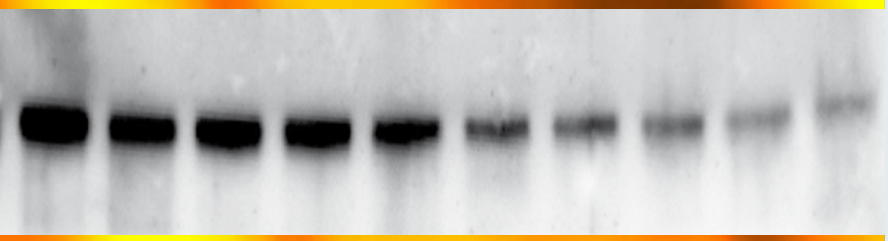
Western Blotting

SERVA
serving scientists



Reagents and Equipment

- Molecular Weight Standards
- SDS PAGE for Western Blotting
- Transfer Buffers
- Transfer Membranes
- Blocking Reagents
- Detection Systems
- Instruments for Western Blotting

All you need for...
Blotting and Detection of Proteins

Blotting of proteins onto a membrane after separation by electrophoresis is a routine method in all protein labs around the world. By applying the Western blot technology proteins first are separated by gel electrophoresis like SDS PAGE, native PAGE, Isoelectric Focusing or 2D electrophoresis. Subsequently, the proteins are transferred by electro transfer (semi-dry, tank blotting) or capillary transfer from the gel onto a membrane, either nitrocellulose, nylon or PVDF. To check transfer efficiency blotted proteins could be reversible stained on the membrane with Ponceau S. Also the use of a prestained protein standard helps to visualize the transfer and to judge the overall qualtity of the blot. After blocking of the membrane specific proteins could be detected by primary/secondary antibodies coupled with a chromogenic or luminescence detection system.

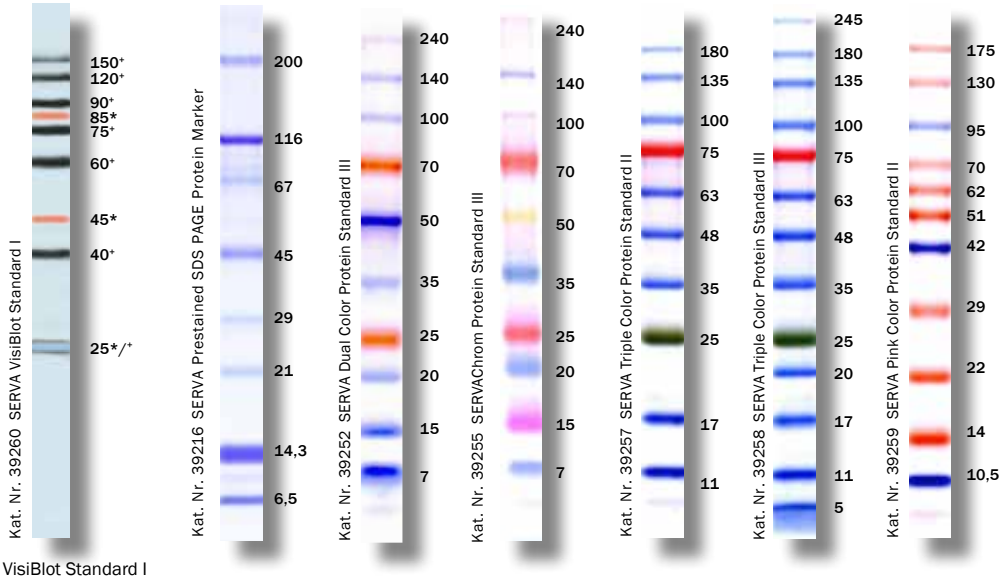
Blotting of proteins onto a membrane after separation by electrophoresis is a routine method in all protein labs around the world.

1 Molecular Weight Markers for Western Blotting

VisiBlot Standard I is a mixture of 10 recombinant proteins with molecular weight range from 25 kDa to 150 kDa. The prestained protein bands of 25 kDa, 45 kDa and 85 kDa allow monitoring of the protein separation during SDS PAGE. The remaining seven proteins contain several IgG binding sites. The binding of primary or secondary antibodies in Western blotting facilitates marker visualization on the transfer membrane. Because the proteins have no chromophore attached, the marker enables accurate molecular weight estimation. Recommended loading volume for a mini gel is 5 µl/lane.

