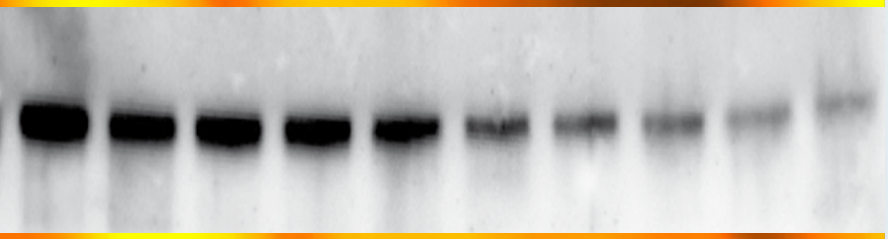
Western Blotting

SERVA
serving scientists



Reagents and Equipment

DOMINIQUE DUTSCHER SAS

- Molecular Weight Standards
- SDS PAGE for Western Blotting
- Transfer Buffers
- Transfer Membranes
- Blocking Reagents
- Stripping Buffer
- 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.

1 Molecular Weight Standards for Western Blotting

2 SDS PAGE for Western Blotting

3 Transfer Buffers

4 Transfer Membranes

5 Blocking Reagents

6 Stripping Buffer

7 Detection Systems

8 Instruments for Western Blotting

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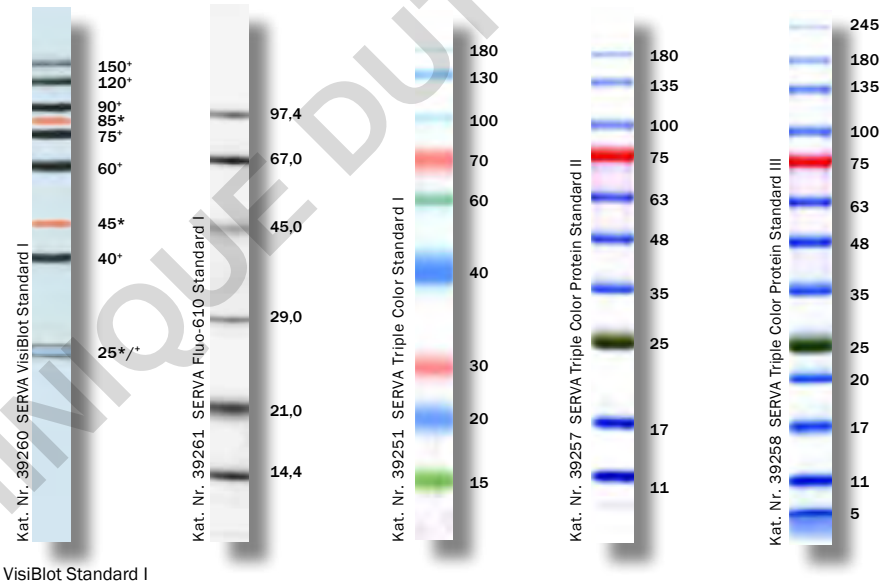
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1 Molecular Weight Markers for Western Blotting

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 Fluo-610 Standard I	500 µl	39261.01
SERVA Triple Color Standard I	500 µl	39251.01
SERVA Triple Color Protein Standard II	500 µl	39257.01
SERVA Triple Color Protein Standard III	500 µl	39258.01

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

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

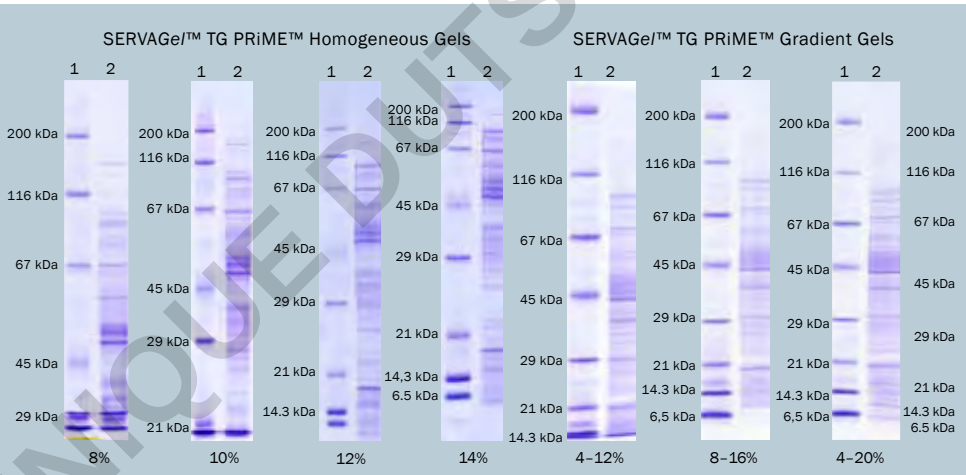
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2 SDS PAGE Gels for Western Blotting

