Transfer Buffer Formulations

Protocol

Bulletin 6211

Transfer Buffer Formulations

The following buffers are recommended for use with all of Bio-Rad's electrophoretic transfer cells. Care should be taken when preparing these buffers because incorrect formulation can result in a current that exceeds the recommended conditions.

TIPS

Use only high-quality, analytical grade methanol. Impure methanol can increase transfer buffer conductivity and yield a poor transfer.

In many cases, ethanol can be substituted for methanol in the transfer buffer with minimal impact on transfer efficiency. Check this using your samples.

Do not reuse transfer buffer since the buffer will likely lose its ability to maintain a stable pH during transfer.

Do not dilute transfer buffers below their recommended levels since this decreases their buffering capacity.

Do not adjust the pH of transfer buffers unless specifically indicated. Adjusting the pH of transfer buffers can result in increased buffer conductivity, manifested by higher initial current output and decreased resistance.

Increasing SDS in the transfer buffer increases protein transfer from the gel but decreases binding of the protein to nitrocellulose membrane. PVDF membrane can be substituted for nitrocellulose when SDS is used in the transfer buffer.

Addition of SDS increases the relative current, power, and heating during transfer, and may also affect antigenicity of some proteins.

Increasing methanol in the transfer buffer decreases protein transfer from the gel and increases binding of the protein to nitrocellulose membrane.

Towbin Buffer, 1 L

25 mM Tris, 192 mM glycine, 20% (v/v) methanol (pH 8.3) (catalog #1610734, without methanol, 1 L, 10×)

Tris base	3.03 g
Glycine	14.4 g
diH_2O	500 ml
Methanol	200 ml

Adjust volume to 1 L with diH_2O.

The pH will range from pH 8.1 to 8.5 depending on the quality of the Tris, glycine, methanol, and diH_2O.

Towbin Buffer with SDS, 1 L

25 mM Tris, 192 mM glycine, 20% (v/v) methanol, 0.025–0.1% SDS (pH 8.3)

Add 2.5–10 ml 10% SDS to 1 L buffer prepared above.

Bjerrum Schafer-Nielsen Buffer, 1 L

48 mM Tris, 39 mM glycine, 20% methanol (pH 9.2)

Tris base	5.82 g
Glycine	2.93 g
diH_2O	500 ml
Methanol	200 ml

Adjust volume to 1 L with diH_2O.

Bjerrum Schafer-Nielsen Buffer with SDS, 1 L

48 mM Tris, 39 mM glycine, 20% methanol, 1.3 mM SDS (pH 9.2)

Add 0.0375 g SDS (or 3.75 ml 10% SDS) to 1 L buffer prepared above.

CAPS Buffer, 1 L

10 mM 3-(cyclohexylamino)-1-propanesulfonic acid, 10% methanol (pH 11.0)

CAPS	2.21 g
diH_2O	500 ml
Methanol	100 ml

Adjust volume to 1 L with diH_2O.

Measure the pH and adjust as needed with NaOH.

Dunn Carbonate Buffer, 1 L

10 mM $NaHCO_3$, 3 mM Na_2CO_3, 20% methanol (pH 9.9)

$NaHCO_3$	0.84 g
Na_2CO_3 (anhydrous)	0.318 g
diH_2O	500 ml
Methanol	200 ml

Adjust volume to 1 L with diH_2O.

0.7% Acetic Acid

Add 7 ml glacial acetic acid to 993 ml diH_2O.

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This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).

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