

BlueLine
Instruments for Electrophoresis

INSTRUCTION MANUAL

Blue Vertical 102

Vertical Electrophoresis Chamber



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WARNING

These units are capable of delivering potentially lethal voltage when connected to a power supply and are to be operated only by qualified technically trained personell.

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

The BlueLine vertical electrophoresis systems are 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.

First check that the apparatus has been received complete and undamaged following shipment. Any faults or losses must be notified to responsible **SERVA Electrophoresis GmbH** distributor immediately. **SERVA Electrophoresis GmbH** Heidelberg 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.

Please retain all packaging materials until the warranty period has expired.

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1. Packing List

BlueVertical 102

Cat. No.: BV 102B

No. of Items	Description	Part. No.
1	Main Unit	BV 102
1	Dummy-Platte	BV 102-6

Cat. No.: BV102

No. of Items	Description	Part. No.
1	Main Unit	BV 102
1	Plain Glass Plate (4 pcs., 3 mm)	BV 102-9
1	Notched Glass Plate (4 pcs., 3 mm)	BV 102-10
2	Spacer-Set, 1.0 mm	BV 101-1.0
2	Comb, 10 sample, 1.0 mm	BV 101-10-1.0
1	Dummy-Platte	BV 102-6

BlueVertical 102 System

Cat.No.: BV102 Syst.

No. of Items	Description	Part. No.
1	Main Unit	BV 102
1	Plain Glass Plate	BV 102-9
1	Notched Glass Plate	BV 102-10
2	Spacer Set, 1.0 mm	BV 101-1.0
2	Comb, 10 sample, 1.0 mm	BV 101-10-1.0
1	Dummy Plate	BV 102-6
1	Casting Module	BV 102/CA

2. Specifications

- Rugged acrylic construction
- All acrylic joints chemically bonded
- Doubly insulated cables, rated safe up to 1,000 volts
- Gold plated electrical connectors, corrosion-free and rated safe up to volts
- Recessed power connectors, integral with the safety lid
- 0.2 mm diameter platinum electrodes, 99.99 % pure

3. Operational conditions and electrophoresis parameter

Model	Buffer volume (ml)	Ca. gel volume (ml)	Max. voltage (Volt)	Max. current (mAmp)	Run condition (1 mm spacer)
BV102	Upper 200 Lower 700	7	500	250	125 - 180 V const. voltage 15 - 30 mA const. current

Suitable environmental conditions

- This apparatus is intended for indoor use only.
- The normal operating temperature range is between 4 °C and 65 °C.
- For operation recommended: Maximum relative humidity 80 % (for temperatures up to 31 °C) decreasing linearly to 50 % relative humidity (for temperatures up to 40 °C), at maximum altitude of 2000 m (NN).

4. Optional accessories (not included in the standard scope of supply):

Combs for BlueVertical 102 (Gel format 8 x 10 cm)

Cat. No.	No. of wells	Comb thickness	Well width	Well depth*)	Sample volume**)
BV 101-10-1.0	10	1.0 mm	5.5 mm	15 mm	75 µl
BV 101-15-1.0	15	1.0 mm	3.0 mm	15 mm	43 µl
BV 101-15-1.5	15	1.5 mm	3.0 mm	15 mm	63 µl
BV 101-10-1.5	10	1.5 mm	5.5 mm	15 mm	110 µl
BV 101-10-0.75	10	0.75 mm	5.5 mm	15 mm	55 µl
BV 101-15-0.75	15	0.75 mm	3.0 mm	15 mm	30 µl
BV 101-P1-1.5	1 Ref.	1.5 mm	3.0 mm	15 mm	62 µl
	1 Prep.	1.5 mm	54 mm	15 mm	1090 µl
BV 101-P2-1.5	1 Ref.	1.5 mm	3.0 mm	15 mm	60 µl
	1 Prep.	1.5 mm	48.3 mm	15 mm	980 µl

*) The depth of a well refers to a gel cast up to its maximum height; the sample volume of a well relates to it. If the filling height is less, the sample volume is reduced accordingly.

***) Ca. sample volumina ($\pm 10\%$), which can be loaded into the wells.

A complete list of accessories for the SERVA BlueVertical Units is contained in the electrophoresis section of the Main Catalogue of **SERVA Electrophoresis GmbH** which is available upon request.