Beside applying prestained standard proteins to visualize blotting efficiency (see table below) the VisiBlot Standard I offers important advantages:

- Ready-to-use, no reconstitution, further dilution or heating required
- Prestained bands for monitoring electrophoresis and membrane transfer efficiency
- Visualization of marker proteins on Western Blots by horseradish peroxidase or alkaline phosphatase-based immuno-detection methods
- Molecular weight determination of proteins detected on transfer membrane



SERVA VisiBlot Standard I
+ proteins with antibody binding sites
* prestained proteins

Product	Size	Cat. no.
SERVA VisiBlot Standard I	500 µl	39260.01
SERVA Prestained SDS PAGE Protein Marker 6.5 - 200 kDa, liquid mix	2 x 250 µl	39216.01
SERVA Dual Color Protein Standard III	500 µl	39252.01
SERVAChrom Protein Standard III	500 µl	39255.01
SERVA Triple Color Protein Standard II	500 µl	39257.01
SERVA Triple Color Protein Standard III	500 µl	39258.01
SERVA Pink Color Protein Standard II	500 µl	39259.01

VisiBlot Standard I covers all musts of a modern protein standard for Western blotting experiments

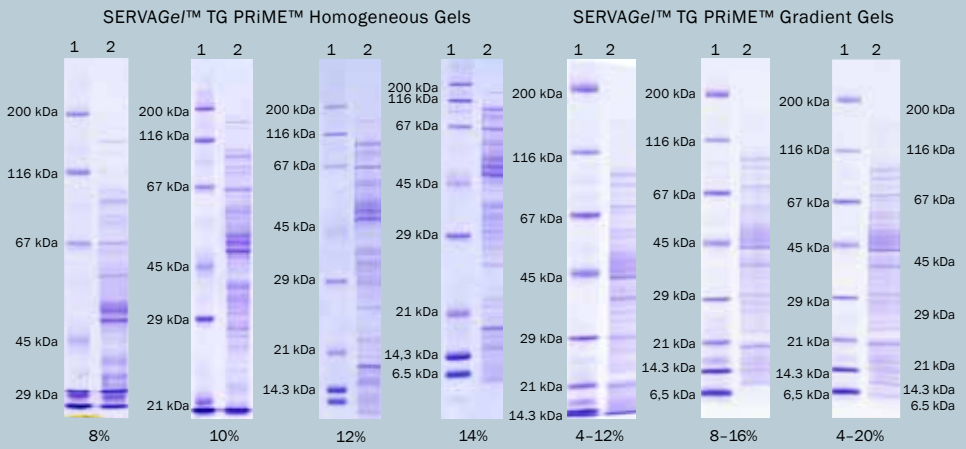
A set of prestained protein markers to check electrophoresis run and blotting efficiency

2 SDS PAGE Gels for Western Blotting

Top resolution, efficient transfer and a sensitive detection system are the most important requirements for best results in Western blotting experiments. By applying SERVA*Gel*™ TG PRiME™ precast gels excellent separation of your protein sample will be achieved. SERVA*Gel*™ Neutral HSE is optimized for very short running times. Use BlueVertical™ PRiME™ mini slab gel unit to run SERVA*Gel*™ precast gels.

SERVA*Gel*™ TG PRiME™ Precast Gels

- High resolution
- Available as homogeneous or gradient gel, with 10, 12 or 15 wells, or 2D well
- Laemmli buffer system, comparable separation patterns to standard Laemmli gels
- In stable plastic cassette (10 cm x 10 cm x 0.7 cm)
- Long shelf life (9 to 12 months, depending on acrylamide concentration)



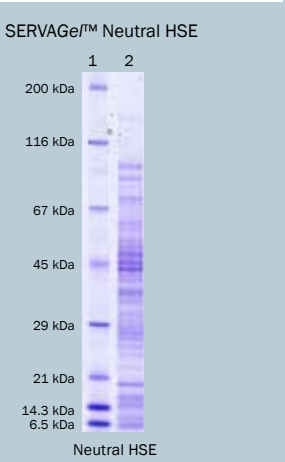
1 SERVA Unstained SDS PAGE Protein Marker (cat. no. 39215) **2** *E. coli* extract

