

**BlueLine**

*Instruments for Electrophoresis*

## **INSTRUCTION MANUAL**

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**BlueFlash Small  
BlueFlash Medium  
BlueFlash Large  
BlueFlash X-Large  
Blue Flash XX-Large**

**Semi-dry Blotting Units**



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## WARNING

This unit is capable of delivering potentially lethal voltage and is to be operated only by qualified technically trained personnel. The BlueFlash Semi-Dry-Blotter is designed to give long service and reproducible results in your laboratory. A few moments spent reading these instructions will ensure that your expectations are reflected in the successful use of the apparatus.

Please read the entire operator's manual thoroughly before operating this unit.

First check that the apparatus has been received complete and undamaged following shipment. Any damages or missing parts must be notified to **SERVA Electrophoresis GmbH** Heidelberg resp. to the responsible distributor immediately. **SERVA Electrophoresis GmbH** cannot accept responsibility for goods returned without prior notification.

Refer to the packing list and check that all components and accessories are present.

**Warranty is 12 months from the date of delivery. This warranty does not include careless or wilful damaging. Please retain all packaging materials until the warranty period has expired.**

Note: In case of repair: Always send in the complete instrument.

## 1. Packing list

### BlueFlash Small, Medium, Large, X-Large und XX-Large (Cat. No.: BF-S, BF-M, BF-L, BF-XL, BF-XXL)

No. of items	Description
1	Main unit consisting of lid with plate cathode and one red and one black cable with connection plug to power supply, (optional cooled) spring mounted, self adjusting plate anode inset and base with safety circuit breaker bracket
1	Manual

## 2. Unit description and technical data

### Lid/Cathode:

- Safety lid with printed quick instructions guide and two safety cables
- Integrated SC plate cathode (-) with blot contact press on-mechanism for homogeneous transfer

### Inset:

- Height adjustable (optional cooled) self-regulating electrode tray with SC plate anode (+)

### Base:

- Stable apparatus tank with safety circuit breaker bracket with gold-plated plugs

When the **BlueFlash semi-dry blotter** unit is empty, i.e. without blot sandwich, the electrodes have no contact. This precludes an inadvertent short-circuit, if the unit is handled properly.

Material of housing: Acrylic (PMMA)  
resistant to ethanol (10 %), not resistant to organic solvents  
(e. g. acetone)

Material of electrodes: synthetic carbon, highly conductive, resistant to corrosion  
(pH 3 – 9 with SDS)  
Attention: Mechanical cleaning only on basis of round grains of sand resp. with “Scotch Brit” sponge or alike.

## **Technical Data:**

	BF-S	BF-M	BF-L	BF-XL	BF-XXL
Blotting area (mm)	100 x 100	150 x 150	280 x 280	385 x 235	385 x 385
Min. voltage	5 V	5 V	5 V	5 V	5 V
Min. amperage	100 mA	100 mA	100 mA	100 mA	100 mA
Operating range of const. current (mA/cm <sup>2</sup> )	0.8 – 3.5	0.8 – 3.5	0.8 – 3.5	0.8 – 3.5	0.8 – 3.5
Max. operating temperature	55 °C	55 °C	55 °C	55 °C	55 °C
Buffer volumes (for gel, blot paper, membrane)	0.2 ml/cm <sup>2</sup> x no. of blotting papers + 1.0 ml/cm <sup>2</sup> transfer membrane + 1.5 ml/cm <sup>2</sup> gel				
Dimensions (cm)	15 x 28 x 8.5	20 x 28 x 8.5	45 x 33 x 8.5	37 x 43 x 8.5	50,5 x 43 x 8.5
Weight	ca. 1.5 kg	ca. 2.5 kg	Ca. 5 kg	ca. 6.5 kg	ca. 6.5 kg

## **3. Western blot after the semi-dry method**

### **3.1. Introduction**

The BlueFlash semi-dry blotter is a horizontal semi-dry blotter, which is used for the electrophoretic transfer of proteins and nucleic acids from polyacrylamide gels to transfer membranes. The procedures described herein address protein blotting.

