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Protocol

Cat. No. H2029

Western Blot Transfer Buffer (Rapid, 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 rapid-type transfer buffer, compatible with Tris-Glycine and Bis-Tris transfer buffer systems. It significantly shortens wet transfer time while maintaining high transfer efficiency, and the cooling ions in the buffer effectively reduce heat generation during the electrotransfer process, making it suitable for routine and medium-to-high-throughput wet transfer applications. Distinguished from standard transfer buffers (transfer time 60–120 min, 200–300 mA, ice-bath required) and ice-free rapid transfer buffers (transfer time approximately 15–40 min, 400 mA, minimal temperature rise during transfer, no cooling measures required), this product enables rapid transfer at room temperature in 20–25 min (at constant current 400 mA), striking an optimal balance between speed and operational convenience, and is effective for simultaneous transfer of proteins ranging from 10 to 245 kDa.

This product is supplied as a ready-to-use powder formulation, featuring an optimized Tris-Glycine system, free of SDS and methanol (anhydrous ethanol is used as a substitute during preparation). Each pouch prepares 1 L of 1× working solution 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 anhydrous ethanol (or 210 mL of 95% ethanol) and mix well to obtain 1 L of 1× transfer buffer.

***Note:** Analytical grade or higher purity ethanol is recommended.

   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: Precool the prepared membrane buffer to 4°C (in a refrigerator or ice bath).

b. Equilibrium treatment: After electrophoresis is complete, balance the gel in the transfer buffer for 5 minutes × 3 times. PVDF membranes should first be soaked in methanol for 15-30 seconds, then rinsed with deionized water, and finally balanced in the transfer buffer for 5 minutes; NC membranes can be directly balanced in the transfer buffer solution.

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 each layer, gently press with a glass rod or roller to thoroughly remove air bubbles between layers; otherwise, bubbles may cause local transfer failure. The gel is near the anode (black side), and the membrane is near the positive electrode (red side).

d. Transfer: Transfer the assembled "sandwich" to the electrotransfer tank and add transfer buffer to completely immerse the assembly. The recommended condition is constant current 400 mA for 20–25 min.

***Note:**

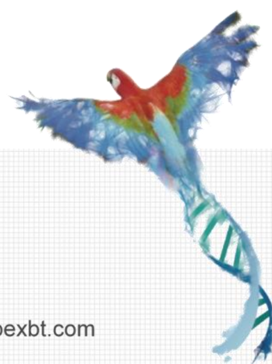
1. For special proteins or experimental conditions, the following adjustments may be made: (1) For target proteins >150 kDa, reduce anhydrous ethanol to 10% (approximately 100 mL) and extend transfer time; (2) For proteins with high molecular weight or strong hydrophobicity, supplement SDS to a final concentration of 0.025–0.1%; (3) For 1.5 mm thick gels, extend transfer time accordingly.
2. During transfer, due to variations in power supply brands and models, some power supplies may trigger overload protection when set to constant current 400 mA. In such cases, reduce the current appropriately and extend the transfer time, or use a power supply with higher capacity.
3. If the ambient temperature is excessively high or the transfer time exceeds 45 min, place an ice pack in the tank to maintain optimal conditions.

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 does not contain SDS or methanol.
3. This product is compatible with Tris-Glycine and Bis-Tris transfer buffer systems.

4. This product is for scientific research use only.



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