BlueLine
Instruments for Electrophoresis

INSTRUCTION MANUAL

HPE™ BlueHorizon

Flatbed Electrophoresis System

(Cat. No.: HPE-BH)

DOMINIQUE DUTSCHER SAS

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Vers. 01/14

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1 Safety

The SERVA HPE™ BlueHorizon comply with the standards and directives mentioned in the applicable CE declaration.

Please take note of the following safety measures:

- Warning: Operation of this instrument requires high voltage.
- Disconnect the high voltage external power supply before opening any drawer.
- Turn off and disconnect any high voltage power supply before opening the safety lid.
- Disconnect the high voltage external power supply and the AC main supply before cleaning or servicing.
- Do not spill or store liquids on top of the unit.
- If liquid is spilled into the HPE™ BlueHorizon, disconnect the high voltage power supply and the AC main power immediately before opening the safety enclosure lid.
- Do not operate or connect power sources to the equipment if there is any mechanical damage.
- The supplied DC cables are rated for 5,000V. Only use cables and adaptors supplied with the HPE™ BlueHorizon or ensure that these have a suitable DC insulation compliance for the used voltages.

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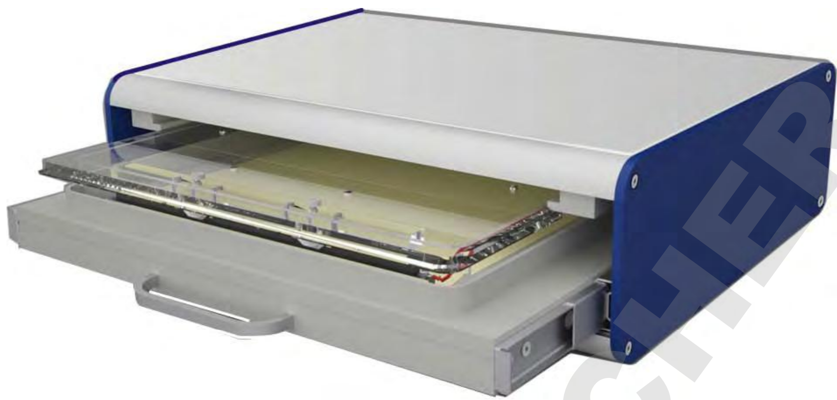
2 Introduction

The HPE™ BlueHorizon is a flatbed system for horizontal electrophoresis. Main applications are isoelectric focusing (IEF), 2D PAGE, SDS PAGE and the separation of nucleic acids in polyacrylamide gels.



The stable and easy-to-clean metal housing allows a space-saving positioning of the power supply on top. The system is cost-saving, because it works without buffer chambers. Instead, fabric wicks are soaked with concentrated electrophoresis buffers.

The integrated drawer holds the cooling plate that is connected to the SERVA HPE™ Cooling Unit (cat. no. HPE-CU1). The cooling plate is made from a special ceramic material (maximum gel size 260 x 205 mm) for efficient heat conductance down to 4 °C resulting in rapid and straight migration and therefore highly focused spots and bands.

The electrode lid comes with one pair of platinum electrodes. They can be installed to three electrode positions serving a wide variety of different gel sizes. A lid with triple electrodes for bi-directional electrophoresis is available optional.

With the drawer / lid arrangement, the plastic-backed gels are protected from dust and light during the run to avoid photo-bleaching of fluorescent labels.

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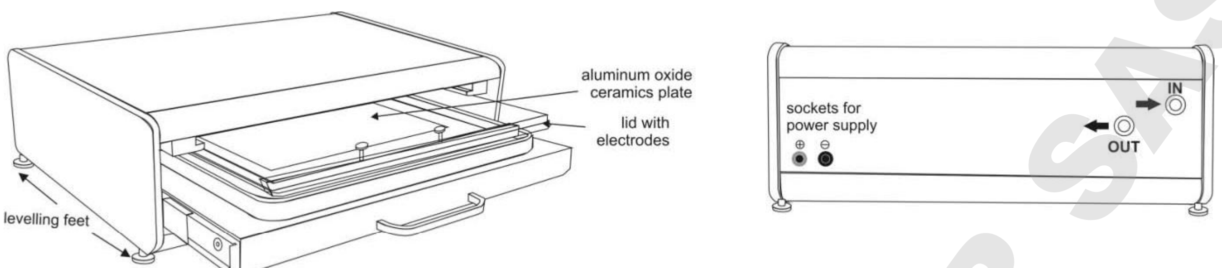
3 Installation



HPE™ BlueHorizon (left: side/front view, right: back view)

The base-unit
Place the base unit on the bench. Unevenness of the lab bench can be corrected by using a spirit level and adjusting the levelling feet.

