranes flat at ambient temperature, away from chemical vapors. Some solvent vapors may partially dissolve the membranes, which will disrupt the pore structure. Keep membranes out of direct sunlight. Sunlight may cause the nitrocellulose to dry and become brittle.

Note: Nitrocellulose membrane filters are highly flammable. Keep away from heat and open flame. Flash point is approximately 200°C. Membranes can be sterilized by steam-autoclaving at 121°C.

Introduction

Nitrocellulose, also known as cellulose nitrate, consists of purified cellulose that is converted to an ester with three nitrate groups per glucose molecule. During membrane preparation, the cellulose nitrate is dissolved in a mixture of organic solvents. The solution is then poured on a smooth surface forming a thin film. The pore size of the membrane is controlled by the rate of evaporation of the solvent mixture used.

The Thermo Scientific Nitrocellulose Membranes have high binding affinity for proteins and are ideal for Western blotting applications, as they are compatible with a variety of detection methods (e.g., chemiluminescent, chromogenic, fluorescent). Some nitrocellulose membranes contain cellulose acetate, which inhibits binding; however, Pierce membranes contain 100% nitrocellulose for excellent binding, biological activity and sensitivity.

Example Procedure for Transferring Proteins to a Nitrocellulose Membrane

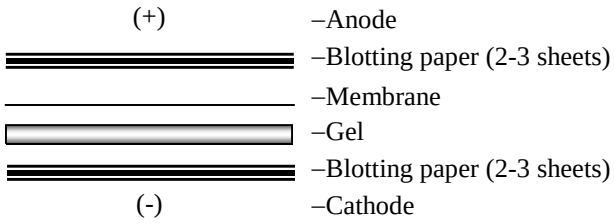
Note: Use clean and dry scissors to cut membrane to the size of the gel. Any small tear may result in a larger tear. Always wear gloves when handling membranes because oils from fingers may prevent proper wetting and proteins from hands may bind to the membrane causing background.

A. Materials required

- Tank transfer system with power supply
- Blotting paper (Product No. 88600)
- Methanol or ethanol
- Thermo Scientific BupH Tris-Glycine Transfer Buffer (Product No. 28380) or other suitable transfer buffer [e.g., 5.8g Tris base, 2.9g glycine, 0.4g SDS (optional), 200mL methanol or ethanol (100%), pH 8.5]. Adjust volume to 1L with ultrapure water. Allow buffer to cool overnight at 4°C.

B. Method

1. Remove gel from the electrophoresis unit and equilibrate in cold Transfer Buffer for 10-30 minutes with gentle shaking. Incubation time is based on a 1.5mm thick gel. Thinner gels will require less incubation time.
2. Cut membrane to the dimensions of the gel. Cut a notch in the membrane corner to correspond to a corner of the gel.
3. Place membrane in container with cold Transfer Buffer until ready to use.
4. Wet the absorbent blotting paper in Transfer Buffer.
5. Use the following component order to form the transfer stack:



6. Ensure that there are no bubbles between the gel and the membrane that would prevent proper transfer.
7. Connect the leads and perform transfer for 45-90 minutes at 0.8mA/cm^2 of gel.

   Note: Transfer time and efficiency will vary depending upon polyacrylamide concentration, gel thickness, the presence of SDS or organic solvents, pH and ionic strength of the transfer buffer and the molecular weight of the protein. Empirically determine optimal transfer conditions.
8. When the transfer is complete, disconnect leads and disassemble the transfer stack to remove the membrane.
9. Keep membrane moist until ready to use. To evaluate transfer efficiency, stain the membrane with Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes (Product No. 24580).

Related Thermo Scientific Products

88600	**Western Blotting Filter Paper, 8cm × 10.5cm,** 100 sheets
34080	**SuperSignal® West Pico Chemiluminescent Substrate,** 500mL
34075	**SuperSignal West Dura Extended Duration Substrate,** 100mL
34095	**SuperSignal West Femto Maximum Sensitivity Substrate,** 100mL
21059	**Restore™ Western Blot Stripping Buffer,** 500mL
34090	**CL-XPosure™ Film (5" × 7"),** 100 sheets
34091	**CL-XPosure Film (8" × 10"),** 100 sheets
21065	**Pierce Background Eliminator Kit**

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

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce