Rapid Transfer Buffer (10X) (Powder)

User Manual

Catalog # CRG1040

Quick, efficient transfer of proteins from SDS-PAGE gels to membranes for Western blotting applications

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Cohesion Biosciences' Rapid Transfer Buffer is a simple one-component system for quick, efficient transfer of proteins from SDS-PAGE gels to membranes for Western blotting applications. Transfer is completed in 10 to 30 minutes using a standard semi-dry or wet transfer apparatus, respectively. Dedicated, expensive transfer equipment is not needed. The transfer efficiency is equivalent to that observed when using a Tris-Glycine-Methanol transfer buffer.

Rapid Transfer Buffer is a methanol-free, non-hazardous formulation that is compatible with both PVDF and nitrocellulose membranes. It works well with most gel types, including Laemmli, pre-cast, and others.

Product Characteristics

※ Fast, efficient transfer in 10 - 30 minutes

※ Compatible with standard wet and semi-dry transfer equipment

※ Methanol-free, non-hazardous formulation

※ Transfers to PVDF and nitrocellulose from poured or pre-cast gels

II. PREPARATION

Stock Solution:

1L size: Dissolve the powder in 1L deionized water for Rapid Transfer Buffer (10X).

100 ml size: Dissolve the powder in 100 ml deionized water for Rapid Transfer Buffer (10X).

Working Solution:

Prepare 1 L of Rapid Transfer Buffer (1X) by diluting 100 mL of Rapid Transfer Buffer (10X) with 800 mL of deionized water, then add 100 ml Ethanol.

III. ASSAY PROCEDURE

Wet Transfer Protocol

1. Prepare membrane and filter paper for transfer:

a. A blotting membrane and 3 pieces of filter paper should be cut to fit dimensions of the gel.

Note: PVDF membranes must be pre-wetted according to the manufacturer's instructions in 100% methanol prior to equilibration in transfer buffer.

b. Equilibrate the membrane and filter paper in 1X Rapid Transfer Buffer for a minimum of 5 minutes.

2. Following protein electrophoresis, assemble the blotting sandwich following the manufacturer's instructions for the transfer apparatus.

3. Place the blotting sandwich in a wet transfer tank filled with 1X Rapid Transfer Buffer.

4. Transfer constant current 300 - 400 mA at room temperature.

Current	Time
300 mA	25 to 30 min
350 mA	20 to 25 min
400 mA	15 to 20 min

Semi-Dry Transfer Protocol

1. Prepare membrane and filter paper for transfer:

a. A blotting membrane and 3 pieces of filter paper should be cut to fit dimensions of the gel.

Note: PVDF membranes must be pre-wetted according to the manufacturer's instructions in 100% methanol prior to equilibration in transfer buffer.

b. Equilibrate the membrane and filter paper in 1X Rapid Transfer Buffer for a minimum of 5 minutes.

2. Following electrophoresis, wash the gel for 2 minutes in deionized water.

3. Pre-equilibrate the gel in 1X Rapid Transfer Buffer for 5 minutes.

4. Assemble the blotting sandwich following the manufacturer's instructions for the semi-dry transfer apparatus.

5. Transfer constant current 300 - 400 mA at room temperature.

Current	Time
300 mA	25 to 30 min
350 mA	20 to 25 min
400 mA	15 to 20 min

Note:

a. For normal PAGE gel, if the molecular weight of the protein is greater than 150 kDa, the transfer time needs to be extended by 5-10 min.

b. For the thicker PAGE gel, such as 1.5 mm thick gels, the transfer time can be extended by 5-10min

c. If use higher current, it is recommended to add ice packs to cool down the buffer.

IV. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Low transfer efficiency	• Insufficient transfer time	Not all proteins transfer at the same rate and efficiency. If needed, transfer time may be increased; empirical testing will be required to determine non-standard protocol conditions.
	• Inadequate equilibration of membrane and/or filter paper in transfer buffer	Completely cover membrane with transfer buffer and incubate for 5 minutes with gentle agitation for best results.
	• PVDF membrane not pre-wetted	Use methanol to wet entire PVDF membrane. Slowly add deionized water (to avoid air bubbles on the membrane). Transfer membrane to Rapid Transfer Buffer for equilibration.
Uneven transfer	• Incomplete contact between membrane and gel	Always take care to roll out air bubbles between the membrane and gel in the transfer sandwich.
	• Incomplete hydration of membrane	Pre-wet the membranes per the manufacturer's instructions.
Poor transfer of protein	• Inefficient binding of some proteins to membrane	Reduce Transfer time to avoid low molecular weight proteins passing through the membrane. Increase transfer time for high molecular weight proteins.
Equipment error	• Incompatible transfer apparatus	Rapid Transfer Buffer does not require, and is not compatible with specialized transfer devices. Use Rapid Transfer Buffer with a standard semi-dry or wet transfer apparatus.
	• Incompatible power supply	The optimal power supply should be capable of running at constant voltage with specifications of 5 - 250V, 0.01 - 3.0A, and 1 - 300W.

V. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VI. NOTES