Connecting the SERVA HPE™ Cooling Unit (Chiller)

Important note: Warm air should not be exhausted towards the BlueHorizon!

Connect the BlueHorizon to the chiller using the provided tubing and fix them with the hose clamps. It is important to tighten these clamps sufficiently to obtain an air-tight seal. The chiller ”Outlet“ must be connected to the BlueHorizon “Inlet” and the chiller “Inlet” to the BlueHorizon “Outlet”. If the flow-direction is wrong, the cooling will work incorrectly. In order to protect the cooling plate from corrosion, the cooling liquid must contain an anti-corrosive additive (Cat. no. 43392)

Air Removal
For efficient cooling, it is vital to remove any air from within the cooling plate. Switch on and leave the chiller running until all of the air in the connecting pipes is removed, this may take several minutes. Then refill the chiller reservoir with cooling liquid (3 parts of water and 1 part anti-corrosive additive) as the BlueHorizon takes up about 1 litre.

Electrode Lid Storage
Remove the protection film and paper. Insert the lid into its “park” position” above the drawer with the connecting plugs of the lid towards the backside of the BlueHorizon. The electrodes are carefully constructed from platinum coated titanium rods and could be damaged if not handled correctly. When not in use, always insert the lid into its “park” position. Never place the lid on the bench with electrode side down to avoid damage.

Connecting the Power Supply
Connect the power supply (black / red cables). Place it on top to save bench space.

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4 Operation

In this section, general instructions for loading and running gels on the HPE™ BlueHorizon are described. Running conditions for specific gel types, videos, and other useful information are provided on the enclosed DVD and on www.serva.de.

Important Information: Always wear powder free disposable gloves when handling gels or stripes. Do not open a drawer during an active electrophoresis run without switching off or pause the power supply. Opening a drawer during running power causes your power supply to detect a “ground leakage”. That may cause a disturbance of the running programme or in the worst case damages the power supply.

4.1 Adjust the electrodes

In the electrode lid, the electrode positions can be adjusted to different gels sizes. When changing the electrode positions, place the electrode lid half way in the park position and loosen the screws of the first electrode. Be careful to catch the nut on the underside of the lid into which the screw fits. Then turn the lid and change the second electrode as described above. Please note: The position for PreCotes is not fixed but adjustable. Before each run with PreCotes or CleanGels IEF, superpose the electrodes and the wicks on the gel.

Gels	PreCotes CleanGel IEF FocusGel	SDS Gel CleanGel	2D HPE Triple / Double Gels	2D HPE Large Gel
Application	IEF	1D PAGE	2D PAGE	HiRes 2D PAGE
Electrode position				

4.2 Apply a gel

Important Information: To avoid water condensation on the gel surface, do not yet switch on the chiller.

A specially formulated cooling fluid is added between the surface of the cooling plate and the gel to ensure good contact, even temperature control and efficient heat dissipation. For a standard format gel spread 3 ml cooling contact fluid onto the centre of the cooling plate. For a large format gel spread 6 ml cooling contact fluid onto the cooling plate in the shape of an “H”:

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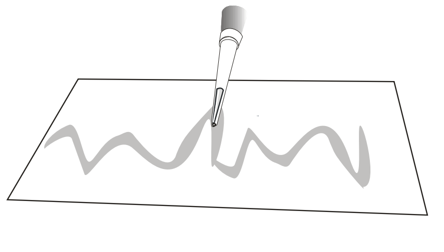
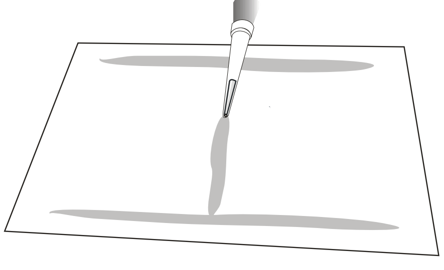
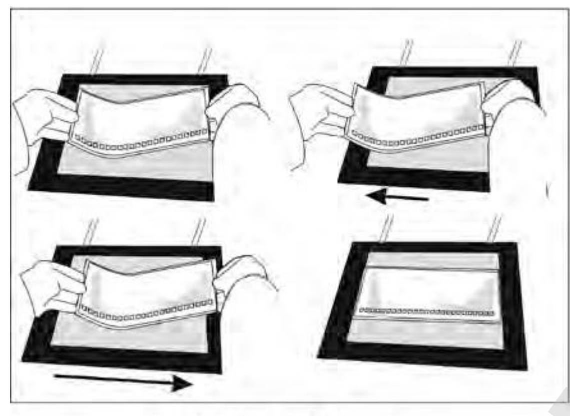


standard gel format

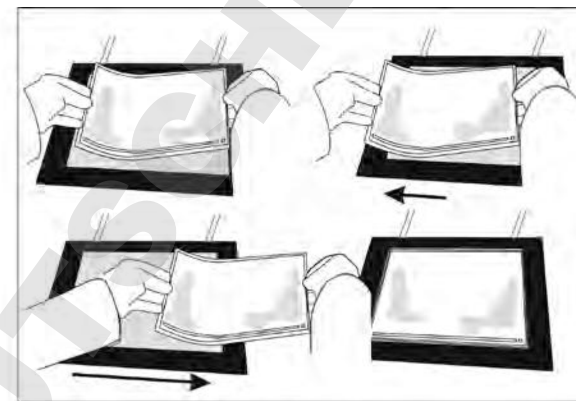


large gel format

To disperse, slide a gel bent into a U-shape from side to side. The sides of the gel are then gently lowered. Avoid air bubbles between the cooling plate and gel. Excess cooling fluid from around the gel is removed using a lint-free tissue.



standard gel format



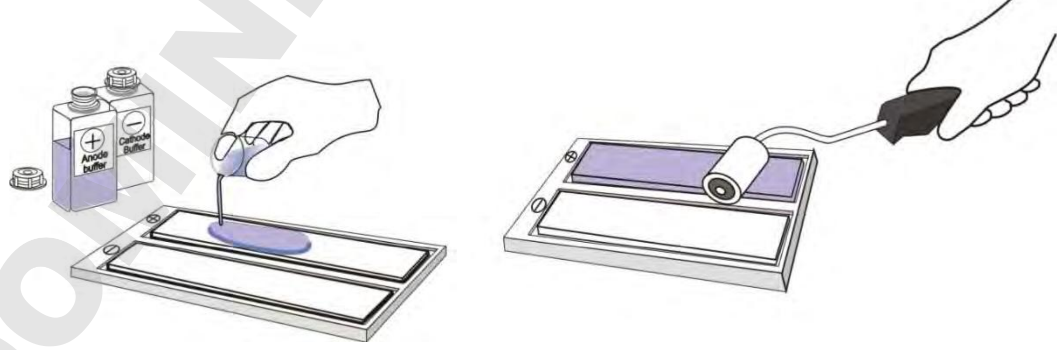
large gel format

4.3 Prepare Electrode Wicks

4.3.1 SDS Gel Kit, CleanGel, HPE Gel

All buffers needed are provided in the SERVA Gel and Buffer Kits. We do not recommend different or self-made components.

Electrode wicks, soaked in an appropriate buffer, provide a convenient alternative to buffer tanks. The wicks should be fully soaked with 45 ml buffer for at least 10 minutes. The wicks should be rolled to remove air bubbles and to distribute the buffer evenly using the supplied roller:



Electrode wicks are applied with the cathode (white) at the front, anode (blue) at the back. Remove excess electrode buffer from the wicks by tilting the electrode wicks along one long edge and dab it on the paper pool bottom. When moving the wicks

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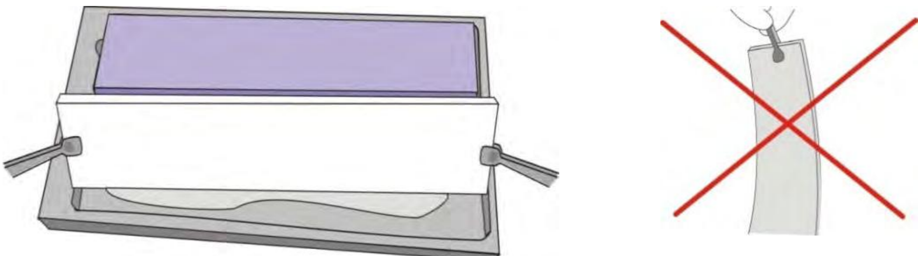
always hold them horizontal, as holding them at a vertical angle can result in unequal buffer concentration.



The electrode wicks should overlap the gel by at least 2 mm. It is important that buffer is not dropped onto the gel surface and therefore avoid moving the buffer soaked wicks over the gel.

4.3.2 PreCotes, PreNets, CleanGel IEF

Place two electrode wicks (5 mm) soaked with anode and cathode buffer. Apply them on the corresponding gel edges: acidic solution on the Anode (+) side, basic solution on the cathode (-) side. Wicks must not extend beyond edge of gel but be aligned parallel to each other and corresponding to where the electrodes will be placed.

4.3.3 FocusGel

On FocusGels, the electrodes are directly placed onto the gel without the need of buffer soaked wicks.