The transfer of electrophoretically separated proteins and nucleic acids from a gel to a solid support (transfer membrane) is one of the most widely used techniques in modern life science. After immobilization of the molecules upon the transfer membrane, a broad range of analytical detection methods is available for qualitative and quantitative analysis of the blotted molecules. The choice of detection method depends on both the kind of molecules (DNA, RNA or proteins) and the selected transfer membrane.

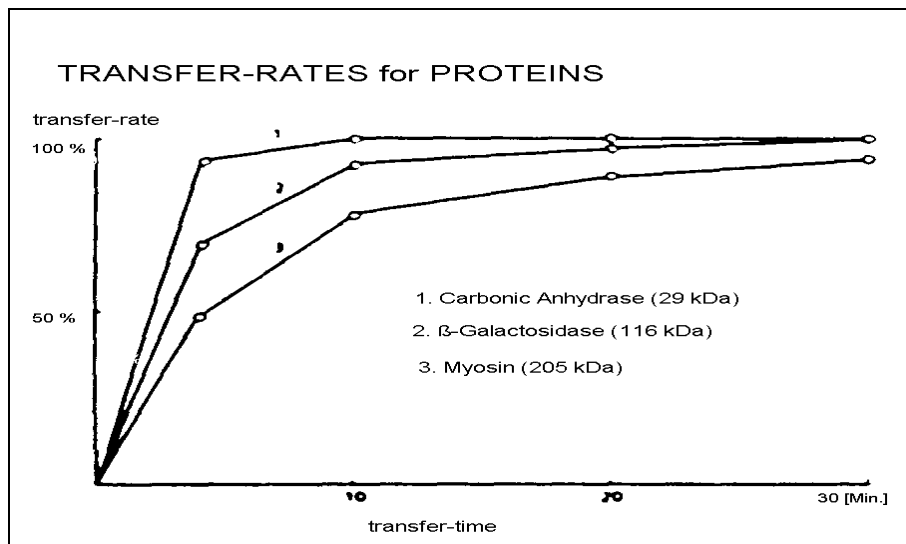
While nucleic acids are usually blotted by capillary transfer, blotting of proteins, the so-called Western Blot, is usually performed by electrophoretic transfer techniques (tank blot, semi-dry blot).

Semi-dry blotting (Kyhse-Anderson, 1984) is widely used as it is easier in handling and requires much less time and buffer as tank blotting. Especially for the transfer of high molecular weight proteins, semi-dry blotting with discontinuous systems (e.g. Kyhse-Andersen, 1984) has proven successful.

The BlueFlash semi-dry blotter has several advantages compared to tank blotters: the buffer requirements are much lower because gel blot paper stacks serve as the buffer reservoir; either the discontinuous transfer buffer (Kyhse-Anderson, 1984) or continuous buffer (Towbin, et al., 1979) systems can be used; with the BlueFlash semi-dry blotter unit 2 gels can be simultaneously transferred, and complete transfer time ranges from 5 to 45 minutes.

### 3.2. Suitable buffers for the semi-dry method

In contrast to tank blotting, either a continuous or a discontinuous buffer system can be used for semi-dry blotting. Quite often a continuous buffer system yields satisfactory results for semi-dry blotting of proteins, especially for lower molecular weight proteins. A continuous buffer system is recommended for stacked blot transfers. However to take full advantage of semi-dry blotting a discontinuous buffer system is recommended. Transfer of proteins in a discontinuous buffer system works according to the principle of isotachopheresis, similar to the stacking effect in discontinuous SDS polyacrylamide gel electrophoresis: In the stacking gel the negatively charged proteins migrate towards the anode in a highly concentrated zone. Thus, transfer of proteins in discontinuous buffer systems is very homogeneous for a wide range of molecular weights, and even large proteins like myosin (205 kDa) can be transferred with an efficiency of 80 % from a 1 mm thick 10 % PAA gel to a NC blot membrane in only 30 minutes.



#### Buffer systems

It is recommended to make buffer in 1-liter batches, which can be stored at room temperature for six months.

#### 1. for the continuous blot (according to Towbin, 1979)

Buffer	25 mM	Tris
	192 mM	glycine
	20 %	methanol

(Towbin Buffer 10x, for Western Blotting, Cat. No. 42558.02)

As an alternative, use ½-concentrated electrophoresis running buffer of your used electrophoretic gel system at anode and cathode.

Maximum amperage: 3.5 mA/cm<sup>2</sup> gel area

## **2. for the discontinuous blot (modified from Kyhse-Andersen, 1984)**

Anode buffer 1: 300 mM Tris/HCl, pH 10.4 in 20 % methanol  
Anode buffer 2: 30 mM Tris/HCl, pH 10.4 in 20 % methanol  
Cathode buffer: 40 mM 6-aminocaproic acid  
25 mM Tris/HCl, pH 9.4 in 20 % methanol  
(Semi-Dry Blotting Buffer Kit for Western Blotting, Cat. No. 42559.01)

Maximum Amperage: 2.5 mA/cm<sup>2</sup> gel area

### **Notes for transfer with methanol/SDS**

Addition of methanol: up to max. 20 % (v/v)

Addition of SDS: up to max. 1 % (w/v)

To avoid negative interference of methanol/SDS with your western blot, the use of these additives has to be tested before routine use.

Methanol:

- enhances binding of proteins to nitrocellulose,
- reduces gel swelling,
- may reduce solubility of proteins, in this case proteins are transferred less efficiently.

SDS:

- provides proteins with a homogenous negative charge,
- may change antigenic properties of proteins,
- may reduce binding of protein to the membrane.

### **3.3. Suitable transfer membranes**

For Western blot applications we recommend besides classical nitrocellulose (e.g. NC 45- resp. NC 2- nitrocellulose membrane) in particular because of the better handling properties (no breaking or tearing) fleece-enforced nitrocellulose membranes (e.g. Nylon-Bind B with 0.45 µm pore size). Nitrocellulose has ideal binding properties for most proteins and unspecific binding is easy to block. Nitrocellulose is suitable for most staining methods. Proteins under 20 kD are partially better bound with 0.2 µm pore size than with 0.4 µm. PVDF membranes (e.g. Fluorobind PVDF membrane) are used for special applications as e.g. binding of highly hydrophobic proteins or for sequencing of proteins after Western blotting. An amphoteric nylon membrane (e. g. Nylon-Bind A) is particularly suitable for the immunodetection of proteins with chemiluminescence markers because of its binding properties and interaction with the chemiluminescence substrate.

## 4. Use of the BlueFlash semi-dry blotter

### Please note:

- After the first blot it is possible that dark resp. dull spots appear on the electrode surface. This is a property of the material and does not affect blotting efficiency.
- For efficient semi-dry blotting we recommend a modification of the discontinuous buffer system of Kyhse-Andersen (1984). However, other buffer systems may be used (Buffer: see 3.2.). We recommend GB004 gel blotting paper (Whatman) for semi-dry blotting. Other gel blotting papers may be used: 1 sheet of GB004 is equivalent to 2 sheets of GB003 or 4 sheets of GB002.

### **ATTENTION:**

The **maximum operating temperature of 55 °C must not be exceeded!** This can happen by use of an electrical power >20 W over a time period of more than 30 min.

### 4.1. Performance of a semi-dry blot

#### Buffer volumes

Following discontinuous and continuous buffer volumes are recommended for pre-wetting and equilibration:

GB004 blotting paper	0.2 ml/cm <sup>2</sup> x no. of sheets
Membrane	1.0 ml/cm <sup>2</sup>
Gel	1.5 ml/cm <sup>2</sup>

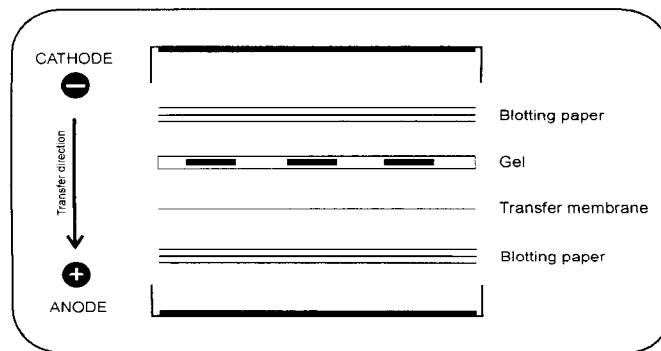
For example for a 10 x 10 cm sandwich blot:

120 ml to prewet 6 sheets of GB004 (Whatman)

100 ml to prewet the membrane

(Please note: PVDF membranes should be prewetted in 100 % methanol prior to equilibration with transfer buffer)

150 ml to equilibrate one polyacrylamide gel



**Fig. 1: Diagram of a semi-dry blot sandwich**

1. Cut transfer membrane and sheets of gel blot paper (GB 004, Whatman) to the size of the polyacrylamide gel.  
*Transfer membrane and gel blotting paper must not be cut larger than the gel to insure optimal flow of current, passing only through the gel.*
2. Carefully lay transfer membrane on bidest. water resp. by performance of a discontinuous blot on anode buffer 2 (s. p. 25) and prewet the membrane for 5 – 10 minutes by capillary forces.  
*The homogeneous and complete wetting of the membrane is a prerequisite for a good transfer! It is necessary to prewet PVDF membranes in 100 % methanol prior to equilibrating with the appropriate cathode transfer buffer.*
3. For a discontinuous blot, equilibrate gel in cathode transfer buffer for 5 minutes, otherwise use gel directly to prevent dissociative band dilation.  
*It is important to be consistent with the gel equilibration time. Shortened or extended times may affect transfer efficiencies.*
4. Prior to stacking the saturated gel blot paper on the anode, the surface of the anode should be wiped with a paper towel that has been saturated with the respective transfer buffer. After assembling of the stack, wipe surface of the cathode with the respective cathode buffer. Place three gel blot papers in Towbin buffer or half-concentrated electrophoresis running buffer, resp. by performance of a discontinuous blot two gel blot papers in anode buffer 1 (s. p. 25), let them drip off briefly and place the wet papers on the anode ((+), base of unit). Wet one gel blot paper in anode buffer 2 (s. p. 25), let it drip off briefly and stack onto the two blotting papers saturated with anode buffer 1 (s. p. 25).  
*Important is a sufficient large buffer reservoir and an air bubble-free paper stack.*
5. Place prewet membrane on top of the saturated stack of gel blot paper. Remove any air bubbles completely by gently rolling a Pasteur pipette over the stack to ensure efficient transfer.
6. Position the equilibrated gel on the transfer membrane, air bubble-free.
7. Saturate three sheets of gel blot paper in Towbin buffer or half-concentrated electrophoresis running buffer, resp. by discontinuous blotting in cathode buffer, let them drip off briefly and place on top of the gel. Remove any air bubbles.

8. Place the lid (cathode) on the base (anode) holding the blot sandwich. Connect both red and black cables to the power supply (black=cathode (-),red=anode(+))



Please make sure that lid electrode contacts have settled properly and completely into basis contacting springs  
Lid has to be completely closed before switching on power supply!  
Don't exceed specified amperage/voltage!

**Transfer conditions:**

Amperage      2.5 mA/cm<sup>2</sup> resp. 3.5 mA/cm<sup>2</sup> (a,b), constant  
Time            30 min.

Under these conditions no external cooling is necessary.

We recommend the following maximum constant amperages for the named gel formats:

- a) **discontinuous blot:** gel size 10 cm x 10 cm, 2.5 mA/cm<sup>2</sup> gel area

<u>gels/blot</u>	<u>area</u>	<u>amperage</u>
1	100 cm <sup>2</sup>	250 mA

- a) **continuous blot:** gel size 10 cm x 10 cm, 3.5 mA/cm<sup>2</sup> gel area

<u>gels/blot</u>	<u>area</u>	<u>amperage</u>
1	100 cm <sup>2</sup>	350 mA
2	200 cm <sup>2</sup>	700 mA

**ATTENTION:**

If using higher amperages/longer transfer times, electrodes and blot sandwich will heat up. **The maximum operating temperature is +55 °C.**

9. After blotting turn off the power supply, disconnect the plugs and remove the BlueFlash Semi-dry blotter cathode lid.
10. Disassemble the blot sandwich carefully. The transfer membrane is ready for immunodetection procedures.

The gel may be stained to check for complete transfer.

