# Blotting Introduction

- Semi-dry blotting
- Tank blotting
- Vacuum blotting
- Dot/Slot blotting

Blotting techniques allow the transfer of proteins and nucleic acids (DNA, RNA) from polyacrylamide or agarose gels onto carrier membranes. Additional these techniques allow immobilisation of those components from solutions onto carrier membranes. On the membrane the proteins and nucleic acids offer open access (compared to in-gel techniques) for detection methods for specific molecules (e.g. antibodies).



Technique	Typical use
Semi-dry blotting	Rapid, high-intensity transfer recommended for proteins up to 150 kD.
Tank blotting	Slower than semi-dry blotting. Efficient and quantitative transfer for small to big proteins. High effective cooling (big buffer volume).
Capillary blotting	Over night transfer of nucleic acids from agarose gels onto a membrane (highly diffuse bands, much lower resolution than vacuum blotting).
Vacuum blotting	Quick and efficient transfer of nucleic acids from agarose gels onto a membrane.
Dot/Slot blotting	Screening systems for immobilisation, concentration and binding of proteins and nucleic acids onto membranes.

#### **Selection Guide**

Product	Sample		Type of blotting	Heat exchanger	Number of gels
	Proteins	Nucleic acids			
Fastblot	+	(+)	electro (semi-dry)	built-in option	1 -≥4*
Tankblot, Tankblot Eco-Line	+	(+)	electro (tank)	built-in option	1 – 4
Vacu-Blot	-	+	vacuum	-	1
Dot/Slot Blot (Dot Blot 96, Hybri•Slot 24)	+	+	vacuum	-	max. 96 samples

\* 1 – 4 stacked, depending on size of gel and apparatus >4 side by side

(+) = limited use

(-) = not recommended

# Fastblot

# Rapid Semi-dry Blotting

- Fast and homogeneous electrophoretic transfer of proteins
- Electrodes:
- Platinum/titanium
- Plasticised carbon
- Two different sizes:
- 16 cm x 20 cm
- 23.5 cm x 38.5 cm
- Recommended for proteins up to 150 kDa
- Cooling available

#### Semi-dry blotting

Electro-blotting is an important method to transfer proteins and nucleic acids from polyacrylamide gels to nitrocellulose or other carrier membranes. Semi-dry blotting allows fast, efficient and homogenous transfer. In contrast to tank blotting little transfer buffer is required and transfer times are dramatically reduced.

Additionally, with semi-dry blotting discontinuous buffer systems can be used, e.g. one cathode buffer and two different anode buffers, to gently blot smaller proteins or to transfer proteins of very different sizes evenly.

#### High capacity

All Fastblot models offer the capacity to transfer multiple gels: stacked (with a dialysis membrane separating the gel sandwiches) or side by side. There is no requirement for additional plastic templates to prevent electrode short circuit as necessary for use with other brands of semi-dry blotters.

#### **Electrode material**

The first graphite electrodes used for protein transfer corroded easily. Biometra permanently improved the blotting electrodes based on a novel bio-inert plasticised carbon material. This type of electrodes (used for models **B33, B34** and **B64**) is stable for years.

Alternatively, Biometra offers corrosionfree metal electrodes without pores for maintenance free use and easy decontamination. These electrodes (used for models **B43** and **B44**) consist of a platinum-coated titanium anode and a stainless steel cathode.

#### **Rapid transfer**

Both, the plasticised carbon electrodes and the metal electrodes can be used for higher currents (max. 5 mA/cm<sup>2</sup> blot). Therefore blotting times are reduced to as little as 10 to 30 minutes. By applying higher current (> 1 mA/cm<sup>2</sup> blot), proteins with higher molecular weights can be blotted faster and with higher efficiency. Even smaller proteins can be transferred faster from thick gels and gels with small pores when blotting with high current.



Platinum / titanium, stainless steel electrodes



Plasticised carbon elektrodes



# Fastblot Rapid Semi-dry Blotting

#### **Cooling option**

Large proteins (>100 kDa) require longer transfer times. The heat generated during the extended transfer time can be removed by using the flow-through cooling system which is available with models **B33** and **B43**. Additionally this option is recommended for use with native gels and temperature sensitive proteins respectively.