4.4 Sample preparation and loading

4.4.1 SDS Gel Kit

1) Add one volume sample to one volume sample buffer (2x) and dilute the sample to loading concentration with the sample diluter (depends on the sensitivity of staining method). Reduce and alkylate your sample.
2) Pipette samples into the sample wells.

4.4.2 CleanGel

Prepare samples according to the related gel instruction manual or specific application procedure.

4.4.3 HPE Gel

1) Equilibrate the IPG strip

   SERVA IPG strip equilibrator (Cat. no. HPE-A04) provides a convenient way to equilibrate IPG strips. After equilibration, the strip can be easily transferred from the slots holding the first equilibration solution (e.g. DTT) to the slots holding the second solution (e.g. IAA).

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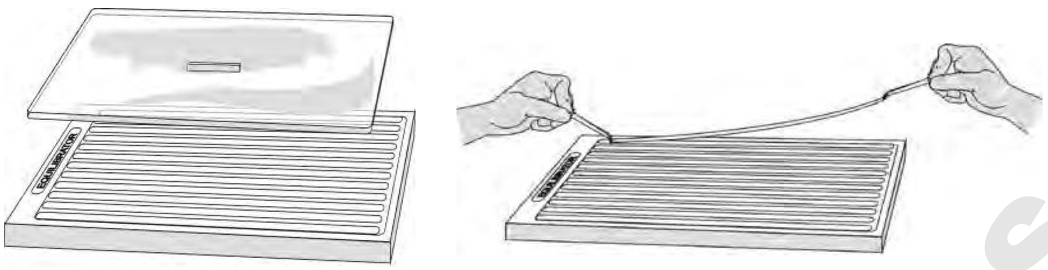


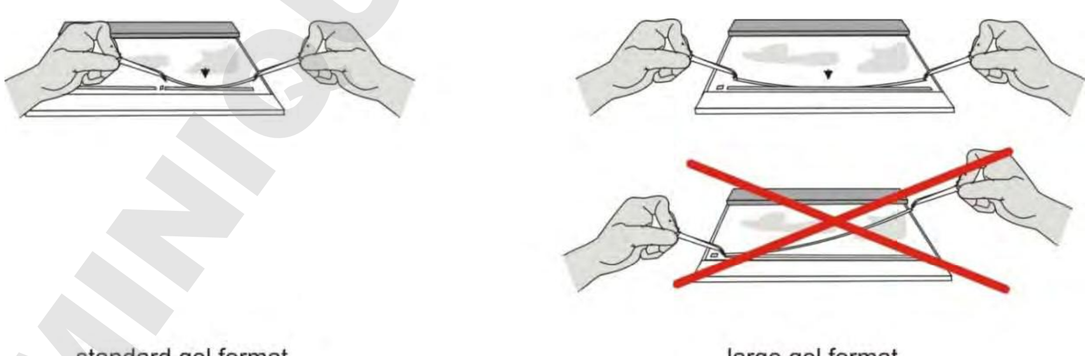
Prepare the two equilibration solutions from the SERVA IPG Strip equilibration buffer:
DTT solution: Weigh urea and Dithiothreitol (DTT), add the equilibration buffer and dissolve completely.
IAA solution: Weigh urea and Iodoacetamide (IAA), add the equilibration buffer and dissolve completely.

Sufficient amount for 1 x 18cm, 1 x 24 cm, 2 x 11cm, 3 x 7cm					
	Urea [g]	DTT [mg]	IAA [mg]	Eq. Buffer [ml]	Total [ml]
DTT solution	1.8	50	-	5	6
IAA solution	1.8	-	125	5	6

Equilibrate each strip in 6 ml (18 or 24 cm strips), 3 ml (11 cm strips) or 2 ml (7 cm strips) solution in an equilibrator on an orbital shaker with 30 –50 /min in DTT for 15 min and in IAA solution for 15 min.

2) Applying the IPG-strip
IPG strips should still contain equilibration buffer on their surfaces. Do not blot IPG strips dry, this can cause vertical streaking in the second dimension due to insufficient protein transfer. Some IPG strips have rather long protruding support film on the ends: In this case the plastic film support on both sides of the IPG-strip must be trimmed just beyond the gel.

The strip should be carried horizontally and applied to the slot center first.



standard gel format　　　　large gel format

The strip should be placed in the IPG slot, gel side down, with the anodal side to the right. To ensure good contact in the slot the back of the forceps is slid gently along the back of the IPG strip.

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4.4.4 PreCotes, PreNets

1) Unpack the gels and remove the protective cover-film from the gel surface. Keep the cover film as it can serve as a protective sheet later.
2) Adjust sample concentration to about 1 - 10 mg protein/ml and desalt by dialysis; by dilution with dest. water or by lyophilization and resuspension in dest. water.
3) Centrifuge the samples for 5 minutes at approx. 12,000 g; use only the supernatant. By omitting this step the separation pattern might become fuzzy and, eventually, precipitates may form within the applicator strip slots.
4) Position the applicator strip on the gel and slightly pressing it with the back of a forceps. Apply the required sample volume using a pipette. Do not leave empty slots between samples. Depending on sample type, it is possible to apply the samples with or without pre-focusing.

4.4.5 CleanGel IEF

1) Unpack and rehydrate the dry gel according to the gel instruction manual.
2) Prepare and apply samples on the gel according to the related gel instruction manual or specific application procedure.

4.4.6 FocusGel

1) Unpack the gels and remove the protective cover-film from the gel surface. If necessary, remove excessive moisture from the gel surface with the edge of a drying cardboard. Keep the cover film as it will serve as a protective sheet later. The gel is ready to use.
   FocusGel 24S and 40S: For some sample types, e.g. serum and CSF the position of the pre-formed wells is optimized for anodal application in a pH gradient 6-11. This well position might also be suitable for other sample types. Nonetheless, the gels can be turned around for cathodal application if needed.

2) Apply the required sample volume using a pipette. Do not leave empty slots between samples. Depending on sample type, it is possible to apply the samples with or without pre-focusing.
3) Take care that the electrodes are placed directly on the FocusGel surface at the gel edges and not on the support film!

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4.5 Start the run

1) Close the lid while lowering the electrodes on the wicks. Plug in the leads.

2) Switch the thermostatic circulator on, set to
   - HPE Gels: 15 °C
   - SDS Gels: 15 °C
   - Clean Gels: dependent on the application (see gel manual)
   - PreCotes, PreNets: 10°C
   - CleanGel IEF: 10°C
   - Focus Gel: dependent on the application (see gel manual)

   Note: During Electrophoresis the electric resistance of the gel is slowly increasing. Therefore, the heat production during the starting phase is rather low. It does not cause overheating when the chiller begins cooling at the same time the electrophoresis is started.

3) Start the run according to the settings described in the gel manual.

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4.6 After the run

After electrophoresis, clean the device as described and follow with staining and detection. We recommend:

Staining	Application	Cat. No.
SERVA HPE™ Silver Staining Kit (MS compatible)	2D	43395
SERVA CSF Silver Staining Kit	CSF IEF	43398
SERVA Silver Staining Kit Native PAGE	Native PAGE	35077
SERVA Silver Staining Kit SDS PAGE	SDS PAGE	35076
SERVA HPE™ Coomassie® Staining Kit	PAGE, 2D	43396
SERVA Blue G	PAGE	35050, 17524
SERVA Blue R	PAGE	35051, 17525
SERVA Blue R Staining Kit	PAGE	42531
SERVA *Densi*Stain Blue G Staining Solution	PAGE	35078
Quick Coomassie™ Stain	IEF, PAGE	GEN-QC-STAIN-1L
SERVA Blue W	IEF	35053
SERVA Violet 17 Staining Kit	IEF, native PAGE	35074
SERVA Violet 17	IEF, native PAGE	35072
SERVASnow Staining Kit	PAGE background stain	35080
SERVA ProteinStain Fluo-R (MS compatible)	IEF, PAGE, 2D	35091
SERVA Purple (MS compatible)	PAGE, 2D	43386
SERVA Lightning Red for 1D SDS PAGE (label)	PAGE	43401
SERVA HPE™ Lightning Red (label)	2D	43400

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5 Maintenance

5.1 Cleaning

Regularly clean the housing of the SERVA HPE^{TM} BlueHorizon.

Precautions for avoiding electric shock

Electronic devices can cause electric shocks in case of an operating error. Never try to repair electric parts. Never open the housing.

- Switch off the instrument and disconnect it from the power supply before starting with cleaning or disinfection works.
- Never let get liquids inside the housing.
- Do not perform spray disinfection.
- Do only connect the instrument with the power supply if it is completely dry.

The repair service may only be performed by authorized staff trained by the manufacturer. A modification of the instrument is not permitted.

Caution when handling aggressive chemicals

Do not use aggressive chemicals e.g. strong and weak bases, strong acids, formaldehyde, acetone, halogenated hydrocarbons, phenol and other organic solvents for cleaning the instrument and its accessories.

- In case of contamination with aggressive chemicals, clean the instrument with a neutral detergent immediately.
- Use neither corrosive detergents nor aggressive solvents or abrasive polishing agents.