**Note:**

Under the conditions suggested above, myosin (205 kD) is transferred to the mem-brane from a 1 mm thick 10 % PAA gel with an efficiency of at least 80 % using the discontinuous buffer system. For different samples/proteins the standard protocol can be adjusted according to individual requirements by decreasing or increasing transfer times. **In this case strictly pay attention to potential heating up of blot sandwich and electrodes.**

## 4.2. Performance of a semi-dry blot with two stacked gels

The simultaneous transfer of two gels can be performed in the BlueFlash semi-dry blotter without the need for dialysis membranes or cellophane sheets between the layers. The **continuous buffer system** is recommended for optimal transfer efficiency.

1. Prepare gels, membranes and gel blot paper as described above, using continuous buffer conditions.
2. Place air bubble-free three sheets of prewetted gel blot paper on top of the transfer stack of the first gel, followed by the second membrane, second gel and three more sheets of prewetted gel blot paper. Hereon put the lid with the cathode.
3. Measure the surface area of the top of the stack to determine proper current. Transfer at 3.5 mA/cm<sup>2</sup> of gel area for 30 minutes.
4. Switch off the power supply after transfer, disconnect the plugs and remove the blotter lid.
5. Carefully disassemble the blot sandwich. Both transfer membranes are now ready for further analysis.

## 4.3. Maintenance of the BlueFlash semi-dry blotter after each transfer

After each transfer clean the electrodes using a damp cloth wetted with deionized water or a mild detergent solution (e.g. 0.1 % SDS). By severe soiling electrodes can be cleaned using scouring agents based on spheric particles (e.g. scouring agents for ceramic glass hobs). Additives of the scouring agent have to be wiped off by ethanol (10 %) after cleaning. For cleaning of the housing use a mild detergent solution or, if necessary, ethanol (10 %).

<b>Important: Never use any organic solvent for cleaning of electrodes or housing!</b>
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## 5. Trouble shooting guide

<u>Problem</u>	<u>Cause</u>	<u>Solution</u>
No transfer	<ul style="list-style-type: none"> <li>• Wrong order of membrane and gel</li> <li>• Wrong connection to the power supply</li> <li>• Acidic buffer system: transfer towards cathode!</li> </ul>	<p>Reverse order of membrane and gel</p> <p>Correct polarity: red = anode (+), black = cathode (-)</p> <p>Place transfer membranes on cathode side (lid) and gel on anode side (base)</p>
Incomplete transfer	<ul style="list-style-type: none"> <li>• Transfer time too short (check by gel staining)</li> <li>• Transfer time too long. „blow through“, i. e. proteins are stained on both sides of the membrane</li> </ul>	<p>Increase transfer time</p> <p>Reduce transfer time, use nitrocellulose with smaller pore size or PVDF membrane.</p>
Irregular transfer	<ul style="list-style-type: none"> <li>• Air bubbles in the blot sandwich.</li> <li>• Generation of air bubbles during long transfer times due to electrolysis and heating up</li> <li>• Irregular current flow as size of blotting papers and transfer membrane are larger than gel size</li> <li>• blotting papers partially dried</li> <li>• Insufficiently soaked transfer membrane</li> <li>• Dirty electrodes</li> </ul>	<p>Remove air bubbles by gently rolling e.g. a Pasteur pipette over the membrane.</p> <p>Reduce transfer time, chill buffers before use.</p> <p>Transfer membranes and blotting papers must be cut exactly to gel size (see page 8)</p> <p>See page 8, reduce transfer time</p> <p>See page 8</p> <p>See page 10, cleaning of electrodes</p>
Proteins are stained on both sides of the membrane	<ul style="list-style-type: none"> <li>• Transfer time too long, „blow through</li> </ul>	<p>Reduce transfer time/use nitrocellulose with smaller pore size or PVDF membrane</p>
Power supply turns off	<ul style="list-style-type: none"> <li>• Power supply does not work at low voltages (inner resistance too high)</li> </ul>	<p>Use power supply which can operate at 10 V</p>
Strong increase of voltage during transfer	<ul style="list-style-type: none"> <li>• Blot sandwich dries out</li> <li>• Generation of electro-lyses gases at the electrodes</li> <li>• Insufficiently soaked transfer membrane</li> </ul>	<p>Reduce amperage, soak blotting papers with more transfer buffer</p> <p>Chill buffers before use, reduce transfer time</p> <p>See page 10</p>
Intensive heating up	<ul style="list-style-type: none"> <li>• Amperage too high</li> <li>• Transfer time too long</li> <li>• Conductivity of buffer too high</li> </ul>	<p>Reduce amperage</p> <p>Chill buffers before use, reduce transfer time</p> <p>Check your buffers, use buffers described on page 5/6.</p>

