



Transfer Buffer for Tank and Semi-Dry Blotting

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

Roti[®]-Blot 2 – für hydrophobe proteins

Both Roti[®]Blot systems consist of two different buffers, anode buffer A and the cathode buffer K. 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 9.2 (acc. to **Bjerrum** and Schaefer-Nielsen (1986)

This buffer may be used with or without additional SDS (0.01-0.1 %). Often, 0.375 % SDS are added. May also be used for blotting of native proteins (with 0.0375 % SDS and 0-10 % methanol).

Formulation: Stock solution 1 litre 10 x buffer:

75.65 g TRIS-HCl or 58.15 g TRIS (base)

29.3 g glycin

dissolve in 600 ml twice distilled water and titrate to pH 9.2

optional: add 5-50 ml SDS solution 20 %

fill to 800 ml with H₂O_{tw.dist}

1 litre working solution is prepared freshly using

100 ml 10 x stock solution

+ 700-800 ml H₂O_{tw.dist}

+ 100-200 ml methanol

Transfer Buffer for Tank Blotting

Only continuous buffer systems may be used in tank-blotting. 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 8.3. This buffer may be used with or without additional SDS (0.01-0.1 %).

Formulation: Stock solution 1 litre 10 x buffer:
39.4 g TRIS-HCl or 30.3 g TRIS (base)
144.1 g glycin
dissolve in 600 ml twice distilled water and titrate to pH 8.3
optional: add 5-50 ml SDS solution 20 %
fill to 800 ml with H₂O_{tw.dist}

1 litre working solution is prepared freshly using
100 ml 10 x stock solution
+ 700-800 ml H₂O_{tw.dist}
+ 100-200 ml methanol

Tris-glycin buffer acc. to Bjerrum

48 mM Tris, 39 mM glycin, 10-20 % methanol, pH 9.2 (acc. to **Bjerrum** and Schaefer-Nielsen (1986))
This buffer may be used with or without additional SDS (0.01-0.1 %). Recommended for blotting of native proteins (with 0.04 % SDS and 0-10 % methanol).

Formulation: Stock solution 1 litre 10 x buffer:
75.65 g TRIS-HCl or 58.15 g TRIS (base)
29.3 g glycin
dissolve in 600 ml twice distilled water and titrate to pH 9.2
optional: add 5-50 ml SDS solution 20 %
fill to 800 ml with H₂O_{tw.dist}

1 litre working solution is prepared freshly using
100 ml 10 x stock solution
+ 700-800 ml H₂O_{tw.dist}
+ 100-200 ml methanol

Sodium carbonate buffer acc. to Dunn

10 mM NaHCO₃, 3 mM Na₂CO₃, 10-20 % methanol, pH 9.9 (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 NaHCO₃
3.2 g Na₂CO₃
dissolve in 600 ml twice distilled water and titrate to pH 9.9
optional: add 5-50 ml SDS solution 20 %
fill to 800 ml with H₂O_{tw.dist}

1 litre working solution is prepared freshly using
100 ml 10 x stock solution
+ 700-800 ml H₂O_{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
<i>Buffer acc. to Towbin</i>	25-40 V	50-100 V	25-50 V
	40-80 mA	200-400 mA	100-200 mA
<i>Buffer acc. to Bjerrum</i>	25-40 V	50-100 V	25-50 V
	40-80 mA	200-400 mA	100-200 mA
<i>Buffer acc. to Dunn</i>	10 V	40-80 V	20-40 V
	40-80 mA	200-500 mA	100-250 mA

General Data

Methanol prevents the gel from swelling during transfer and improves the absorption of proteins on the membrane. No or only very little methanol should be used with native gels or the transfer of native proteins.

SDS improves the transfer efficiency of large proteins in particular. However, in the event of very small proteins/peptides, especially with nitro-cellulose membranes, it may result in penetration of peptides through the membrane and consequently lead to a loss. SDS can be omitted during the transfer of smaller proteins, particularly in semi-dry-blottin (SDB).

Recommendations (approximate values)

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

Recommended Reagents

Reagent	Ord. No.
TRIS-HCl	9090
TRIS (Base)	AE15
Glycin	T873
Roti-Stock 20 % SDS	1057
Methanol	4627
Roti®-Blot 1	L509
Roti®-Blot 2	P039

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