5. Using the vertical gel electrophoresis unit

5.1. Safety precautions

- **Read** the instructions before using the apparatus.
- Always isolate electrophoresis units from their power supply before removing the safety cover. Isolate the power supply from the mains **first** then disconnect the leads.
- **Do not** attach the safety lid to the internal gel running unit while it is out of the bottom buffer chamber.
- **Do not** exceed the maximum operating voltage or current (see table 1).
- **Do not** operate the electrophoresis units in metal trays. Acrylamide is a volatile, cumulative neurotoxin and suspected carcinogen. Wear effective protective clothing and follow recommended handling and disposal procedures.
- Polymerised gels contain some unpolymerised monomer. Handle with gloves only. Following the replacement of a platinum electrode have the unit inspected and approved by your safety officer prior to use.
- **Do not** fill the unit with running buffer above the maximum fill lines.
- **Do not** move the unit when it is running.
- **Caution:** During electrophoresis very low quantities of various gases are produced at the electrodes. The type of gas produced depends on the composition of the buffer employed. To disperse these gases make sure that the apparatus is run in a well ventilated area.

5.2. General care and maintenance

- To remove the safety lid, push thumbs down on the plastic lugs and lift the lid vertically with your fingers.
- To remove the gel running unit, slide the unit backwards and then lift vertically.
- Before use clean and dry the apparatus with **distilled water only**. **Important: Acrylic plastic is not resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 30 %) and acids (over 25 %).**
- Before use, and then on a monthly basis, check the unit for any leaks at the bonded joints. Place the unit on a sheet of dry tissue and then fill with **distilled water only** to the maximum fill line. Any leakage will be seen on the tissue paper. If any leakage is seen **do not attempt to repair or use the apparatus**, but notify

SERVA Electrophoresis GmbH Heidelberg resp. the **SERVA Electrophoresis GmbH** distributor immediately.

- The replacement platinum electrodes are partially shrouded for protection. However, when cleaning the main tank **do not** use cleaning brushes in the electrode area. Usually a thorough rinse with distilled water is all that is required.
- Ensure that the connectors are clean and dry before usage or storage.

5.3. Storage of the unit

- The units can be stored with water in the base core but 0.02% sodium azide should be added to prevent algal growth.
- Store in a dark cupboard or cold room.
- Alternatively, drain the unit. A small quantity of water will remain in the base core.
- If algal growth does build up over a period of time fill the tank with **neutral** Decon overnight and then flush through with clean water.

5.4. Filling the base cooling core

The base cooling core will already contain a small quantity of water from control tests. The base cooling core can be used in two ways. Static water can be used as a simple heat sink or the tank can be actively regulated using flowing water from a tap or water bath.

Static water cooling regulation

1. Attach a short length of rubber hose to each connector.
2. Incline the tank at an angle of approximately 45 degrees with the ports uppermost.
3. Use a funnel to fill the tank with deionised water containing 0.02% (w/v) sodium azide (preservative to prevent bacterial and algal growth).
4. When filled, keep the unit inclined and attach clamps to each piece of rubber hose.
5. The unit can be cooled before an electrophoresis run if required.
Do not freeze.

Active water cooling regulation

1. Attach a short length of rubber hose to each connector.
2. Attach one end of the rubber hose to the outlet port of a water bath, and the other end to the inlet port.
3. **The maximum recommended water flow rate is 1 l/min. Do not exceed this figure.**
4. If you are using a circulating waterbath which exceeds this flow rate you can attach a T-connector in line. One branch of the connector can return water to the bath and the other can flow to the cooling core and incorporate a flow regulator such as an adjustable tubing clamp. Measure and adjust the flow rate before attaching the line to the gel unit.

No responsibility will be accepted by **SERVA Electrophoresis GmbH** Heidelberg for damage resulting from misuse.

5.5. Self-casting of gels

Gel plate preparation

1. Clean the plates, spacers and combs in mild laboratory detergent. **Do not** use abrasive creams or scourers. If a particularly clean finish is required (e.g. for silver-stained gels) glass plates can be soaked in chromic acid overnight, rinse with water then wipe successively with ethanol, acetone and ethanol again. **Never** allow organic solvents or chromic acid come into contact with the plastic components.
2. The notched glass plate can be siliconised in a fume hood with a Dimethyldichlorosilane or treated with with non-toxic BlueSlick (SERVA cat.no. 42500) which does not require a hood. The hydrophobic coating will assist in plate separation after the run.
3. Handle clean plates with gloved hands (remove any fingerprints with acetone).

