Electroblotting

Method

Blotting offers a simple and rapid technique for the sensitive detection of biomolecules such as proteins and nucleic acids.

After separation in an electrophoresis gel molecules are transferred by means of an electrical field onto a membrane e.g. SERVA nitrocellulose, SERVA Nylon Bind A, Nylon Bind B or SERVA Fluorobind membranes.

Immobilized onto the surface of a membrane, molecules can be further processed, i.e. exposed to nucleic acid probes (hybridization), stains or labelled antibodies (specific immunodetection). The tank-blotting procedure is applicable to all polyacrylamide and agarose gels e.g. Tris-Glycine, Tris-Tricine, IEF-, TBE-gels. Vertical Gels (precast cassette gels) can be used as well!

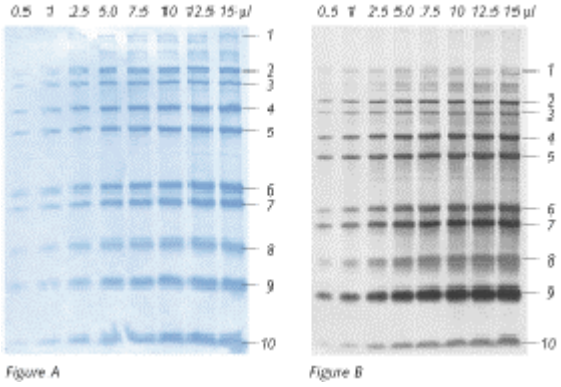


Figure A Figure B

Volumes of 0.5, 1.0, 2.5, 5.0, 7.5, 10, 12.5 and 15 µl of a SERVA Protein Markers liquid mix (conc. = 0.5 mg/ml, cat.no. 39212.05) were separated on a 10% Tris-Tricine Vertical precast gel and then blotted onto a SERVA nitrocellulose membrane, 0.45 µm (cat. no. 42516).

Total protein was detected using Amido Black 10 B (figure A) and India Ink (figure B).

The protein detection limit using Blue Blot Wet 100 with Amido Black 10 B staining and India Ink staining was 40 ng/band and 20 ng/ba well width: 4 mm).

Table 1

Protein	Source	M.W.	Band
Myosin	rabbit muscle	200.0	1
ß-Galactosidase	E. coli	116.4	2
Phosphorylase b	rabbit muscle	97.2	3
Albumin	bovine serum	66.0	4
Glutamate dehydrogenase	bovine liver	55.5	5
Lactate dehydrogenase	porcine muscle	36.5	6
Carbonic anhydrase	bovine erythrocytes	29.0	7
Trypsin inhibitor	soybean	20.0	8
Lysozyme	chicken egg	14.0	9
Trypsin inhibitor	bovine lung	6.5	10

Tank Blotting – gentle & efficient

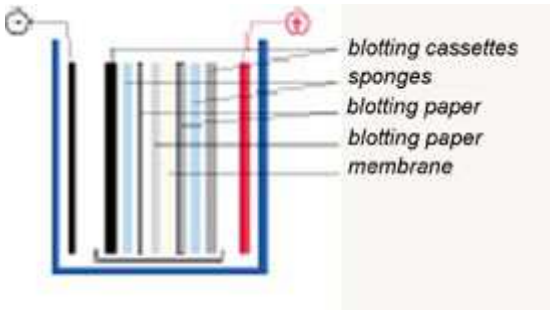
Tank blotting using BlueBlot Wet offers a rapid and efficient method for the gentle transfer of sensitive biomolecules. Driven by an applied electrical field, molecules migrate according to their net charge out of the gel and towards the blotting membrane. A proper contact between gel and membrane over the entire surface of the gel cassette has shown to be the most critical factor for highly efficient and short transfers. The tank is filled with transfer buffer, which is thermostatted by a cooling device in order to counteract the Joule's heat generated during electrotransfer. The recommended buffer temperature during the transfer is below 20℃. Transfer efficiency has been determined to be >90% for proteins and nucleic acids. A variety of unspecific staining of proteins exists today (see tab. 1) which provide rapid visualization of bands (e.g. Ponceau S, cat. no. 33427) and even at low concentration (Amido Black 10 B, cat. no. 12310, and India Ink, see fig. A and B).

Benefits of Tank-Blotting:

- applicable to most biomolecules (e.g. DNA, RNA, proteins & peptides)
- ideally suited for sensitive samples
- transfer rates better than 90%

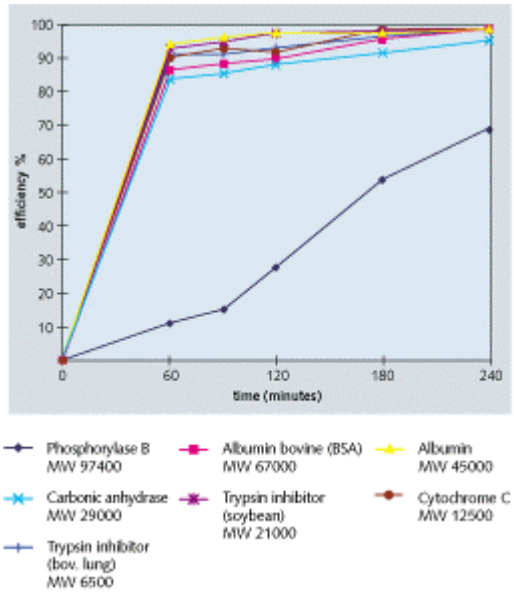
Procedure

For blotting proteins onto membranes, the gels are carefully removed from the electrophoresis unit, pre-equilibrated in transfer buffer (e.g. Towbin Buffer, cat. no. 42558) and assembled in layers in the following order:
The transfer of proteins takes place under slightly alkaline conditions 1) so that the negatively charged proteins migrate towards the positively charged anode. For DNA and RNA transfer, acidic buffers containing phosphate and citrate are used 2)



A total of 10 µg of Protein Test Mixture 4 and 5 (cat. no. 39208.01 and 39209.01 resp.) was electrophoresed on a 10% polyacrylamide gel respectively and blotted subsequently onto a SERVA Fluorobind membrane (cat. no. 42571.01).
Blotting efficiency was determined by scanning SERVA Blue R (cat. no. 35051.01) stained gels with a densitometer. Values were calculated as percentage of the non-blotted part of the same gel.

Protein detection on the SERVA Fluorobind membranes was performed using Ponceau S solution for electrophoesis (cat. no. 33427.01).



References:
1. Towbin, H., Staehlin, T., Gordon, J. Proc. Natl. Acad. Sci. USA, 76 : 4350; 1979
2. Smith, M.R., Devine, C.S., Cohn, S.M., Lieberman, M.W. Anal. Biochem., 137 : 120 - 124; 1984