Cleaning

When running the BlueHorizon for the first time, or if the cooling plates became soiled, clean them using a 0.1% SDS solution, followed by isopropyl alcohol and finally distilled water. Disconnect the instrument from the power supply before you start cleaning to obtain the best results it is recommended to clean the cooling plates and electrodes before and immediately after use with distilled water. The cooling plate can be gently rubbed and dried using a lint-free tissue. Abrasive cleaners and other solvents must not be used. Cleaning of the platinum electrodes after each run is particularly important to prevent crystallization of buffer salts, which can result in uneven contact. Electrodes should be cleaned with distilled water-moistened lint-free tissue.

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Disinfection

1. Disconnect the instrument from the power supply before you start disinfecting.
2. Let the instrument cool down.
3. Clean the instrument as described above.
4. Select a disinfection method compliant to the applicable local legal regulations and directives.
5. Wipe off all outer parts of the instrument with the disinfectant and a lint-free cloth.

Decontamination before shipment

If you need to send the instrument back to us, decontaminate all parts. Document this in our Decontamination Certificate (Download on www.serva.de) and include it within the shipment.

6 Technical data

	SERVA BlueHorizon
Max. Spannung, Strom / Max Voltage, Current	3000V, 25mA
Max. Gelgröße / Max gel size	260 x 205 mm
Temperatur-Arbeitsbereich / Temperature-working range	+4°C to +30°C
Elektrodenabstand / Electrode distance	270, 195, 115 mm
Abmessungen (B x T x H) / Dimensions (W x D x H)	450 x 500 x 120 mm
Gewicht (ohne Block) / Weight (without block)	6 kg

7 Explanations

	Attention! Electric shock!
	Attention!

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8 Trouble Shooting

Symptom	Cause	Remedy
7.1 Effects during electrophoresis		
No water flow after chiller is switched on.	Wrongly connected tubing or kinked tube.	Straighten tubing. Check tubing: Chiller-“Out“ connected to chamber-“In“.
Air bubbles between film-backing and cooling plate.	Insufficient volume of cool contact fluid	Lift up gel on one side and apply a higher volume
Excess cooling fluid around the film support.	Too much cooling fluid applied on the cooling plate.	Remove excess fluid with lint-free tissue paper.
Water droplets on gel surface.	Gel was pre-cooled without lid at high humidity conditions leading to water condensation.	Do not switch on chiller during gel application and sample loading.
No electric current, drawer control lamps do not illuminate after starting the power supply.	Electronic control detects wrong orientation of electric field.	Plug power supply cables in correctly: black cable to cathode, red cable to anode.
	Electronic control detects that one or more draws do not contain a gel.	When less than four gels are run, unplug the non-used electrode lids and place them into the parking position.
	Lids not properly positioned or not plugged-in.	Re-position lids and check connections between lids and drawers.
Condensation inside of electrode lid.	The gel gets hot during electrophoresis because of insufficient heat dissipation.	Do not forget to switch chiller on!
		Check chiller temperature and ensure no other apparatus is connected to the same chiller.

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Front is curved instead of straight.	The gel gets hot during electrophoresis because of insufficient heat dissipation.	See above
	BlueHorizon is subject to hot exhaust from chiller or other apparatus.	Relocate chiller or other apparatus.
	The gel gets hot during electrophoresis because too much power is applied per gel.	If you run less than four gels at a time, reduce the mA and W settings in the power supply accordingly. Follow strictly the manual.
Migration of front is very slow and will not reach the anode in time.	The electric field is too low.	Adjust the mA and W settings according to the number of gels run.
Front is slanted, not straight.	Uneven buffer concentration within the electrode wicks.	Always hold electrode wicks horizontal when carrying them to the gel.
Condensation water develops inside electrode lid near to IPG strip(s).	Local heat production at IPG strip(s) because of electroendosmotic effect.	Remove IPG strip(s) from gel after the first 70 minutes and then continue the run. Follow strictly the manual.
Minor disturbance(s) in the front.	Buffer drop(s) fell on the gel surface.	Avoid passing wicks over gel surface.
	Air bubbles inside the wicks.	Gently roll wicks in PaperPool to remove air.
2D Gels: Irregular bulging of the front on one side.	Equilibration buffer unequally distributed within the IPG strip(s).	Hold the IPG strip(s) horizontal, start in the middle when placing the strip into the slot.