Gel plate assembly

1. On a clean level bench position the two side spacers flush with the edges of the large glass plate and overlay the small or notched plate, if used.
2. The gel plates can be sealed either with tape, or by using a gel casting module (BV102/CA for BV102).
3. For tape sealing hold or clamp the plates firmly and seal the edges of the gel cassette with gel sealing tape. The tape should be applied smoothly with no wrinkles. Reinforce the corners by overlapping extra pieces of tape onto the glass. Grease or fingerprints will prevent a good seal being formed.

Gel casting - Using the gel casting module (BV102/CA)

1. Place the inner core running unit on an even surface (e.g., a large glass plate but not the casting module).
2. Clean the glass plates as described above. Make sure that the plates including the ground edges are dry. This is important to provide proper sealing when casting.
3. Lay the notched glass plate on a clean surface and place the spacers down each side flush with the edges of the plate.
4. Overlay the spacers with the plain glass plate.
5. Uplift the wedges in the inner core running unit and fix them in the upper position by tightening the bolt.
6. Place the glass plate sandwich into the electrophoresis unit, with the notched plate innermost. Allow the glass plates and spacers to sit flush with the bottom of the running unit.
7. Affix the glass plate sandwich to the inner core running unit by pushing down the wedges until they sit tight and hereby seal the glass plate sandwich. Lock the wedges by tightening the bolt. Check if the glass plates and spacers still sit flush with the bottom of the running unit. This is important for leakage-free casting.
8. When running only one gel, a dummy plate is required on the other side of the unit, to retain the top buffer level. Insert the dummy plate with the smooth side directed away from the wedges.
9. Place the inner core running unit onto the silicon pads of the gel casting stand. Insert the cams through the holes in the ends of the casting module. The flattened side of the cams has to point upwards. Use the cams to tighten the assembly down onto the silicone pads by turning simultaneously with both hands the set of two front cams gradually (say, by 90 degrees). Then move to the two cams in the back and proceed in the same way. Repeat this procedure until the flattened side of the cams points downwards (max. position!) **(see fig. 1, A). Important: do not try to overtighten** the cams; the casting adapter will now seal the glass plates properly.