Gel type	15 sample wells	12 sample wells	10 sample wells	2D well	Size
SERVA*Gel*™ TG PRiME™ 8 %	43284.01	43260.01	43261.01	-	10 gels
SERVA*Gel*™ TG PRiME™ 10 %	43285.01	43263.01	43264.01	-	10 gels
SERVA*Gel*™ TG PRiME™ 12 %	43286.01	43266.01	43267.01	43268.01	10 gels
SERVA*Gel*™ TG PRiME™ 14 %	43287.01	43269.01	43270.01	43271.01	10 gels
SERVA*Gel*™ TG PRiME™ 4-12 %	43288.01	43273.01	43274.01	-	10 gels
SERVA*Gel*™ TG PRiME™ 4-20 %	43289.01	43276.01	43277.01	-	10 gels
SERVA*Gel*™ TG PRiME™ 8-16 %	43290.01	43279.01	43280.01	43281.01	10 gels

- **Short set-up times, gels are ready-to-use**
- **Separation distance: 7 cm**

SERVAGel™ Neutral HSE

- High Speed Electrophoresis (HSE) – run your gel in 20 minutes
- Best suited for tank and semi-dry blotting due to lower acrylamide concentration compared to standard Laemmli gels
- Top resolution, pattern comparable to SERVAGel™ TG PRiME™ gradient gel 4 – 20 %
- Long shelf life (15 months)



Product	Sample Wells	Size	Cat. no.
SERVAGel™ Neutral HSE	10	10 gels	43246.01
SERVAGel™ Neutral HSE	12	10 gels	43245.01
SERVAGel™ Neutral HSE	15	10 gels	43249.01
SERVAGel™ Neutral HSE	2D well	10 gels	43247.01

BlueVertical™ PRiME™

The BlueVertical™ PRiME™ is a dual mini tank system with the option to operate one or two precast gels. It accomodates SERVAGel™ TG PRiME™, all other types of SERVAGel™ and all other commercially available precast gels with an outer cassette dimension of 10 x 10 x 0.7 cm. The fixture of the inner core unit has been re-engineered to provide four robust clamps (two on both sides) which fix two precast gel cassettes properly and tightly in their correct position. When quality becomes an issue – choose BlueVertical™ PRiME™.

- Dual mini tank systems
- Accommodates 1 – 2 gels in cassettes with outer dimensions of 10 cm x 10 cm x 0.7 cm
- Unique, leak-free clamp system

Product	Size	Cat. no.
BlueVertical™ PRiME™	1 unit	BV 104

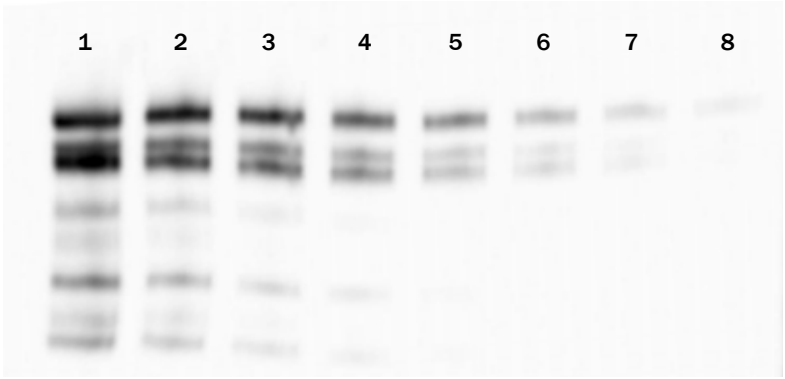
- **SERVAGel™ Neutral HSE gels - Best suited for Blotting**
- **BlueVertical™ PRiME™: High quality electrophoresis tank for top results in electrophoresis**

3 Transfer Buffers

Xpress Blotting Kit

The SERVA Xpress Blotting Kits allow fast and efficient semi-dry transfer of proteins in only 15 minutes. With the ready-to-use SERVA Xpress Blotting Buffer the efficient simultaneous semi-dry blotting of high and low molecular weight proteins is possible. The use of SERVA´s newly developed Blotting Fleece instead of blotting paper enables an undisturbed transfer in a short time. The buffer system is compatible with nitrocellulose and PVDF membranes. Any semi-dry blotter with a capacity of 400 mA can be used. The kit includes 250 ml 10x SERVA Xpress Blotting Buffer and 20x Blotting Fleece sheets (size 80 mm x 85 mm). Kits including additionally precutted NC or PVDF membranes (10x, 80 mm x 85 mm) are available.

- Fast and efficient transfer in only 15 min
- Ready- to-use – no preparing of buffer or cutting of blotting papers or membranes
- Blotting Fleece instead of several layers of blotting paper for undisturbed transfer



Lane: 1: 5 µg Collagenase, 2: 2.5 µg Collagenase, 3: 1.25 µg Collagenase, 4: 0.625 µg Collagenase, 5: 0.313 µg Collagenase, 6: 0.156 µg Collagenase, 7: 78 ng Collagenase, 8: 39 ng Collagenase

Other Transfer Buffers

As an alternative, for standard procedures Towbin Buffer for Western Blotting and Semi-Dry Blotting Buffer Kit consisting of three buffers for efficient transfer of small and large proteins are available.

Product	Size	Cat. no.
Xpress Blotting Buffer (10x)	1 L	42661.01
Xpress Blotting Kit	1 kit	42662.01
Xpress NC Blotting Kit	1 kit	42663.01
Xpress PVDF Blotting Kit	1 kit	42664.01
Towbin Buffer for Western Blotting, 10x concentrated	1 L	42558.02
Semi-Dry Blotting Buffer Kit	3x 500 ml	42559.01

Xpress Blotting Kit for fast and efficient blotting of proteins

4 Transfer Membranes

For blotting of proteins SERVA offers all types of membranes: Nitrocellulose, Nylon and PVDF.

- The “supported” nitrocellulose membranes are fibre-reinforced, enabling easier handling and cutting, stripping and repeated hybridization as well as automated immobilizing
- Nylon-Bind membranes feature low background, high sensitivity and high binding capacity
- The PVDF membranes are non-fluorescent with excellent mechanical stability and compatibility with most staining and immunological methods

Product	Pore size	Format	Size	Cat. no.
NC 2 Nitrocellulose Membrane (roll)	0.22 µm	30 cm x 3 m	1 roll	71224.01
NC 2 Nitrocellulose Membrane (sheets)	0.22 µm	20 cm x 20 cm	5 sheets	71223.01
NC 2 Supported Nitrocellulose Membrane (roll)	0.22 µm	30 cm x 3 m	1 roll	71226.01
NC 45 Nitrocellulose Membrane (roll)	0.45 µm	30 cm x 3 m	1 roll	71208.01
NC 45 Nitrocellulose Membrane (sheets)	0.45 µm	88 mm x 88 mm	10 sheets	42516.01
NC 45 Supported Nitrocellulose Membrane (roll)	0.45 µm	30 cm x 3 m	1 roll	71225.01
Nylon-Bind B Membrane, positive surface (roll)	0.45 µm	30 cm x 3 m	1 roll	42569.01
Fluorobind Membrane, surface PVDF (roll)	0.22 µm	25 cm x 3 m	1 roll	42571.01
Immobilon™-P-Membrane (roll)	0.22 µm	26.5 cm x 3.75 m	1 roll	42574.01
Immobilon™-P-Membrane (roll)	0.45 µm	26.5 cm x 3.75 m	1 roll	42581.01
PVDF 0.2 Transfer Membrane (roll)	0.22 µm	30 cm x 3 m	1 roll	42515.01
PVDF 0.45 Transfer Membrane (roll)	0.45 µm	30 cm x 3 m	1 roll	42514.01

5 Blocking Reagents

It is important to block all free membrane surfaces by unspecific molecules to avoid false signals from antibodies. The protein-free BlueBlock PF (10x) solution is the best choice to achieve an excellent signal-to-noise ratio in Western blotting. Unspecific proteins like albumins or skim milk powder may also be used as blocking reagent.

- BlueBlock PF
- Albumin bovine (BSA)
- Skim Milk powder

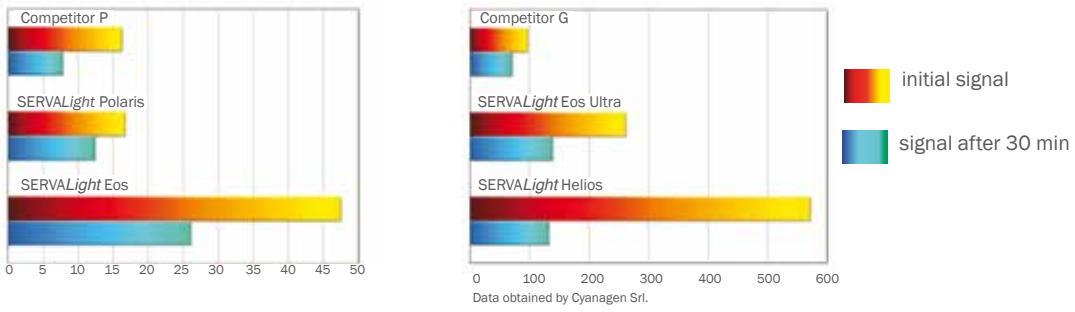
Product	Size	Cat. no.
BlueBlock PF (10x)	250 ml	42591.01
	1 L	42591.02
Albumin Bovine Fraction V, pH 7.0 (BSA)	10 g	11930.01
	25 g	11930.02
	100 g	11930.03
Skim Milk Powder	500 g	42590.01
	1 kg	42590.02
	5 kg	42590.03