#### **Blotting of nucleic acids**

Nucleic acids can also be electro-blotted using the Fastblot systems. For transfer of DNA from polyacrylamide gels onto a membrane electro-blotting is the only way. However, vacuum blotting is the method of choice for transferring nucleic acids from agarose gels onto membranes e.g. with the Biometra Vacu-Blot Apparatus (please refer to page 110).

#### **Proteomics blotter**

The XXL sized blotter **B64** offers passive cooling and is specially designed for the transfer of big (2D) gels in proteomics research or multiple gels side by side at the same time.



#### Literatur:

- Towbin, H., Staehlin, T., Gorden, J. (1979); Proc. Nat. Acad. Sci. 76, 4350 – 4356.
- Bittner, M., Kupferer, P., Morris, C.
  F. (1980); Anal. Biochem. 102, 459 – 471.
- Burnette, W. N. (1981); Anal. Biochem. 112, 195 – 203.
- Kyse-Andersen, J. (1984); J. Biochem.
  Biophys. Meth. 10, 203 209.

All Fastblots need a high capacity power supply. The ideal choice is the versatile Biometra Standard Power Pack P25, which can also be used for every type of standard electrophoresis application (see page 97).

Fastblot B64

# Fastblot Order Information



#### **Electrophoretic transfer of proteins**

SDS-PAGE, acrylamide concentration: 10 %; Nitrocellulose blotting membrane, pore size 0.45 µm; current: 5 mA per cm<sup>2</sup> gel; thickness of gel: 1.0 mm; transfer buffer: Tris/Glycin/SDS (Using B43/B44 can reduce the transfer time in the range of 20 %.)

Blotting stack assembly for discontinuous buffer systems

Item	Order No.
Electrode surface 16 cm x 20 cm:	
Fastblot B33, plasticised carbon electrodes, with flow-through cooling	014-100
Fastblot B34, plasticised carbon electrodes, without cooling	014-200
Fastblot B43, anode: platinum-coated titanium, cathode: stainless steel, with flow-through cooling	015-100
Fastblot B44, anode: platinum-coated titanium, cathode: stainless steel, without cooling	015-200
Electrode surface 23.5 cm x 38.5 cm:	
Fastblot B64, plasticised carbon electrodes, with passive cooling	015-600
Accessories	
Whatman 3MM Chr. 580 mm v 680 mm 0.34 mm thick 100/pkg	B3030031

Whatman 3MM Chr, 580 mm x 680 mm, 0.34 mm thick, 100/pkg	B3030931
Whatman 17Chr, 460 mm x 570 mm, 0.92 mm thick, 25/pkg	B3017915
Whatman GB005, 200 mm x 200 mm, 1.2 mm thick, 25/pkg	B10426981

See also chapter: Blotting Membranes and Whatman Paper



### Tank Blot Apparatus and Blotting Modules

For Gentle Blotting of Large and Temperature-Sensitive Proteins

- Best suited for large proteins and native enzymes
- Fast, efficient and reproducible transfer of proteins
- Effective cooling thanks to built-in cooling
- Simultaneous blotting of up to four gels

During tank blotting proteins are transferred in a vertical buffer tank between electrodes arranged on the sidewalls onto membranes. Tank blotting is recommended particularly for blotting of large molecules (> 100 resp. 200 kDa, depending on the proteins characteristics) or of proteins which are difficult to transfer with other blotting techniques. It is the method of choice also for native and temperature-sensitive proteins. Transfer can be done fast or overnight. Thanks to the higher buffer volume transfer times of more than 24 h can be realised, e.g. for gentle blotting of very big proteins.

Different models are available: The highly sophisticated **Tankblot** for mini-gels offers the most effective cooling by the laterally integrated cooling jacket. **Tankblot Eco-Mini** and **Tankblot Eco-Maxi** are part of the modular Eco-Line and offer highly effective cooling of small and large gels by integrated cooling bases. The **Mini-V8-10 Blot Module** is designed for use in the buffer tank of the Mini-V8-10 vertical gel electrophoresis apparatus.