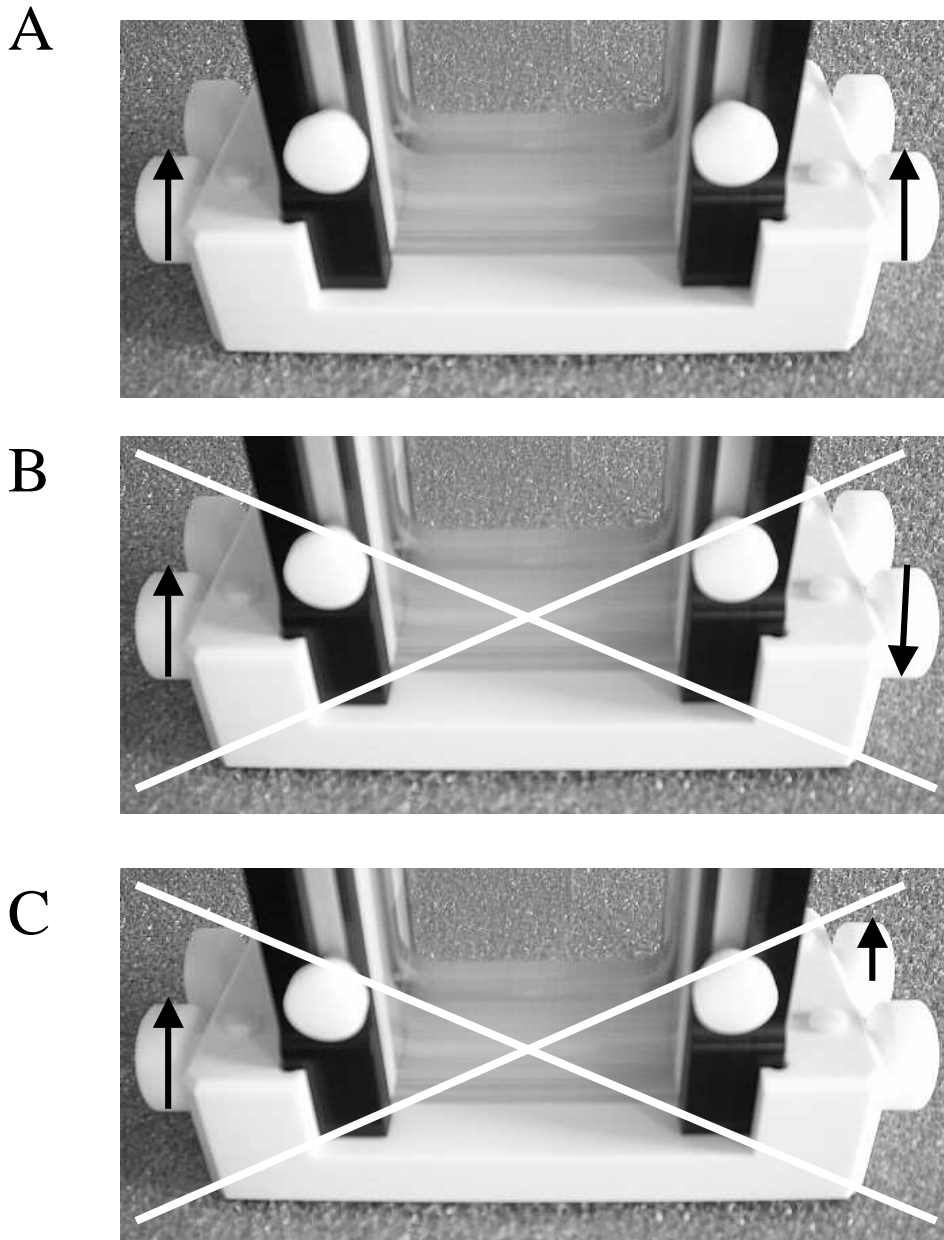


Fig. 1: A shows the correct handling. If you tighten the screws as displayed in B and C, leakage can occur.

10. Cast the gel(s). When the gel(s) is (are) polymerised remove the gel casting adapter, by loosening the cams.
11. Place the running unit with the gels into the bottom buffer chamber, using the locating pegs as guides. Slide the gel running unit forward, to fix it into position and to allow the safety lid to be attached.
12. Add the appropriate volume of running buffer to the upper and lower chambers (see Table 1). **Important: do not** fill over the maximum fill lines.

Gel pouring

For reproducibility and uniform polyacrylamide crosslinking **we recommend deionising, degassing and filtration of acrylamide gel solutions prior to use.** Acrylamide solutions should be stored in a cool, dark environment such as a refrigerator and allowed to reach room temperature prior to pouring. Avoid exposure to heat and sunlight.

1. Run the acrylamide separating gel mix slowly down the inside edge of the gel cassette. **Avoid aeration.** If a stacking gel is to be used, carefully overlay the separating gel to a depth of 3 - 5 mm with 1 x separating gel buffer or water-saturated butanol.
2. Following polymerisation of the separating gel pour off the overlay layer (rinse off butanol with electrophoresis gel buffer) and pour a stacking gel. Insert the comb ensuring bubbles are not trapped. Once the stacking gel has polymerised use the gel immediately.

5.6. Gel and buffer volumes/running conditions

Some guidelines for operating conditions are given in Table 1 but conditions vary according to the number of gels, their composition, length and cross sectional area. The current required will increase in proportion to the number of gels or gel thickness providing that the voltage is not limiting, e.g. 2 gels require twice the current of 1 but the same voltage. Longer gels require proportionally higher voltages.

By increasing the gel concentration the electrical resistance is increased and the rate of migration decreases. Higher voltages can be applied but be careful not to overheat the gel. The conductivity of non-dissociating buffer systems gels vary enormously and conditions have to be determined empirically.

Table 1. Operating conditions for 10 cm and 20 cm long vertical gels with 1 mm spacers

Model	Buffer volume (ml)	Ca. gel volume (ml)	Max. voltage (Volt)	Max. current (mAmp)	Run condition (1 mm spacer)
BV102	Upper 200 Lower 700	7	500	250	125 - 180 V const. voltage 15 - 30 mA const. current

1. The run conditions are to be taken as a guideline only and apply to SDS Tris-glycine gels. If the plates become hot increase the water flow rates within the recommended limits or reduce the power settings.
2. If a native gel is being used, pre-electrophorese the gel for 15 - 40 minutes prior to loading.
3. For SDS gels do not pre-electrophorese the gel.

5.7. Performing electrophoresis

Sample loading

1. Centrifuge samples at 12,000 x g for 5 minutes. If this stage is omitted samples may streak during electrophoresis.
2. Carefully remove the sample comb and immediately flush the wells with electrophoresis buffer from a syringe.