6 Detection Systems

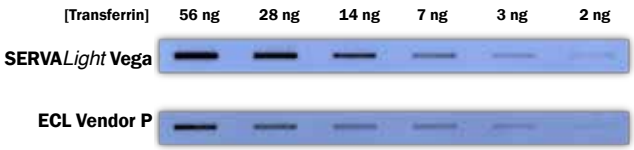
SERVA*Light* HRP Chemiluminescence Kits are a family of highly sensitive ready-to-use kits for chemiluminescence detection of membrane bound antigens (Western Blot) or nucleic acid sequences (Southern and Northern Blot), labelled directly with Horseradish Peroxidase (HRP) or indirectly with HRP-conjugated antibodies/streptavidin. They are easy to use, have an excellent stability, extended signal duration and save money and precious antibodies due to high dilution of antibodies.

SERVA*Light* CL HRP WB Substrate Kits

- SERVA*Light* Vega - directly compatible with protocols of standard ECL western blot substrates of other vendors, no change of protocol but superior performance and less costs
- Extended signal duration – all substrates show long light emission, but signal duration is optimized for SERVA*Light* EosUltra with an outstanding light emission for 18 – 20 hours at a very high signal
- Easy to use – simply mix the two components, a luminol/enhancer solution and a stabilized peroxide solution in a one-to-one ratio.
- Excellent stability – at least one year stable when stored at room temperature
- Economical - save money and precious antibodies due to high dilution of antibodies



Data obtained by Cyanagen Srl.



Sensitivity

SERVA*Light* type	Signal intensity	Signal duration	Protein quantity	Detection limit
Vega	Standard	Short	High abundance	Low picogram
Polaris	Medium	Good	High abundance	Low picogram
Eos	High	Long	Medium abundance	High femtogram
EosUltra	Very high	Very long	Low abundance	Mid femtogram
Helios	Extreme	Moderate	Very low abundance	Low femtogram

Specifications

SERVA*Light* type	Primary Ab dilution	Secondary Ab dilution	Working solution stability	Concentration
Vega	1:100 – 1:5,000	1:1,000 – 1:15,000	5 days	0.1 ml/1 cm²
Polaris	1:500 – 1:5,000	1:20,000 – 1:100,000	24 h	
Eos	1:1,000 – 1:15,000	1:25,000 – 1:150,000	24 h	
EosUltra	1:5,000 – 1:50,000	1:50,000 – 1:250,000	8 h	
Helios	1:5,000 – 1:100,000	1:100,000 – 1:500,000	8 h	

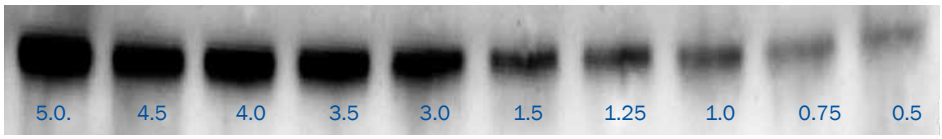
- **Easy and safe to use, excellent stability and an extended signal duration for best results in chemilumninescence detetction**
- **Five different substrate kits for standard application with the Vega kit up to highest sensitivity with the Helios kit**

Detection Reagents for Western Blotting

The SERVAColor blot solutions are ready-to-use and non-toxic. Outstanding features are rapid precipitate formation due to high activity, a very low background, long term stability at room temperature as well as no significant fading after reaction stop.