#### Tankblot

In the Tankblot a cooling jacket is integrated into the sidewalls. This results in convective chilling by circulation: the temperature is distributed more homogeneously ( $\pm$  0.5 °C at 6 °C buffer temperature) than with other designs that use a magnetic stirrer circulating the buffer. Additionally this special design prevents formation of ion gradients without the need for a magnetic stirrer.

Depending on the selected buffer, transfer can be fast (between 30 min. and 4 h) or, under exceptionally gentle conditions, overnight (ca. 12 h). Thanks to the buffer volume of 1,200 ml long transfer times can be realised.



**Cooling system of the Tankblot** 

#### Compatibility of gel sizes

Apparatus	Blotting area (W x L, cm)	Tankblot 10.0 x 10.0	<b>Tankblot Eco-Mini</b> 9.4 x 8.0	Tankblot Eco-Maxi 22.0 x 19.0	<b>Mini-V8.10 Blot Module</b> 9.0 x 7.5
Minigel-Twin		+	+	+	(+)
Eco-Mini		+	+	+	(+)
Mini-V8·10		+	+	+	+
Multigel		-	-	+	-
Multigel-Long		-	-	+	-
Model V15·17		-	-	+	-
Maxigel		-	-	+	-
Eco-Maxi		-	-	+	-

+ = recommended

(+) = limited use -

# Tank Blot Apparatus and Blotting Modules

For Gentle Blotting of Large and Temperature-Sensitive Proteins

#### Tankblot Eco-Line

Wire electrodes are placed on the side walls to provide uniform, reproducible protein transfer over a wide molecular weight range. The lower buffer chamber with built-in cooling base absorbs heat generated during rapid transfers and allows temperature controlled runs. A stir bar may be added to the buffer chamber to further improve buffer circulation and heat exchange. The colour coded blotting cassettes in combination with the colour coded connectors for the Bigfoot Safety Lid ensure accurate orientation of the gel sandwich during the transfer. The tankblot is available as stand-alone apparatus or as a module compatible with the buffer chamber EBC (with integrated cooling base) or EB (without cooling option).

The **Electrophoresis Module** for Eco-Mini and Eco-Maxi are available separately and will convert the corresponding tank blotting systems into powerful PAGE electrophoresis systems.

#### Tankblot Eco-Mini

Tankblot Eco-Mini is designed to transfer up to four PAGE mini-gels (up to 9.5 cm x 8.5 cm) in separate blotting cassettes simultaneously.

The special design of the blotting cassettes allows easy assembly and loading into the blot module. Additional the assembly defends blotting sandwiches from squeezing.



For more details see page 47

#### Tankblot Eco-Maxi

Tankblot Eco-Maxi is the large version for gel sizes up to 22 cm x 19 cm. The system offers the capacity of multiple transfers: several small gels or 1 large gel per cassette. Up to 2 large gels can be transferred simultaneously in separate blotting cassettes.

The special design for fixing the blotting sandwich in the blotting cassette prevents squeezing and allows easy handling.



For more details see page 49

#### Mini-V8·10 Blot Module

The Mini-V8·10 Blot Module operating in the buffer tank of the Mini-V8·10 electrophoresis apparatus allows the blot transfer of proteins from one or two 9.0 cm x 7.5 cm gels at a time. The special design of blot support frame and blot restrainer allows easy handling. Slipping is eliminated and close contact between gel and membrane is guaranteed. The buffer volume of approx 1,000 ml is sufficient for cooling even at higher current. The Mini-V8·10 Blot Module is available separately or in combination with the Mini-V8·10 Gel Electrophoresis Apparatus.





