Transfer Buffers and General Tips on Blotting Procedures

Transfer Buffer for Semi-Dry Blotting

In semi-dry blotting systems both, continuous buffer systems (identical buffers at the anode and cathode) as well as discontinuous buffer systems (different buffers at the anode and cathode), can be used. The transfer performance of discontinuous systems is higher as a rule as the two buffers are prepared separately according to the needs of the two electrodes.

We recommend the following discontinuous buffer system

ROTI®Blot 1 – for standard proteins

The system consists of two different buffers, anode buffer A (pH 7.8±0.1) and the cathode buffer K (pH 8.5±0.1). The blotting stack is set up by impregnating the top blotting papers with cathode buffer and the bottom ones with anode buffer. Detailed instructions for use can be found alongside the products on the Internet.

Continuous Tris-glycin buffer acc. to Bjerrum

48 mM Tris, 39 mM glycin, 0-20 % methanol, pH approx. 9.2 (do not adjust!)
(acc. to **Bjerrum** and Schaefer-Nielsen (1986))

This buffer may be used with or without additional SDS (0.01-0.1 %). Often, 0.0375 % SDS are added. May also be used for blotting of native proteins (with ~0.04 % SDS and 0-10 % methanol).

Formulation: Stock solution 1 litre 10 x buffer:

58.15 g TRIS (base)

29.3 g glycin

dissolve in 600 ml twice distilled water

optional: add 5-50 ml SDS solution 20 %

fill to 1000 ml with $H_2O_{tw.dist}$

1 litre working solution is prepared freshly using

100 ml 10 x stock solution

+ 700-800 ml $H_2O_{tw.dist}$

+ 100-200 ml methanol

Continuous CAPS buffer

Recommended for blotting of basic proteins and for blotting prior to N-terminal sequencing.

CAPS stock solution: Dissolve 2.2 g CAPS (3-[cyclohexylamino]-1 propane sulfonic acid) in 90 ml distilled water

If necessary, adjust pH to 10.5-11.0 with NaOH and fill to 100 ml with distilled water. Store at +4 °C.

Transfer buffer: To each 10 ml CAPS stock solution add 80 ml of distilled water and 10 ml of methanol.

Mix well. Cool to 4 °C prior to use.

Transfer Buffer for Tank Blotting

Only continuous buffer systems may be used in tankBlotting. The best-known buffer is Towbin buffer. Alternatives are Bjerrum or Dunn buffers.

Tris-glycin buffer acc. to Towbin

In the majority of cases, the buffer system acc. to **Towbin** (Towbin et al. (1979) *PNAS USA* 76:4350-4) is the buffer of choice for tank blotting: 25 mM Tris, 192 mM glycin, 10-20 % methanol, pH approx. 8.3 (do not adjust!). This buffer may be used with or without additional SDS (0.01-0.1 %).

Formulation: Stock solution 1 litre 10 x buffer:

30.3 g TRIS (base)

144.1 g glycin

dissolve in 600 ml twice distilled water

optional: add 5-50 ml SDS solution 20 %

fill to 1000 ml with $H_2O_{tw.dist}$

1 litre working solution is prepared freshly using

100 ml 10 x stock solution

+ 700-800 ml $H_2O_{tw.dist}$

+ 100-200 ml methanol

Tris-glycin buffer acc. to Bjerrum (recommended for native proteins)

48 mM Tris, 39 mM glycin, 10-20 % methanol, pH approx. 9.2 (do not adjust!)
(acc. to **Bjerrum** and Schaefer-Nielsen (1986))

This buffer may be used with or without additional SDS (0.01-0.1 %).
(Use for blotting of native proteins with 0.04 % SDS and 0-10 % methanol).

Formulation: Stock solution 1 litre 10 x buffer:

58.15 g TRIS (base)

29.3 g glycin

dissolve in 600 ml twice distilled water

optional: add 5-50 ml SDS solution 20 %

fill to 1000 ml with $H_2O_{tw.dist}$

1 litre working solution is prepared freshly using

100 ml 10 x stock solution

+ 700-800 ml $H_2O_{tw.dist}$

+ 100-200 ml methanol

Sodium cabonate buffer acc. to Dunn (recommended for basic proteins)

10 mM $NaHCO_3$, 3 mM Na_2CO_3, 10-20 % methanol, pH approx. 9.9 (do not adjust!) (acc. to **Dunn**, 1986)

This buffer may be used with or without additional SDS (0.01-0.1 %).

