Nitrocellulose Membrane (NC)

N766354 N766340 N766351 N766329

Store at room temperature.

Introduction:

The NC transfer membrane is a nitrocellulose microporous membrane, which is used to transfer proteins from various gel matrices. Compared with the PVDF membrane, the NC membrane is a hydrophilic membrane and does not require pre-treatment for hydrophilicity. This hydrophilic membrane has two pore size models of 0.45μm and 0.22μm, and is capable of binding proteins with a wide range of molecular weights. The 0.45μm pore size is suitable for most proteins and has high detection sensitivity. The 0.22μm pore size is suitable for proteins with a molecular weight of less than 20kDa, and can better adsorb and retain small molecular proteins. The PVDF from Aladdin has excellent protein retention rate, high physical strength and broad chemical compatibility, making it well applied in immunodetection.

This product is compatible with a variety of dyes, such as Ponceau-S red, Amido black, CPTS, Colloidal gold, and India ink. Among them, after staining with dyes such as Ponceau-S red and CPTS, the dyes can be washed off, and the membrane can be used for further analysis; while dyes such as Amido black and India ink are irreversible, and the membrane cannot be used for further analysis after staining.

This nitrocellulose membrane is suitable for detection methods such as chemiluminescence (such as ECL, etc.), conventional color development (such as DAB, TMB), staining, isotope and fluorescence.

Usage method:

I. Membrane Wetting

1. Wet the dry membrane in methanol for 10-20 seconds, or until it changes from an opaque white to a uniformly translucent gray.
2. Immerse the membrane in ultrapure water for 1-2 minutes to displace the alcohol.
3. Equilibrate the membrane in the transfer buffer for 2-3 minutes or until it is ready for use.
4. Note: Once the membrane is wet, do not let it dry. It can be kept in the buffer until the protein transfer. If the membrane dries (turns opaque white), even partially, it must be wetted again (steps 1-3).

II. Semi-dry Transfer

1. Disperse the protein mixture on the polyacrylamide gel.
2. Immerse the gel in the transfer buffer and equilibrate it for 10-15 minutes.

3. Assemble the transfer stack according to the instructions of the transfer device manufacturer.
4. Note: To ensure uniform transfer, carefully roll a clean pipette or blotting roller over the surface of each layer in the stack. Do not apply excessive pressure, as this may damage the gel and the membrane.
5. Transfer the proteins according to the instructions of the transfer device manufacturer.
6. Remove the blot from the transfer system and briefly rinse the membrane in ultrapure water to remove gel debris. The blot can be air-dried for storage or used immediately for the immunodetection step.
7. Note: Drying the blot before immunodetection may enhance the binding of certain proteins and reduce the background color.

III. Standard Immunodetection

1. If the blot is dry, re-wet it in methanol for 15 seconds or until it changes from an opaque white to a translucent gray.
2. Rinse the blot in ultrapure water for 1 minute.
3. Place the blot in the blocking buffer and incubate it for 1 hour with gentle stirring. Dilute the primary antibody in a commercial antibody diluent, washing buffer, or blocking buffer.
4. Place the blot in the diluted primary antibody solution and incubate it at room temperature for 1 hour with gentle stirring (or incubate overnight at 4°C).
5. Wash the blot 3-5 times with the washing buffer (buffer with Tween-20 surfactant (TBST or PBST) for 5 minutes each time. Prepare a commercial antibody diluent and dilute the secondary antibody with the washing or blocking buffer.
6. Place the blot in the diluted enzyme-labeled secondary antibody solution and incubate it at room temperature for 1 hour.
7. Wash the blot 3-5 times with the washing buffer for 5 minutes each time.
8. Place the blotting membrane in a clean container and add the appropriate detection reagent.
9. Incubate for 1-5 minutes according to the instructions of the detection reagent manufacturer.
10. For HRP or AP chemiluminescent reagents, expose the blot to X-ray film or acquire an image using a digital imaging system. For colorimetric detection, add the reagent and wait for the signal to appear.

Matters needing attention:

1. Use blunt forceps to prevent damage to the membrane.
2. During cutting or handling, keep the protective paper (light blue paper) together with the membrane, but the protective paper should be discarded when wetting the membrane.
3. Handle with care to avoid scratches on the surface of the membrane, and do not fold the membrane.

4. The hydrophilic NC transfer membrane does not need to be wetted in advance. Once the membrane is wetted, it will change from opaque to translucent. After the protein transfer, wash the blot with ultrapure water to remove residual debris.
5. The blotting membrane piece can be air-dried and stored at 4°C for several months (for future use), or it can be used immediately.