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2D Gels: Run stops, front does not continue to migrate, sparking at the IPG strip(s).	Strong electroendosmosis effect at the IPG strip(s), because it has not been removed after the first 70 minutes.	Remove IPG strip(s) from gel after the first 70 minutes and then continue the run. Follow strictly the manual.
7.2 Effects during scanning		
Gel edges curl up during scanning.	Gel edges start to dry out.	Apply Scan-Frame on the edges of the gel during scanning.
12.4 Effects seen in the result		
2D Gels: Horizontal streaking	The first dimension IEF separation in the IPG strip did not work well because of inappropriate sample preparation IEF separation problems.	Check the trouble shooting guides supplied by the providers of the IPG strips and web forums.
2D Gels: Vertical streaking	Insufficient equilibration of the IPG strip.	Use the equilibration buffer supplied with the HPE gels, weigh-out the correct amounts of DTT and IAA (should be of highest reagent quality), follow the manual.
	IPG strip has become too dry.	Ensure that there is still a thin layer of buffer on the surface.
Local disturbances in the pattern	Air bubble in the cooling contact fluid layer	Use sufficient cooling contact fluid on the cooling plate (3 for standard size gels, or 6 ml for large gels), distribute is evenly by sliding the gel with the film-backing several times left and right.
	Air bubble in a buffer wick	Distribute the buffer solutions evenly in the wicks by thoroughly rolling

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SERVAPurple staining: Unsufficient sensitivity of staining	Wrong protocol (for non-backed gels) has been applied, or the solution volumes were too small	Follow the protocol of this HPE Horizon manual which has been optimized for the HPE Flatbed gels.

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9 Addendum: Example Applications

1D SDS PAGE 25 slots
SDS Gel Kit 10% 25S; Cat. No. 43359
Marker, muscle, *E. coli* extracts
Coomassie® staining
SERVA Blue R Staining Kit; Cat No. 42531

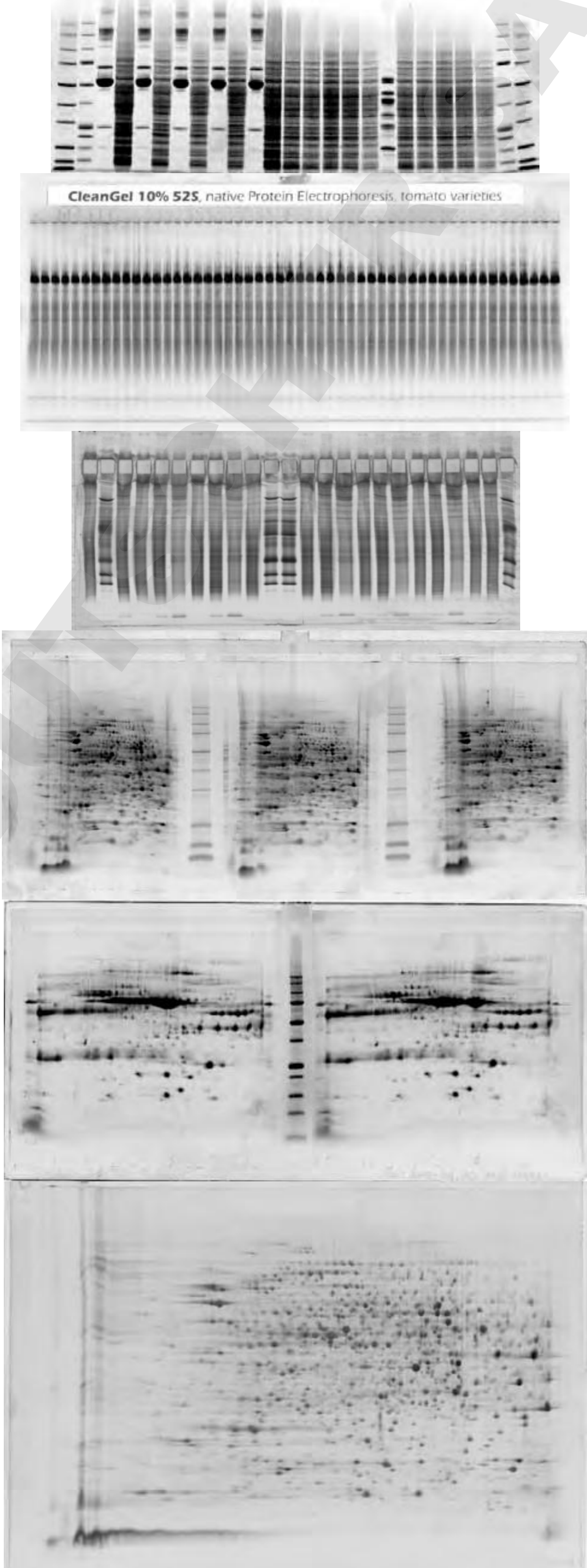
1D native PAGE 52 slots
CleanGel 10% 52S; Cat. No. 43340
Native Buffer; Cat. No. 43352
Tomato seeds
Coomassie® staining
SERVA Blue R Staining Kit; Cat No. 42531

