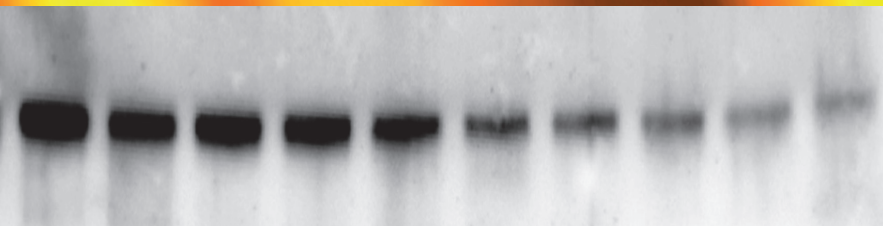


Western Blotting



Reagents and Equipment

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 nitro-

cellulose, nylon or PVDF. To check transfer efficiency blotted proteins could be reversibly 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 quality 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

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

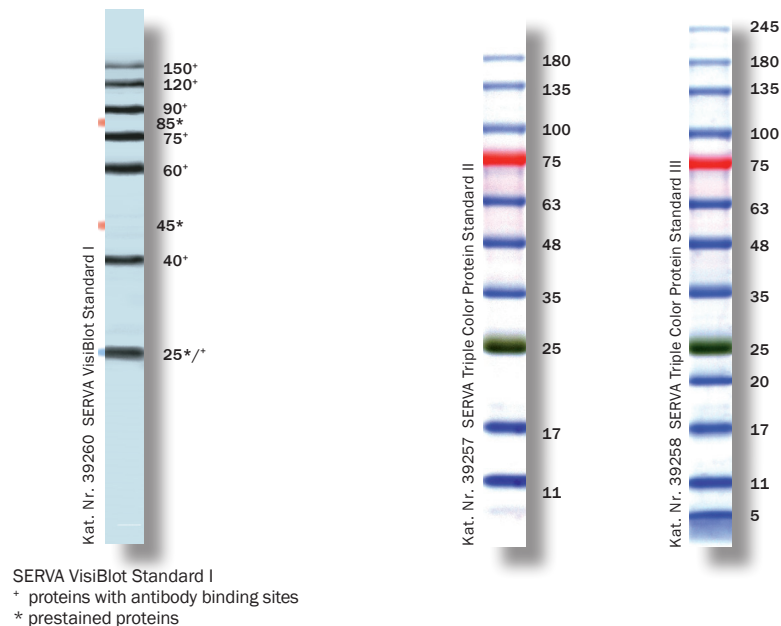
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Molecular Weight Markers for Western Blotting

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



Product	Size	Cat. no.
SERVA VisiBlot Standard I	500 µl	39260.01
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 prestained protein markers to check electrophoresis run and blotting efficiency

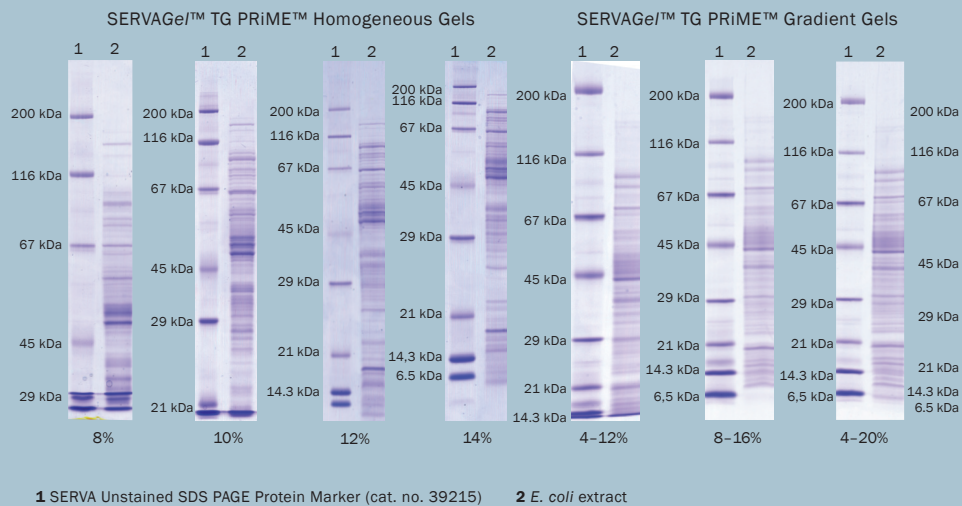
SDS PAGE Gels for Western Blotting

Top resolution, efficient transfer and a sensitive detection system are the most important requirements for best results in Western blotting experiments. By applying

gentle separation of your protein sample will be achieved. SERVAGE™ Neutral HSE is optimized for very short running times. Use BlueVertical™ PRiME™ mini slab gel unit to run SERVAGE™ precast gels.