# Tank Blot Apparatus and Blotting Modules

Order Information

Item	Order No.
<b>Tankblot</b> , complete instrument with integrated cooling jacket, 1 gel cassette for up to four 10 cm x 10 cm gels, 2 fixation rings and 2 fiber pads	013-300
Accessories	
Gel cassette (blotting cassette) for up to four 10 cm x 10 cm gels, 1/pkg	013-301
Fixation rings for gel cassette, 4/pkg	013-304
Fiber pads, 4/pkg	013-305
<b>Tankblot Eco-Mini C</b> , complete instrument with integrated cooling base, Blot Module, 4 Blotting Cassettes and 8 foam pads Note: The instrument is compatible with Eco-Mini Electrophoresis Module	018-100
<b>Tankblot Eco-Mini</b> , complete instrument (without cooling base), Blot Module, 4 Blotting Cassettes and 8 foam pads Note: The instrument is compatible with Eco-Mini Electrophoresis Module	018-101
Accessories	
Buffer chamber EB (without cooling base) for Eco-Mini and Tankblot Eco-Mini, without Bigfoot Safety Lid	017-170
Buffer chamber EBC (with integrated cooling base) for Eco-Mini and Tankblot Eco-Mini, without Bigfoot Safety Lid	017-171
Bigfoot Safety Lid with cables and safety plugs for Eco-Mini and Tankblot Eco-Mini	017-172
Blot Module Eco-Mini, incl. 4 Blotting Cassettes, colour coded (black/red) each for 1 gel with size 9.4 x 8.0 cm and 8 foam pads	018-105
Blotting Cassette, colour coded (black/red), for Tankblot Eco-Mini Blot Module, 1/pkg	018-111
Foam pads for Tankblot Eco-Mini Blot Module, 4/pkg	018-113
Electrophoresis Module for Eco-Mini (1 - 4 gels)	017-175
<b>Tankblot Eco-Maxi C</b> , complete system with buffer chamber EBC (with integrated cooling base), Bigfoot Safety Lid, 2 Blotting Cassettes (black/red), each for 1 gel with size 22 cm x 19 cm, 4 foam pads Note: The instrument is compatible with Eco-Maxi Electrophoresis Module	018-400
<b>Tankblot Eco-Maxi</b> , complete system with buffer chamber EB (without cooling base), Bigfoot Safety Lid, 2 Blotting Cassettes (black/red), each for 1 gel with size 22 cm x 19 cm, 4 foam pads Note: The instrument is compatible with Eco-Maxi Electrophoresis Module	018-401
Accessories	
Buffer chamber EB (without cooling base) for Eco-Maxi and Tankblot Eco-Maxi, without Bigfoot Safety Lid	017-471
Buffer chamber EBC (with integrated cooling base) for Eco-Maxi and Tankblot Eco-Maxi, without Bigfoot Safety Lid	017-472
Bigfoot Safety Lid with cables and safety plugs for Eco-Maxi and Tankblot Eco-Maxi	017-474
Blot Module Eco-Maxi, incl. 2 Blotting Cassettes colour coded (black/red), each for 1 gel with size 22 cm x 19 cm and 4 foam pads	018-405
Blotting Cassette, colour coded (black/red), for Tankblot Eco-Maxi Blot Module, 1/pkg	018-411
Foam pads for Tankblot Eco-Maxi Blotting Module, 4/pkg	018-413
Electrophoresis Module for Eco-Maxi (1 or 2 gels)	017 - 475
Mini-V8-10 Blot Module, complete with blot restainer, blot support frame, clamping knob and three transfer pressure pads	21078019
Accessories	
Transfer pressure pad, 6/pkg	11958048

t⊕r ⊖

# Tankblot Application

#### Homogenous blotting of heterogenous protein mixtures

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Often, when establishing a Western blot system, certain proteins cannot be blotted quantitatively. They are either remaining in the gel (particularly large molecules) or they are blotting through the membrane (especially small molecules). In both cases a quantitatively false low detection signal will result, especially obvious when simultaneous blotting molecules which vary in size highly. The search for proteins of unknown size might thus be complicated.

Using gradient gels is a simple and effective way to circumvent these problems as well as to ensure homogenous blotting of molecules with different sizes. Following SDS gel electrophoresis in gradient gels small molecules are located in areas of small mesh size, large ones in areas of wide mesh size.