Precast Horizontal Gel for Isoelectric Focusing
FocusGel 6-11 24 slots; Cat. No. 43329
Cerebrospinal fluids and serum
Ammoniacalic silver staining with SERVA CSF Silver Staining Kit; Cat. No. 43398

2D Electrophoresis
2D HPE™ Triple-Gel NF 12.5 % Kit; Cat. No. 43300
E. coli extracts
ServaPurple™ staining; Cat. No. 43386

2D Electrophoresis
2D HPE™ Double Gel NF 12.5 % Kit, Cat. No. 43302
Human serum proteins
ServaPurple™ staining; Cat. No. 43386

2D Electrophoresis
2D HPE™ Large Gel 12.5 % Kit,Cat. No. 43310.
E.coli proteins
ServaPurple™ staining; Cat. No. 43386



10 Pack List and Order Information

These spare parts are packed with the SERVA HPE™ BlueHorizon devices:

- The BlueHorizon base unit
- 1 lid packed with installed electrodes
- 2 mm power supply cables (2 pcs.)
- 1 m thick wall silicone tubing
- 4 hose clamps
- Manual

Order Information:

Product	Cat. no.
HPE™ BlueHorizon™	HPE-BH
PaperPool	HPE-A02
Roller	42991
BluePower™ 3000x4 Power Supply	BP-3000x4
HPE™ Cooling Unit (Chiller)	HPE-CU1
Anti Corrosive Additive (4X)	43392
Cooling Fluid	43371

Optional: BluePower™ Supply Control Kit (Cat. no. BP-PCSV01)

The BluePower™ Supply Control Kit provides all needed to linkany SERVA BlueLine Power Supply via an USB - serial converter to a personal computer with documentation software. It has basically two functions:

1. Monitoring and documenting the voltage, voltage-hour integral, current and power during the time course of an electrophoresis run.

2. Programming, loading, storage, and documentation of multistep power supply settings.

Optional: SERVA HPE™ ScreenPicker (Cat. no. HPE-SP1)

The semi-manual spot picking system for Mass Spectrometry Analysis SERVA HPE™ ScreenPicker is a device to semi-manually pick fluorescent 2D gels (DIGE, ServaPurple, Serva Lightning Red, and others…) without the need of post staining and/or sophisticated robot and software. It consists of an embedded screen connected to a small computer to display the scanned image of the gel, an XY

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carriage to guide the picker accurately over the spot position, and software which can read images and picking lists from most evaluation packages.

Optional: SERVA Gel Remover (Cat. no. HPE-GR01)

For electrophoretic transfer of proteins on a blotting membrane the film-backing must be removed from the gel. The easiest and safest way to remove the film-backing without damaging the gel layer is cutting it with a thin wire using the SERVA Gel Remover.

IEF gels have larger pore sizes than electrophoresis gels, and can therefore be successfully blotted with pressure blotting according to Towbin H, Özbey Ö, Zingel O. Electrophoresis. 22 (2001) 1887-1893.

Further information is available from www.serva.com

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EG – Konformitätserklärung

EC Declaration of Conformity / Déclaration de Conformité CE

Hiermit erklären wir, dass das nachfolgend bezeichnete Produkt den einschlägigen, grundlegenden Sicherheits- und Gesundheitsanforderungen der nachstehend aufgeführten Richtlinien und Normen entspricht. Bei einer nicht mit uns abgestimmten Änderung des Produktes verliert diese Erklärung ihre Gültigkeit.

We declare herewith that the product described below conforms to the relevant basic safety and health requirements of the directives listed below. Any modification of the product not approved by us renders this declaration invalid.

Par la présente, nous déclarons que les produits désignés ci-dessous répondent aux critères de base relatifs à la sécurité et à la santé qui ont été définis dans les directives sous-indiquées. En cas de modification du produit sans notre consentement préalable, cette déclaration devient nulle.

Flachbett Elektrophoresekammer Flatbed electrophoresis unit / Chambre à plat électrophorèse	
Art. Nr. / Cat. No. / No. de réf.	**Typ /** Type **/** Type
HPE-BH	HPE™ BlueHorizon™

EU-Richtlinien / EC Directives / Directives CEE

Angewendete harmonisierte Normen, nationale Normen Applicable harmonised standards, national standards Normes harmonisées appliquées, Normes nationales appliquées
EN 61010-1 IEC 61010-1 + Corr.

Heidelberg, 24.02.2014

Dr. Barbara Müller
Geschäftsführerin
Chief executive officer
Présidente-directrice générale

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