SERVAGE™ 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)



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

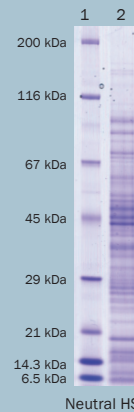
■ Short set-up times, gels are ready-to-use

■ Separation distance: 7 cm

SERVAGe/™ 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 SERVAGe/™ TG PRIME™ gradient gel 4 – 20 %
- Long shelf life (15 months)

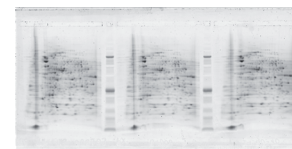
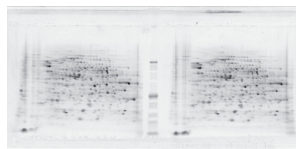
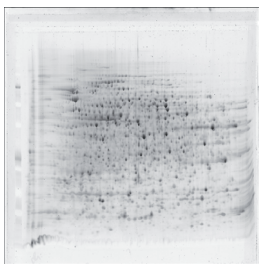
SERVAGe/™ Neutral HSE



Product	Sample Wells	Size	Cat. no.
SERVAGe/™ Neutral HSE	10	10 gels	43246.01
SERVAGe/™ Neutral HSE	12	10 gels	43245.01
SERVAGe/™ Neutral HSE	15	10 gels	43249.01
SERVAGe/™ 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

- SERVAGe/™ Neutral HSE gels for mini vertical gel tank and semi dry blotting
- 2D HPE™ BlotGels for large format horizontal 2D gel semi dry blotting

3

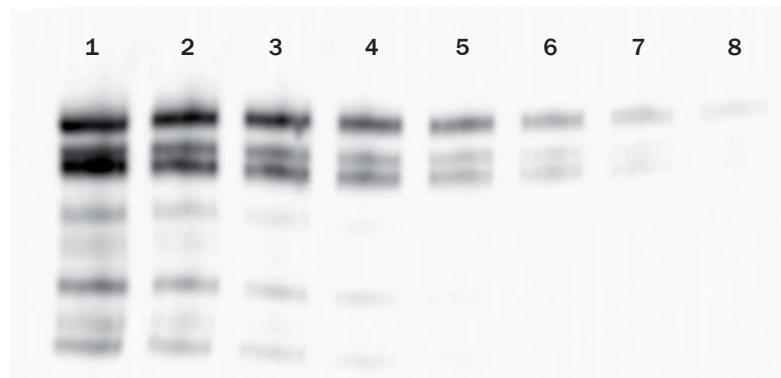
Transfer Buffers

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 pre-cutted 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

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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

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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 β-ME or DTT
- Suitable for NC and PVDF membranes



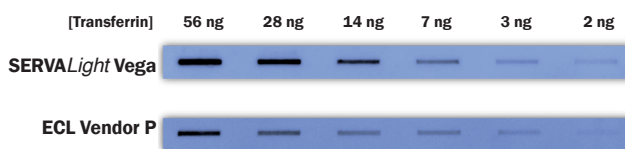
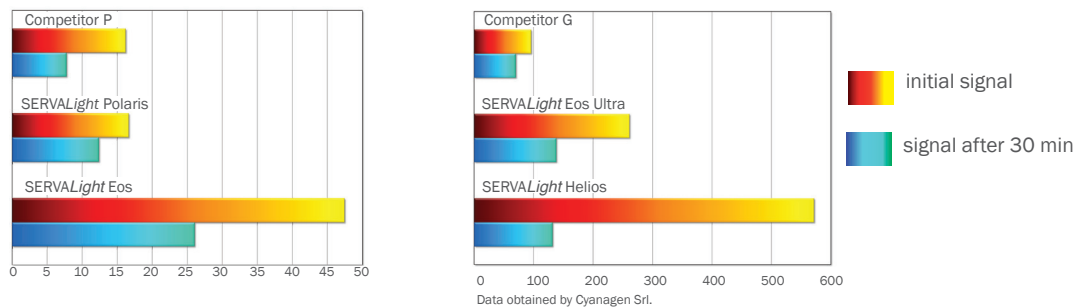
Product	Size	Cat. no.
BlueClear SB Antibody Stripping Buffer	250 ml	42599.01
	1 L	42599.02

Detection Systems

SERVALight 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 Horse-radish 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.