During blotting large molecules leave the "low percentage" gel quite easily, in contrast, small molecules are retarded in the "high percentage" gel (figure). Thus, the resulting blot becomes very homogenous: The blotting membrane is reached both by small and by large proteins quantitatively (appr. 10 to 180 kDa). For demonstration SDS molecular weight markers (MWM) and coronavirus JHM proteins are separated in a gradient gel system using a resolving gel of only 5 cm height (stacking gel T= 5, C= 0.6; resolving gel T= 7, C= 0.6 up to T= 20, C= 0.6; ca. 25 °C; stacking gel 20 mA, 50 V; resolving gel 30 mA const., 70 – 200 V; ca.1h). After transfer in the Tankblot (25 mM Na<sub>2</sub>HPO<sub>4</sub>: pH 9.4, 1000 mA const., 30 V, 4 h) the marker proteins were stained unspecifically with amido black. Virus proteins (E=14 kDa, N= 56 kDa, S = 90 and 180 kDa) were labelled via specific antibodies and enzyme mediated colour conversion.



Item	Order No.
Blotting paper	
Whatman 3MM Chr, 580 mm x 680 mm, 0.34 mm thick, 100/pkg	B3030931
Whatman 17 Chr, 460 mm x 570 mm, 0.92 mm thick, 25/pkg	B3017915
Whatman GB005, 200 mm x 200 mm, 1.2 mm thick, 25/pkg	B10426981
Whatman GB005, 580 mm x 580 mm, 1.2 mm thick, 25/pkg	B10426994

See also chapter: Blotting Membranes and Whatman Paper.



# Vacu-Blot For Efficient Southern and Northern Blotting

- Fast and efficient transfer of DNA or RNA
- Membrane sizes up to 20 cm x 20 cm
- Large buffer tank permits easy recycling of transfer buffer
- Optimised sealing system
- Reliable and quiet pump with adjustable vacuum

#### **Fast Transfer**

Transfer of nucleic acids to membranes during Southern or Northern analysis was traditionally done by capillary blotting, a time-consuming procedure that usually takes up to 12 hours. With vacuum blotting, transfer times can be reduced to 15 – 60 minutes, depending on the size of the DNA.

#### Durable

The **Vacu-Blot** is built for long life. It features a solid and durable construction made of acrylic glass. A reliable sealing system guarantees fast and efficient transfer on the whole gel. The large tank allows easy recycling of buffer and protects the pump from buffer aspiration. Therefore no additional separator or trapping bottle (e.g. Woulff's bottle) is required for use as necessary with other brands of vacuum blotting systems.

#### Flexibility

The Vacu-Blot was designed for maximum flexibility. The window gaskets (rubber sheets) can be cut to accept gels of all thicknesses and sizes up to 20 cm x 20 cm.

Vacuum should never be more than 50 mbar – 100 mbar below ambient pressure to prevent gel torsion and reduced transfer efficiency, as in an "collapsed" gel the DNA or RNA would be trapped.

The use of model MP86 vacuum pump (included in the Vacu-Blot System) is recommended as this pump offers controlled and adjustable vacuum.





#### Efficiency

Vacuum blotting guarantees reproducible blotting results. Compared to traditional transfer methods vacuum blotting offers increased transfer efficiency and allows the detection of low amounts of DNA during hybridisation.

## Vacu-Blot

### Application (Example) and Order Information

#### Vacuum blotting of Drosophila DNA: Faster and more efficient detection of the SD Responder Sequence

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The SD (segregation distorter) system of Drosophila melanogaster causes directed deviation of the expected gamete ratio of 1:1 in spermiohistogenesis. To detect the SD responder sequence, genomic DNA was transferred onto a nylon membrane and hybridised with a specific probe. DNA transfer from the gel onto the membrane was performed both by capillary transfer (left fig.) and by vacuum blotting with a Biometra Vacu-Blot (right fig.). Vacuum blotting with the Vacu-Blot proved to be far superior to capillary transfer because:

- 1. Transfer time could be reduced by 85 % (from 15 h down to 2 h).
- 2. DNA transfer showed improved homogeneity.
- Transfer efficiency was increased, resulting in a higher detection limit in subsequent hybridisation.



Comparison of lane B2 and A2 indicates that DNA transfer with vacuum blotting (right) is more efficient and precise than capillary transfer (left).