- SERVAColor BCIP/NBT Blot Solution
  - Highly sensitive substrate solution for detection of alkaline phosphatase (AP)
- SERVAColor TMB Blot Solution
  - Highly sensitive substrate solution for detection of horseradish peroxidase (HRP
- Beside SERVA*Light* and SERVAColor kits SERVA offers stand alone detection reagents like Luminol, TMB, BCIP and NBT

Product	Size	Cat. no.
SERVA*Light* Vega CL HRP WB Substrate Kit	50 ml	42588.01
	250 ml	42588.02
	500 ml	42588.03
SERVA*Light* Polaris CL HRP WB Substrate Kit	100 ml	42584.01
	250 ml	42584.02
	500 ml	42584.03
SERVA*Light* Eos CL HRP WB Substrate Kit	50 ml	42585.01
	250 ml	42585.02
	500 ml	42585.03
SERVA*Light* EosUltra CL HRP WB Substrate Kit	20 ml	42586.01
	100 ml	42586.02
SERVA*Light* Helios CL HRP WB Substrate Kit	20 ml	42587.01
	100 ml	42587.02
	200 ml	42587.03
SERVAColor BCIP/NBT Blot Solution	250 ml	15245.01
SERVAColor TMB Blot Solution	100 ml	37071.01
	250 ml	37071.02
3,3',5,5'-Tetramethylbenzidine (TMB)	5 g	35926.02
	25 g	35926.03
Luminol	5 g	28085.01
5-Bromo-4-chloro-3-indolyl-phosphate:p-toluidine salt (BCIP)	100 mg	15247.02
	500 mg	15247.03
Nitro Blue Tetrazolium Chloride (NBT)	250 mg	30550.01
	1 g	30550.02
	5 g	30550.03
Amido Black 10 B	25 g	12310.01
Ponceau S	5 g	33429.01
	25 g	33429.02
Ponceau S Solution for Electrophoresis	500 ml	33427.01



Human Transferrin was diluted (5 to 0.5 ng) and electrophoresis was performed. The gel was transferred to PVDF membranes, blocked and incubated with 1:20,000 rabbit anti-transferrin. After washing, the membranes were incubated with 1:100,000 of HRP-conjugated goat anti-rabbit antibody. The membrane was washed again and then incubated with SERVA*Light* EOSUltra. Exposure time was 300 sec.

For your convenience: SERVAColor blot solutions ready-to-use

Ponceau S for reversible staining of proteins bound to membranes

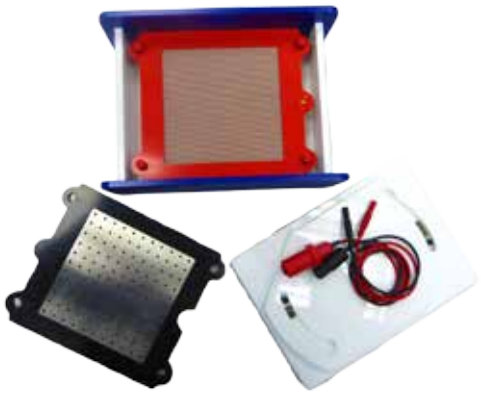
7 BlueBlot Semi-Dry Blotter

The BlueBlot semi-dry blotter forms a homogeneous electrical field that guarantees fast and efficient transfer of proteins from gel to membrane. As associated with semi-dry blotting compared to tank blotting less heat is generated for gentle protein transfer. It is fast and requires less buffer. By applying the Xpress blotting buffer (cat. no. 42662) semi-dry transfer of high and low molecular weight proteins is done fast and efficient within 15 minutes. Moreover, all common continuous and discontinuous buffer systems can be applied without any limitations.

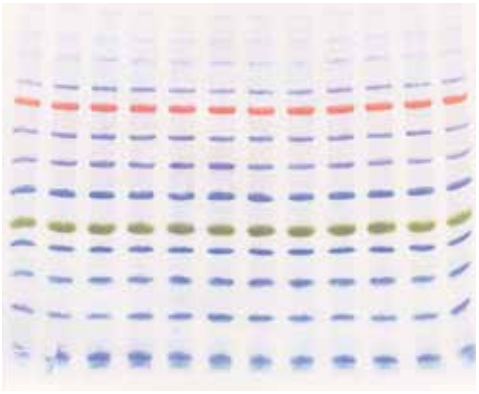
- Platinum-covered steel net as anode, spring-mounted
- Stainles steel plate as cathode
- Versatile blotting areas (11 cm x 11 cm, 17 cm x 17 cm, 24 cm x 26 cm)
- Deployable for thicker gels and blotting stacks

The anode is made from a steel net covered by platinum, the cathode is a stainless steel plate. The spring-mounted anode allows blotting of thicker gels and gel stacks. To avoid air bubbles within the blotting system the cathode carries drill holes to transport gas generated by the electro-chemically blotting process from inside to outside. The electrodes are built into a stable acrylic housing that is resistant to 10 % ethanol and easy to clean. The long-lasting electrodes can be detached and cleaned separately.