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

SERVAGel™ 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

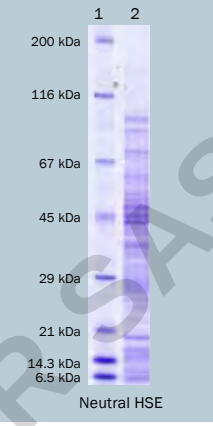
Gel type	15 sample wells	12 sample wells	10 sample wells	2D well	Size
SERVAGel™ TG PRiME™ 8 %	43284.01	43260.01	43261.01	-	10 gels
SERVAGel™ TG PRiME™ 10 %	43285.01	43263.01	43264.01	-	10 gels
SERVAGel™ TG PRiME™ 12 %	43286.01	43266.01	43267.01	43268.01	10 gels
SERVAGel™ TG PRiME™ 14 %	43287.01	43269.01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

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SERVAGel™ Neutral HSE

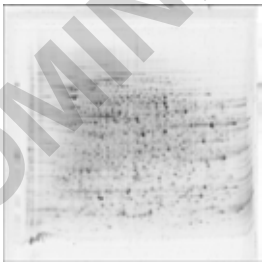
- High Speed Electrophoresis (HSE) – run your gel in 20 minutes
- Best suited for tank and semi-dry blotting due to lower acrylamide concentration compared to standard Laemmli gels
- Top resolution, pattern comparable to SERVAGel™ TG PRiME™ gradient gel 4 – 20 %
- Long shelf life (15 months)

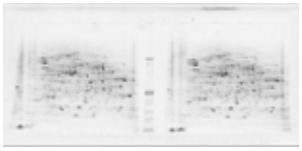


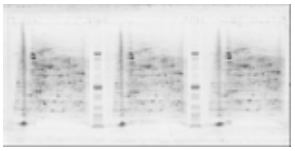
Product	Sample wells	Size	Cat. no.
SERVAGel™ Neutral HSE	10	10 gels	43246.01
SERVAGel™ Neutral HSE	12	10 gels	43245.01
SERVAGel™ Neutral HSE	15	10 gels	43249.01
SERVAGel™ Neutral HSE	2D well	10 gels	43247.01

2D HPE™ BlotGels

- Analysing target proteins by large format, horizontal 2D Western blotting
- Perform 2D WB HCP analysis or other applications requiring blotting of 2D gels
- How the transfer from 2D gel to membrane simply works:
  - A narrow edge strip is covalently bonded to the backing film
  - Most of the gel matrix adheres only non-covalent
  - Before the semi-dry blot, the covalently bound strip is simply cut off
  - Gel is ready for 2D Western Blot
- Gel size is 250 mm x 110 mm x 0.65 mm, available in three formats:
  - „Large“ for one 24 cm IPG strip
  - „Double“ for two 11 cm IPG strips
  - „Triple“ for three 7 cm IPG strips
- Best suited for semi dry blotting on BlueBlot Semi Dry Blotter SD 26 (see p. 11)







Product	Size	Cat. no.
2D HPE™ Triple BlotGel NF 12.5 % Kit	1 kit	43429.01
2D HPE™ Double BlotGel NF 12.5 % Kit	1 kit	43430.01
2D HPE™ Large BlotGel NF 12.5 % Kit	1 kit	43432.01

SERVAGel™ Neutral HSE gels for mini vertical gel tank and semi dry blotting

2D HPE™ BlotGels for large format horizontal 2D gel semi dry blotting

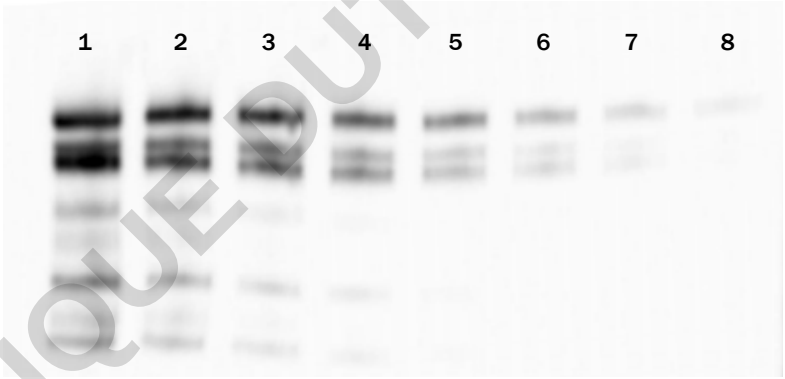
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3 Transfer Buffers