Formulation: Stock solution 1 litre 10 x buffer:

8.4 g $NaHCO_3$

3.2 g Na_2CO_3

dissolve in 600 ml twice distilled water

optional: add 5-50 ml SDS solution 20 %

fill to 1000 ml with $H_2O_{tw.dist}$

1 litre working solution is prepared freshly using

100 ml 10 x stock solution

+ 700-800 ml $H_2O_{tw.dist}$

+ 100-200 ml methanol

Please note: Transfer parameters depend on the exact buffer system used.
Examples:

Transfer buffer	**Blot over night**	**Blot for 1 h**	**Blot for 3 hs**
Buffer acc. to Towbin	25-40 V	50-100 V	25-50 V
	40-80 mA	200-400 mA	100-200 mA
Buffer acc. to Bjerrum	25-40 V	50-100 V	25-50 V
	40-80 mA	200-400 mA	100-200 mA
Buffer acc. to Dunn	10 V	40-80 V	20-40 V
	40-80 mA	200-500 mA	100-250 mA

Transfer Buffer for Southern Blotting

1x (or 0.5x) TAE

or 1x (or 0.5x) TBE

or 20x SSC

For adequate stock solutions see below “Recommended Reagents”.

Transfer Buffer for Northern Blotting

20 mM morpholinopropanesulfonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA, pH 7.0

We recommend to prepare 10x stock solution with DEPC treated water, and adjust pH with NaOH.

Sterilize by passing through a 0.45-µm filter.

Store at RT protected from light.

The buffer gets straw-coloured with time, discard if it gets darker.

General Data

Make sure that **contact** between papers, gel, membrane and electrodes is optimal over the whole blot surface.

At no point touch the membrane with bare fingers as this will cause changes in the surface properties of the membrane and cause inconsistent sample binding as well as subsequent false signals.

In no case reuse transfer buffers !

Transfer buffers must be made accurately using high grade reagents. Methanol, in particular, must be used in analytical quality (p.a., Ph. Eur.), since metallic contaminants may plate on the electrodes and increase conductivity of the transfer buffer. pH and buffer quality will vary according to the purity of the reagents used. Best blotting results are obtained using buffers of low ionic strength and low conductivity.

For blotting of **basic proteins** we recommend use of transfer buffers with higher pH values, e.g. the Sodium carbonate buffer acc. to Dunn or CAPS buffer systems (see above). In Towbin buffer (Tris, glycine, methanol, pH ~8.3), transfer of basic proteins may be hindered due to the electric neutrality of the molecules under these conditions. We recommend use of a transfer buffer the pH of which is 2 ph units higher than the IEF of the proteins. Make sure to denature such proteins thoroughly prior to gel loading, since else the proteins may migrate to the cathode.

Do not adjust pH of the buffer, until it is definitely recommended. During transfer, H^+ ions form at the anode and OH^- ions at the cathode, which have to be dissipated via the wetted blotting papers. Adding acid or base to the buffer will result in higher conductivity, thus often leading to increased formation of heat as well as ions (OH^- at the cathode and H^+ at the anode), and, therefore, to a strong increase or decrease of the pH values at the electrode plates. This increase in ion concentration cannot be compensated by the blotting paper, thus resulting in brown, “burnt” looking blotting paper as well as, if worse comes to worst, damage to the electrode plates.

Do not use **cathode** buffers with pH values of under 8.3, since electrodes may take damage with frequent use.

Methanol prevents the gel from swelling during transfer and improves the absorption of proteins on the membrane. In addition, pore size is decreased and proteins may precipitate in the gel due to high methanol concentrations.

No or only very little methanol should be used with native gels or the transfer of native proteins. Also during transfer of large proteins (>100.000 Da) (particularly onto PVDF membranes) methanol concentration should be decreased (or omitted), since transfer efficiency of large SDS-coated proteins is low under presence of alcohols. This effect may be due to a) the methanolic-tight net structure of gel and membrane, and b) to a “stripping” of SDS from the proteins by alcohols.

For transfer of gradient gels methanol should be added in any event, since otherwise those gels swell to a trapezium.

