Western Blot Transfer Buffer (Standard, Powder)

Introduction

Western blot transfer buffer is a core reagent used in Western blot experiments to transfer proteins from polyacrylamide gels to solid-phase membranes (such as PVDF or nitrocellulose membranes). Its working principle is based on electroblotting technology: under the influence of an electric field, negatively charged proteins migrate out of the gel and are adsorbed onto the surface of the solid-phase membrane, forming a "blot" that faithfully reflects the protein distribution pattern within the gel.

This product is a standard basic-type transfer buffer, suitable for routine protein transfer. Compared with rapid transfer buffer (wet transfer time ~20–25 min, 400 mA) and ice-free rapid transfer buffer (wet transfer time ~15–40 min, 400 mA, minimal temperature rise during transfer, no ice bath required), this product requires longer transfer time under conventional conditions, but offers milder conditions and broader applicability. It is recommended to perform wet transfer at 4°C to minimize protein degradation and band diffusion.

This product features the classic Towbin formulation (Tris-Glycine system) and is supplied as a ready-to-use powder, free of methanol and SDS. Each pouch prepares 1 L of 1× working solution (200 mL methanol needs to be added before use) and remains stable for 2 years when stored at room temperature.

Protocol

1. Preparation of Transition Buffer (1×)

    a. Transfer the entire contents of one pouch into a clean beaker.

    b. Add approximately 600 mL of deionized water or double-distilled water and stir to dissolve.

    c. Bring the volume to 800 mL with additional water and mix thoroughly.

    d. Immediately before use, add 200 mL of methanol (guaranteed reagent or analytical grade) and mix well to obtain 1 L of 1× transfer buffer.

***Note:** Guaranteed reagent (GR) grade methanol is recommended for optimal transfer results; analytical reagent (AR) grade methanol is also acceptable.

    e. Adjustment (optional): For target proteins with high molecular weight (>150 kDa) or strong hydrophobicity, SDS may be supplemented to a final concentration of 0.025–0.1% to enhance transfer efficiency.

2. Transfer Procedure (Wet Transfer)

    a. Preparation: Pre-chill the prepared transfer buffer to 4°C (refrigerator or ice bath).

b. Equilibration: After electrophoresis, equilibrate the gel in transfer buffer for 5 min × 3 times. PVDF membranes should be pre-wetted with methanol for 15–30 seconds, rinsed with deionized water, and then equilibrated in transfer buffer for 5 min; NC membranes can be equilibrated directly in transfer buffer.

c. Assembly of the transfer "sandwich" (from anode to cathode):

(+) Anode (red plate)

- Filter paper (2–3 layers, pre-soaked in transfer buffer)
- PVDF membrane (equilibrated)
- Gel (equilibrated)
- Filter paper (2–3 layers, pre-soaked in transfer buffer)

(–) Cathode (black plate)

*Note: After placing each layer, gently roll with a glass rod or roller to thoroughly remove any trapped air bubbles between layers, as bubbles can cause localized transfer failure. The gel should face the cathode (black side) and the membrane should face the anode (red side).

d. Transfer: Transfer the assembled "sandwich" to the electrotransfer tank and add pre-chilled transfer buffer to completely immerse the assembly. The recommended conditions are constant current 200–400 mA or constant voltage 80–100 V for 60–120 min, with the exact duration adjustable according to the molecular weight of the target protein.

***Note:** The entire transfer process must be performed at 4°C (e.g., in a cold room or with an ice bath surrounding the transfer tank) to effectively dissipate the heat generated during electrotransfer, prevent protein degradation, and ensure optimal transfer results.

e. Post-transfer processing: Remove the membrane. Ponceau S staining may be used to preliminarily confirm transfer efficiency before proceeding to blocking and subsequent antibody incubation steps.

Note

1. Storage and shipping conditions: Store at room temperature; stable for 2 years. Ship at room temperature.
2. This product is for scientific research use only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com