SERVALight CL HRP WB Substrate Kits

- SERVALight 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 SERVALight 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



Sensitivity

SERVALight type	Signal intensity	Signal duration	Protein quantity	Detection limit
Vega	Standard	Long	High abundance	Mid picogram
Polaris	Medium	Long	High abundance	Low picogram
Eos	High	Very long	Medium abundance	Mid femtogram
EosUltra	Very high	Very long	Low abundance	Mid to low femtogram
Helios	Extreme	Moderate	Very low abundance	Below femtogram

Specifications

SERVALight 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 cm ²
Polaris	1:1,000 – 1:5,000	1:20,000 – 1:100,000	3 days	
Eos	1:1,000 – 1:15,000	1:25,000 – 1:150,000	3 days	
EosUltra	1:5,000 – 1:50,000	1:50,000 – 1:250,000	3 days	
Helios	1:10,000 – 1:200,000	1:300,000 – 1:1,000,000	8 h	

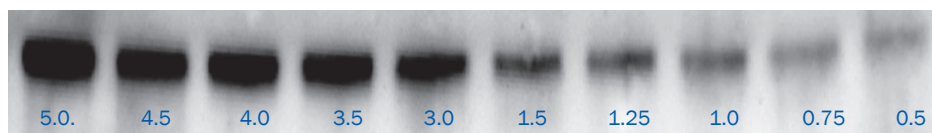
- Easy and safe to use, excellent stability and an extended signal duration for best results in chemiluminescence detection
- Five different substrate kits for standard application with the Vega kit up to highest sensitivity with the Helios kit

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 *SERVALight* and *SERVAColor* kits *SERVA* offers stand alone detection reagents like Luminol, TMB, BCIP and NBT

Product	Size	Cat. no.
<i>SERVALight</i> Vega CL HRP WB Substrate Kit	250 ml	42588.02
	500 ml	42588.03
	100 ml	42584.01
<i>SERVALight</i> Polaris CL HRP WB Substrate Kit	250 ml	42584.02
	500 ml	42584.03
<i>SERVALight</i> Eos CL HRP WB Substrate Kit	50 ml	42585.01
	250 ml	42585.02
<i>SERVALight</i> EosUltra CL HRP WB Substrate Kit	20 ml	42586.01
	100 ml	42586.02
	20 ml	42587.01
<i>SERVALight</i> Helios CL HRP WB Substrate Kit	100 ml	42587.02
	200 ml	42587.03
<i>SERVAColor</i> BCIP/NBT Blot Solution	250 ml	15245.01
<i>SERVAColor</i> 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
	250 mg	30550.01
Nitro Blue Tetrazolium Chloride (NBT)	1 g	30550.02
	5 g	30550.03
	5 g	33429.01
Ponceau S	25 g	33429.02
	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 *SERVALight* 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

Instruments for Western Blotting

BlueVertical™ PRiME™

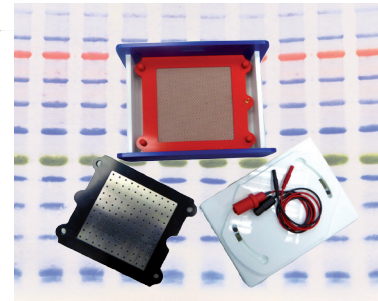
- Dual mini tank systems
- Accommodates 1 – 2 gels in cassettes with outer dimensions of 10 cm x 10 cm x 0.7 cm
- Leak-free inner core unit with a unique clamp system
- Gel Casting Stand for casting two gels
- Unique, easy-to-handle blotting insert for tank blotting
- Quality designed and made in Germany



Product	Size	Cat. no.
BlueVertical™ PRiME™	1 unit	BV-104
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
- Stainless 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 programmes with 9 steps each)
- 4x 2 outlets



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

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 240 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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