NC Membrane, 0.45 μm

Introduction

Nitrocellulose (NC) transfer membrane is widely used for protein and nucleic acid detection in research and medical diagnostics, and is one of the classic blotting supports. NC membrane offers advantages such as relatively high protein binding capacity, no pre-activation requirement, and low background, making it a common choice for routine Western blot applications. It is suitable for various applications including Western blotting, Northern blotting, Southern blotting, and dot/slot blotting.

NC membrane is inherently hydrophilic and can be directly wetted in transfer buffer without the need for pre-wetting with organic solvents such as methanol, offering ease of operation and relatively improved safety. Its protein binding capacity is typically approximately 0.08–0.11 mg/cm² (80–110 μg/cm², exact values vary slightly by brand and model), with binding mechanisms primarily involving hydrophobic interactions and electrostatic forces, and minimal further perturbation to protein spatial conformation. NC membrane generally exhibits clean background and high signal-to-noise ratio, making it suitable for routine single-use Western blot detection, as well as various colorimetric, chemiluminescence, or fluorescence detection systems. The disadvantages of NC membrane include becoming brittle after drying, lower mechanical strength and chemical stability compared to PVDF membrane, poor tolerance to many organic solvents, and unsuitability for frequent or vigorous stripping and multiple rounds of reprobing.

NC membranes are commonly available in 0.22 μm and 0.45 μm pore sizes: the 0.22 μm pore size is more suitable for small molecular weight proteins (typically < 20 kDa), while the 0.45 μm pore size is recommended for most routine proteins (> 20 kDa). Compared with PVDF membranes, NC membranes are more economical and represent a cost-effective choice that balances cost and operational convenience.

Components and storage

Components	Size 1	Size 2	Size 3
NC Membrane, 0.45 μm	1 roll (30 cm x 300 cm)	20 sheets (6.5 cm x 8.5 cm)	100 sheets (6.5 cm x 8.5 cm)
Shipping: Room temperature		**Shelf life: 2 years at 10°C to 25°C**	

Protocol

1. Pre-transfer Preparation

a. Cutting: Cut the NC membrane to the same size as the gel, and clip a small notch in one corner of the membrane to align with the corresponding corner of the gel (for orientation and side identification).

b. Pre-wetting the membrane: Place the cut NC membrane into a container filled with pre-chilled transfer buffer and soak until ready for use.

c. Wetting the filter paper: Thoroughly wet the thick blotting paper with transfer buffer.

2. Transfer Sandwich Assembly (Wet Transfer)

Assemble the transfer "sandwich" in the following order from anode to cathode:

(+) Anode

Filter paper (2–3 layers, pre-soaked with transfer buffer)

NC membrane (equilibrated)

Gel

Filter paper (2–3 layers, pre-soaked with transfer buffer)

(-) Cathode

*Note: After placing each layer, gently roll over with a glass rod or roller to thoroughly expel any air bubbles between layers; trapped bubbles will cause localized transfer failure.

3. Electrotransfer (Membrane Transfer)

a. Place the assembled transfer sandwich into the electrotransfer tank and add sufficient transfer buffer.

b. Connect the electrodes and perform the transfer at a constant current of 0.8 mA/cm² (based on gel area) for 45–90 minutes.

c. After transfer is complete, disconnect the electrodes, disassemble the transfer sandwich, and carefully remove the NC membrane with forceps.

***Note:** Transfer time and efficiency vary depending on polyacrylamide concentration, gel thickness, presence or absence of SDS and methanol, pH and ionic strength of transfer buffer, and molecular weight of the target protein. Optimal transfer conditions should be determined experimentally. In general, larger proteins (> 100 kDa), higher percentage gels, or thicker gels require extended transfer time; SDS facilitates migration of large proteins from the gel, but high concentrations may reduce protein binding efficiency to the membrane; methanol promotes protein binding to the membrane but simultaneously shrinks gel pores, hindering migration of large proteins; the Tris-Gly buffer system at pH 8.3 is routinely used for transfer. Transfer conditions should be optimized according to the specific experimental system.

4. Post-transfer Processing

a. Turn off the power, disassemble the transfer sandwich, and carefully remove the NC membrane.

b. Keep the membrane wet until subsequent blocking, antibody incubation, and other steps.

c. (Optional) To evaluate transfer efficiency before blocking, the NC membrane may be stained with Ponceau S or other stains to check for complete protein transfer.

Note

1. This product is for scientific research use only.

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