SDS improves the transfer efficiency of large proteins in particular. However, it increases relative current, power and the development of heat. Additionally, in the event of very small proteins/peptides, especially onto nitro-cellulose membranes, it may result in penetration of peptides through the membrane (“blowout”) and consequently lead to a loss. SDS can be omitted in transfer of smaller proteins onto NC membranes, particularly in semi-dryBlotting (SDB). When PVDF membranes are used, however, we recommend supplementation with SDS (0.01-0.05 %) in all cases, since blotting efficiency onto PVDF is significantly enhanced in the presence of SDS.

Recommendations for SDS and Methanol (approximate values) (SDB: Semi Dry Blotting)

	Denaturing		Native	
	Methanol	SDS	Methanol	SDS
small proteins /peptides (approx. <20 kDa)	20 %	Tank: 0.01 % SDB: 0 % (NC) 0.01 % (PVDF)	5-10 %	Tank: 0.01 % SDB: 0 % (NC) 0.01 % (PVDF)
middle sized proteins/peptides (approx. 20-80 kDa)	10 %	Tank: 0.05 % SDB: 0.01 %	0-5 %*	Tank: 0.05 % SDB: 0.01 %
large proteins/peptides (approx. >80 kDa)	10 % (NC) 0-5 % (PVDF)	Tank: 0.1 % SDB: 0.05 %	0 %*	Tank: 0.1 % SDB: 0.05 %

* Only recommended for PVDF membranes

Membranes:

	PVDF	**NC**
Equilibration	Obligatory: In **methanol**	In water
During transfer	**SDS** may be reduced but not omitted. Presence of SDS increases blotting efficiency. **Methanol** may be omitted.	**Methanol** may be reduced but not omitted. Presence of methanol increases protein absorption. **SDS** may be omitted. Small proteins in particular should be blotted w/o SDS (risk of “blowout”).

Make sure that there is at least 2 mm **blotting paper** at each side of the sandwich. Too little paper limits the amount of buffer ions available for the blot, resulting in lower transfer efficiency and, in the long run, leading to damage of the electrode surface. Make sure the paper is adequately wettet.

The actual **run time** will depend on the size of the molecules to be transferred. Large proteins and long nucleic acids will need 2 hours, small molecules less than 1 h. We recommend transfer time of 1 hour for the first blot performed with a special set of molecules.

The **efficiency** and **quality** of transfer depends on the type of buffer used, also the type of samples, and the parameters used for the run. Generally, better results can be obtained by reducing the power settings and increasing the blot time.

If **more than one gel** is to be blotted under the same conditions, simply stack them as previously described, with a piece of dialysis membrane presoaked in distilled water. This prevents small proteins from traveling through the blotting membrane and contaminating the one below.

Pre-equilibrate all gels in transfer buffer prior to blotting (15 mins. up to 1 hour). Pre-equilibration will remove residues of electrophoresis buffers and salts, hence preventing formation of too much heat due to an increase of conductivity during transfer. It also reduces the concentration of SDS in the gel that may hinder absorption to membranes (mainly to NC membranes) and cause penetration of the peptides through the membrane. Equlibration also allows the gel to form its natural and final size in the buffer, preventing swelling of the gel during transfer that may lead to unsharp bands. Gradient gels should definitely be equilibrated until they don’t change form and size anymore.

During **tank blotting**, the buffer should be kept in permanent **agitation** by a stir bar placed into the tank.

Cooling of transfer buffers prior to use, as well as cooling of the unit during transfer, prevents overheating and damage of the proteins and blotter.

Recommended parameters for Power Supplies

Blot under continuous **intensity of current**.

Semi Dry blotting: 2-5 mA / cm^2 gel size

Tank blotting: 2-4 mA / cm^2 gel size

Recommended Reagents

Reagent	Art. No.
Capronic acid	8799
CAPS	9168
EDTA disodiumsalt dihydrate, p.a.	8043
Glycin, Blotting-Grade	0079
Methanol, Blotting-Grade	0082
Morpholinopropanesulfonic acid (MOPS)	6979
Natriumacetate trihydrate, p.a.	6779
ROTI®Stock 20 % SDS	1057
ROTI®Blot 1 for proteins in general	L509
ROTI®-Stock 20x SSC	1054
ROTIPHORESE® 10x TAE Buffer	T845
ROTIPHORESE® 10x TBE Buffer	3061
ROTIPHORESE® 50x TAE Buffer	CL86
SDS, Blotting-Grade	0183
TRIS, Blotting-Grade	0188

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