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

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4 Transfer Membranes

- The PVDF membranes are non-fluorescent with excellent mechanical stability and compatibility with most staining and immunological methods
- Fluorobind™ for fluorescent detection

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 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
Fluorobind Membrane, surface PVDF (roll)	0.22 µm	25 cm x 3 m	1 roll	42571.01
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

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), for Blotting and ELISA	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
	500 g	11930.04
Skim Milk Powder	500 g	42590.01
	1 kg	42590.02
	5 kg	42590.03

6 Stripping Buffer

- Remove even highly specific antibodies from membranes
- Ready and easy-to-use:
  - Incubate the membrane in BlueClear SB, then
  - Wash – Block – Probe again
- Without ß-ME or DTT
- Suitable for NC and PVDF membranes



Product	Size	Cat. no.
BlueClear SB Antibody Stripping Buffer	250 ml	42599.01
	1 L	42599.02

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7 Detection Systems

SERVA*Light* HRP chemiluminescence substrates are a family of highly sensitive reagents for detection of membrane bound antigens (Western Blot), labelled directly with Horseradish Peroxidase (HRP) or indirectly with HRP-conjugated antibodies/streptavidin. They are easy to use, have an excellent stability of at least one year, and save money and precious antibodies due to high dilution of antibodies at a working concentration of 0.1 ml/cm² during the detection process. Most important, the signal duration is significantly extended compared to tother comercially available systems.

SERVA*Light* CL HRP substrates are available in **two formats:**

- **PreMixed** as One-Tube-Solution – ready to use with no need to mix two components with excellent signal duration between 2 and 4 h (see table below)
- **Two-component system** – up to sixfold extended signal duration form 8 h up to 24 h, working solution stable for at least three days

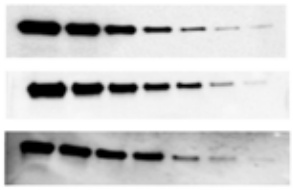
SERVA*Light* CL HRP substrates are available **in five detection ranges** - fine tune your signal intensity adapted to your experimental needs!

- **Vega** for mid-picogram protein quantities/high abundance
- **Polaris** for low picogram protein quantities/high abundance
- **Eos** for mid-femtogramm protein quantities/medium abundance
- **Eos Ultra** for Mid to low femtogram protein quantities/low abundance
- **Helios** for below femtogram protein quantities/very low abundance

SERVA*Light*	Signal intensity	Signal duration	Primary Ab dilution	Secondary Ab dilution
PreMix Vega	Standard	3 h	1:100 – 1:5000	1:1000 – 1:20,000
Vega	Standard	Long	1:100 – 1:5000	1:1000 – 1:15,000
Polaris	Medium	8 h	1:1000 – 1:5000	1:20,000 – 1:100,000
PreMix Eos	High	4 h	1:1000 – 1:15,000	1:25,000 – 1:150,000
Eos	High	>24 h	1:1000 – 1:15,000	1:25,000 – 1:150,000
Eos Ultra	Very high	>24 h	1:5000 – 1:50,000	1.50,000 – 1:250,000
PreMix Helios	Extreme	2 h	1:5000 – 1:100,000	1:100,000 – 1:500,000
Helios	Extreme	11 h	1:5000 – 1:100,000	1:100,000 – 1:500,000

SERVA*Light* CL HRP Substrates:

- Optimized for attaining low background and high signal to noise ratio
- Extended signal duration
- Excellent stability – at least one year stable when stored at room temperature
- Economical - save money and precious antibodies due to high dilution of antibodies

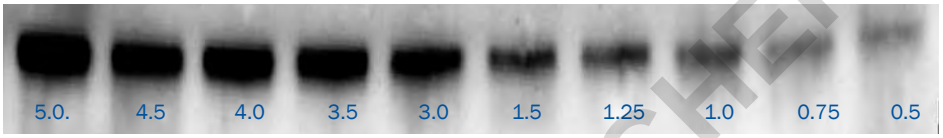


SERVA*Light*	Primary Ab	Secondary Ab
SERVA*Light* PreMix Vega	1:2000	1:20,000
SERVA*Light* PreMix Eos	1:5000	1:75,000
SERVA*Light* PreMix Helios	1:10,000	1:100,000

Easy and safe to use, excellent stability and an extended signal duration for best results in chemilumninescence detetction

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Product	Size	Cat. no.
SERVA*Light* Vega CL HRP WB Substrate Kit	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
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
SERVA*Light* PreMix Vega CL HRP WB Substrate	250 ml	42655.01
SERVA*Light* PreMix Eos CL HRP WB Substrate	250 ml	42656.01
SERVA*Light* PreMix Helios CL HRP WB Substrate	250 ml	42657.01



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.