Product	Blot Area	Size	Cat. no.
BlueBlot Semi-Dry Blotter SD 11	11 cm x 11 cm	1 unit	BB-SD11
BlueBlot Semi-Dry Blotter SD 17	17 cm x 17 cm	1 unit	BB-SD17
BlueBlot Semi-Dry Blotter SD 26	24 cm x 26 cm	1 unit	BB-SD26
Electrode Set for BB-SD11	11 cm x 11 cm	1 unit	BB-E11
Electrode Set for BB-SD17	17 cm x 17 cm	1 unit	BB-E17



The BlueBlot Semi-Dry Blotter consists of a spring-mounted anode and a drill holes carrying cathode made from stainless steel. The housing is closed with the lid and secured by a newly developed rotating device mechanism. Due to the design of the cable pins wrong connection is excluded.



Best blot results for small and large proteins.

8 Additional Instruments for Western Blotting

Gravity Blotter

The SERVA Gravity Blotter allows to blot film-based IEF, SDS PAGE and 2D gels at high efficiency. The unit consists of a base plate with a transfer area of 14 x 29 cm. The pressure is provided by aluminum plates that are placed on top of the blotting stack. The results are comparable to tank or semi-dry transfer methods. Transfer time is 4 h or overnight.

BluePower™ Power Supply

The BluePower™ 300 BLOT power supply easily handles all your blotting applications including large format gels up to 24 cm x 26 cm. BP-300-BLO is fully programmable, offering up to 9 multi-step settings and saving up to 9 programs (300 V, 2000 mA, 300 W, 4 pairs of outlet terminators). For running and blotting of mini gels the BluePower™ 600 PRIME™ power supply (BP-600-PRI, 600 V, 1000 mA, 300 W, 4 pairs of outlet terminators) is best suited.

BIO-5000P

The BIO-5000 Plus VIS Gel Scanner is a dual platform scanner specially designed for scanning of electrophoresis gels and blots by visual detection. It is equipped with energy-saving LEDs and an optical CCD whose resolution is up to 4,800 dpi, including CRF 21 Part 11 conformity if required.

SERVA Musketeer

The SERVA Musketeer is an advanced gel documentation system for white and UV light, fluorescence and chemiluminescence image capturing. This imaging system consists of a scientific CCD camera (16 bit, cooled -55 °C +/- 5 °C to ambient, 6X6 Bin mode), motor driven lens, and white backlight board. The chemiluminescence treatment such as ECL can easily be observed and captured by the SERVA Musketeer, including CRF 21 Part 11 conformity if required.

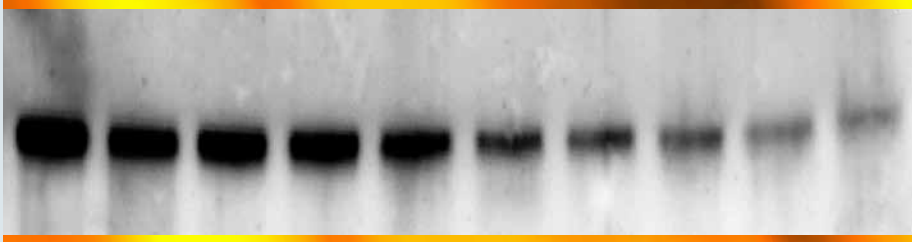
Product	Size	Cat. no.
Gravity Blotter	1 unit	GB-14X29
BluePower™ 300 BLOT Power Supply	1 unit	BP-300-BLO
BluePower™ 600 PRIME™ Power Supply	1 unit	BP-600-PRI
BIO-5000 Plus VIS Gel Scanner	1 unit	BIO-5000P
SERVA Musketeer	1 unit	MSK-01

SERVA

serving scientists

SERVA WORLDWIDE

www.serva.de



SERVA Electrophoresis GmbH
Carl-Benz-Str. 7
69115 Heidelberg / Germany
Fon: +49 6221 13840-0
Fax: +49 6221 13840-10
E-mail: info@serva.de · www.serva.de

Version 18/06