## 6. Literature

- 1) Kyhse-Andersen, J. 1984. J. Biochem-Biophys. Methods 10: 203-209
- 2) Towbin, H; Staehelin, T.; Gordon, J. 1979. Proc. Natl. Acad. Sci. USA 76: 4350-4354
- 3) Westermeier, R. 1990. Elektrophoresepraktikum VCH, D-69451 Weinheim

## 7. Recommended products for the protein transfer

SERVA reagents for electrophoresis underlie stringent quality and application control to ensure best performance and results. We recommend the usage of SERVA electro-phoresis reagents especially along with BlueLine electrophoresis instruments as the quality of consumables is fine-tuned to the equipment (applications test).

Product	Cat. No.	Packaging size
$\epsilon$ -Aminocaproic Acid	12548	100 g
Glycin analytical grade	23390	100 g 500 g 1 kg 5 kg
Tris	37190	250 g 1 kg 5 kg
SDS Sodium dodecyl sulfate	20760	100 g 250 g 1 kg
Sodium dodecyl sulfate in pellets	20765	100 g 250 g 1 kg
Methanol for HPLC	45630	2.5 l
Semi-dry blotting buffer kit (for Western Blotting)	42559	3 x 500 ml
Towbin buffer 10x, for Western Blotting	42558	2 l
Nitrocellulose NC 45, 0.45 $\mu$ m	42516	10 sheets 8.8 x 8.8 cm
Nitrocellulose NC 45, 0.45 $\mu$ m	71208	1 roll 30 cm x 3 m
Nitrocellulose NC 2, 0.2 $\mu$ m	71223	5 sheets 20 x 20 cm
Nitrocellulose NC 2, 0.2 $\mu$ m	71224	1 roll 30 cm x 3 m
SERVA Fluorobind, PVDF, 0.2 $\mu$ m	42572	10 sheets 20 x 20 cm
SERVA Fluorobind, PVDF, 0.2 $\mu$ m	42573	20 sheets 10 x 10 cm
SERVA Fluorobind, PVDF, 0.2 $\mu$ m	42571	1 roll 25 cm x 3 m
SERVA Nylon-Bind A, amphoteric, 0.2 $\mu$ m	42566	1 roll 30 cm x 3 m

<b>Product</b>	<b>Cat. No.</b>	<b>Packaging size</b>
SERVA Nylon-Bind A, amphoteric, 0.45 µm	42517	10 sheets 8,0 x 8,3 cm
SERVA Nylon-Bind A, amphoteric, 0.45 µm	42564	20 sheets 10 x 10 cm
SERVA Nylon-Bind A, amphoteric, 0.45 µm	42567	1 roll 30 cm x 3 m
SERVA Nylon-Bind A, amphoteric, 0.45 µm	42568	10 sheets 20 x 20 cm
SERVA Nylon-Bind B, positive, 0.45 µm	42518	10 sheets 8.0 x 8.3 cm
SERVA Nylon-Bind B, positive, 0.45 µm	42565	20 sheets 10 x 10 cm
SERVA Nylon-Bind B, positive, 0.45 µm	42569	1 roll 30 cm x 3 m
SERVA Nylon-Bind B, positive, 0.45 µm	42570	10 sheets 20 x 20 cm
Fluorobind Membrane, PVDF, 0.2 µm	42571	1 roll 25 cm x 3 m
Fluorobind Membrane, PVDF, 0.2 µm	42572	10 sheets 20 cm x 20 cm
Fluorobind Membrane, PVDF, 0.2 µm	42573	20 sheets 10 cm x 10 cm
Immobilon™-P-membrane, PVDF, 0.2 µm	42574	1 roll 26,5 cm x 3,75 m
Immobilon™-P-membrane, PVDF, 0.2 µm	42579	10 sheets 9 cm x 12 cm