Genomic DNA both undigested (lane 1) and digested (lane 2) with the restriction enzyme Xba I (19 U/µg DNA) of the mutant cn bw (cinnabar brown) of Drosophila melanogaster was separated on a 1 % agarose gel. Transfer was performed in 20 x SSC onto a nylon membrane using both transfer methods. To detect the SD responder sequence, the transferred DNA was hybridised with a digoxigenin-labeled oligonucleotide (26mer, 100 pMol each). The nucleotide sequence of this probe is contained in all variants of the 240 bp unit of the SD responder sequence (Wu et al. (1988) Cell, 54, 179-189).

Detection of the hybridised probes was carried out with anti-digoxigenin antibodies coupled to alkaline phosphatase by color reaction (NBT and BCIP).

Item	Order No.
<b>Vacu-Blot System</b> for blots up to 20 cm x 20 cm, 230 V Complete system consisting of transfer unit, 3 rubber sheets, 8 clips, membrane vacuum pump MP86 with manometer, adjustable vacuum gauge and tubing	053-000
dto., 115 V	053-090
dto., 100 V	053-091
Vacu-Blot without pump, incl. 3 rubber sheets, 8 clips and tubing	053-100
Accessories	
Rubber sheets (280 mm x 280 mm) for Vacu-Blot, 3 pieces	053-002
Porous filter plate for Vacu-Blot	053-004
Clips (1 set), 6 pieces	010-057
Whatman 3MM Chr, 580 mm x 680 mm, 0.34 mm thick, 100/pkg	B3030931
See also chapter: Blotting Membranes and Whatman Paper	
Membrane pump	

Membrane vacuum pump MP86, 230 V (50 Hz) with adjustable vacuum gauge and manometer, end vacuum 100 mbar, max. delivery 6 l/min	049-000
dto., 115 V (60 Hz)	049-090
dto., 100 V (50/60 Hz)	049-091

#### Accessories

Vacuum tubing for MP86	(2 x 1 m)
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049-002



# **Dot Blot 96** Trouble-free Handling Guaranteed

- Innovative sealing system
- No cross-contamination
- Multichannel pipet compatible for fast sample loading
- High capacity for up to 96 samples
- Precisely adjustable vacuum

#### Reproducible

The **Dot Blot 96** system provides easy and reproducible methods for immobilising, concentrating and binding proteins, DNA or RNA in solution onto membranes. Typical applications are Dot Blot hybridisation of plasmid DNA, small RNA and DNA fragments, cloned bacteria, lymphomas and viral nucleic acids, screening of recombinant clones, screening of cell surface antigens, as well as filtration and immobilisation of small volumes onto immobilising matrices.





#### Easy to use

The Dot Blot 96 works without movable O-rings, making it extremely easy to set up. Its innovative sealing system eliminates lateral leakage that can cause cross-contamination between wells. Each well is numbered and lettered compatible to the standard 96-well plate format. Multichannel pipets for rapid handling of samples can be used.

#### **High capacity**

Up to 96 samples can be loaded in short time. The maximum sample volume is 350 µl per well.

#### Durable

Dot Blot 96 features a buffer tank design that eliminates the need for trapping bottles (separators), facilitates buffer recycling, and protects any vacuum pump from buffer aspiration. The use of model MP86 vacuum pump (included in the Dot Blot 96 System) is recommended as this pump offers controlled and adjustable vacuum to prevent damage of the transfer membrane.

# Dot Blot 96 Order Information

Item	Order No.
<b>Dot Blot 96 System</b> , 230 V, complete system consisting of transfer unit, 1 sealing sheet, membrane vacuum pump MP86 with manometer, adjustable vacuum gauge and tubing	053-400
dto., 115 V	053-490
dto., 100 V	053-491
Dot Blot 96 without pump, transfer unit, 1 sealing sheet and tubing	053-401
Accessories	
Silicone sealing sheet	053-402
Whatman 3MM Chr, 580 mm x 680 mm, 0.34 mm thick, 100/pkg	B3030931
See also chapter: Blotting Membranes and Whatman Paper	
Membrane pump	
Membrane vacuum pump MP86, 230 V (50 Hz) with adjustable vacuum gauge and manometer, end vacuum 100 mbar, max. delivery 6 l/min	049-000
dto., 115 V (60 Hz)	049-090
dto., 100 V (50/60 Hz)	049-091
Accessories	
Vacuum tubing for MP86 (2 x 1 m)	049-002