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.
SERVAColor BCIP/NBT Blot Solution	250 ml	15245.01
SERVAColor TMB Blot Solution	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
Ponceau S	5 g	33429.01
	25 g	33429.02
Ponceau S Solution for Electrophoresis	500 ml	33427.01

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

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8 Instruments for Western Blotting

BlueVertical™ PRiME™ & TankBlotter

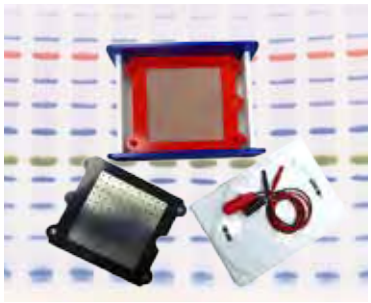
- Dual mini tank systems
- Accommodates 1 – 2 gels in cassettes with outer dimensions of 10 cm x 10 cm x 0.7 cm
- Leak-free inner core unit with a unique clamp system
- Unique, easy-to-handle blotting insert for tank blotting
- Gel Casting Stand for pouring two gels
- Quality designed and made in Germany



Product	Size	Cat. no.
BlueVertical™ PRiME™	1 unit	BV-104
BlueVertical™ PRiME™ TankBlot	1 unit	BV-104-TB
BlueVertical™ PRiME™ Casting Stand	1 unit	BV-104-CS
BlueVertical™ PRiME™ Blot Module	1 unit	BV-104-B

BlueBlot Semi-Dry Blotter

- Best blot results for small and large proteins
- Platinum-covered steel net as anode, spring-mounted
- Stainles steel plate as cathode
- Easy to clean
- Versatile models:
  - 11 cm x 11 cm for mini gels
  - 17 cm x 17 cm for mini and midi gels
  - 26 cm x 24 cm for 2D HPE™ BlotGels and other large format gels
- Deployable for thicker gels and blotting stacks



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

BluePower™ Power Supply for Blotting

- 300 V, 2000 mA, 300 W
- Semi dry blotting applications including large format gels up to 24 cm x 26 cm
- Fully programmable (9 programms with 9 steps each)
- 4x 2 outlets



Product	Size	Cat. no.
BluePower™ 300 BLOT Power Supply	1 unit	BP-300-BLO

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HPE™ BlueHorizon™



- Highly sophisticated flatbed system for horizontal electrophoresis
- High capacity cooling plate for highest resolution and band sharpness
- Suitable for gels up to 260 mm x 205 mm
- For 1D and 2D SDS PAGE, for high voltage applications like IEF
- Wide range of horizontal precast gels available
- Platinised electrodes with variable spacing - depending on the application
- Samples easy to load, low buffer consumption
- Stackable, up to four units under the control of one power supply and one chiller
- Smart design – made in Germany

Product	Size	Cat. no.
HPE™ BlueHorizon™	1 unit	HPE-BH
BluePower™ 3000 HPE™ Power Supply	1 unit	BP-3000-HPE
HPE™ Cooling Unit	1 unit	HPE-CU

Gravity Blotter



- Pressure blotting of gels covalently bound to carrier foil
- For horizontal IEF, SDS PAGE and 2D gels
- High efficiency protein transfer from gel to membrane within 4 hours or overnight
- Transfer area: 14 cm x 29 cm

Product	Size	Cat. no.
Gravity Blotter	1 unit	GB-14X29

BIO-5000 Plus VIS Gel Scanner



- Leak-free holder for scanning wet electrophoresis gels in transmission mode
- Scanning of stained blot membranes in reflection mode
- CCD image sensor
- Resolution up to 4,800 dpi
- Dynamic range over approx. 3.7 O.D. units
- Auto-focus for highest image quality
- Easy-to-use scanning software
- Scanning area up to 216 mm x 254 mm

Product	Size	Cat. no.
BIO-5000 Plus VIS Gel Scanner	1 unit	BIO-5000P

GeneGnome XRQ



- Imaging system for chemiluminescence
- Automatically captures a high quality image of each Western blot
- By built-in white light LEDs, colorimetric markers can be captured as well

Product	Size	Cat. no.
GeneGnome XRQ, no monitor, no processor included	1 unit	GGNOME-XRQ-NPC

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SERVA
serving scientists

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