Table 2. Protein amount for a 5 mm wide, 1 mm resp. 1.5 mm thick gel slot

Comb	Single Band	Multiple Bands	Sample Volume
1 mm x 5 mm wide	1 – 6 µg	30 – 60 µg	<40 µl
1.5 mm x 5 mm wide	1 – 10 µg	50 – 100 µg	<60 µl

The volumes of sample wells can be estimated by simply multiplying the comb tooth width and thickness by the depth of the well.

3. Load the samples using a gel loading pipette tip. If possible avoid taking liquid from the pellet area at the bottom of the tube. During sample loading the pipette tip should be 1 - 2 mm above the bottom of the well to minimise dilution of the sample and to keep the sample as a tight layer.
4. Fill unused wells with the equivalent volume of sample buffer to maintain uniform electrical resistance across the gel.

Insertion of separately casted gels and pre-cast gel cassettes into the inner core running unit:

1. Remove the gel tape from the bottom of the gel and from any region that could affect the seal between the glass and the silicone gasket. Clean both the silicone gasket, located on the upper buffer chamber, and the outside of the gel plates. If the gasket becomes unseated from it's groove simply press it back into place.
2. Insert the glass plate sandwich (with the notched plate innermost) or the pre-cast gel cassette into the inner core running unit and affix the position by pushing down the wedges and them by tightening the bolt.
3. When running only one gel, a dummy plate is required on the other side of the unit, to retain the top buffer level. Insert the dummy plate with the smooth side directed towards the wedges.

4. Place the gel running unit into the bottom buffer chamber, using the locating pegs as guides. Slide the gel running unit forward, to fix it into position and to allow the safety lid to be attached.
5. Add the appropriate volume of running buffer to the upper and lower chambers (see Table 1). **Important: Do not** fill over the maximum fill lines.

Start of electrophoresis

6. Replace the safety lid firmly making sure that the electrical connectors form a good contact.
7. Connect the electrophoresis apparatus to the power pack and connect the power pack to the mains supply (e.g SERVA BluePower 500 (BP 500), BluePower Plus (BP Plus). Turn all settings to zero before turning on the mains supply. Adjust the controls to the desired settings. Follow manufacturers instructions.

5.8. End of run

1. Turn the power supply settings to zero, turn off mains supply and disconnect the power leads.
2. Turn off water supply before detaching the rubber tubing from the connectors.
3. Remove the safety lid by gripping the handles and pressing on the locating lugs with your thumbs.
4. Remove the internal gel running unit by sliding backwards and then lifting vertically.
5. Turn the bolt of the wedge left (ca. 90 °C) and slide the wedge to the top. Lock the wedge in this position by tightening of the bolt. Remove the gel sandwich from the running unit. Separate the plates with a strong, broad blade. If you are using notched or eared plates do not prise them apart at the ears. Spread the load over a wide area.
6. After removing the gel for staining, clean the plates thoroughly and rinse in distilled water. A clean sheet of foam rubber placed in the bottom of the sink serves as a useful support and minimises the risk of glass plate damage.
7. Empty the buffer chambers with a vacuum line and trap or carefully decant the buffer away from the electrical connectors. Rinse the chambers with distilled water then dry the electrode connectors with tissue. Never use organic solvents. Avoid direct contact of objects with the platinum electrodes. Ensure that the connectors are clean and dry before usage or storage.

6. Recommended reagents for vertical electrophoresis

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

Product	Cat. No.
Acrylamide 4X	10674
Acrylamide 4x solution, 40 % w/v)	10677
N,N'-Methylene bisacrylamide 2x	29195
N,N'-Methylene bisacrylamide solution (2 % w/v)	29197
Acrylamide/Bis Solution, 19:1, (40 % (w/v))	10679
Acrylamide/Bis Solution, 29:1, (40 % (w/v))	10680
Acrylamide/Bis Solution, 37.5:1, (40 % (w/v))	10681
Ammonium persulfate (APS)	13375
N,N,N',N'-Tetramethyl-ethylenediamine (TEMED)	35925
SERVAGel™ TG 8 % Tris-Glycine	43208
SERVAGel™ TG 10 % Tris-Glycine	43210
SERVAGel™ TG 12 % Tris-Glycine	43212
SERVAGel™ TG 14 % Tris-Glycine	43214
SERVAGel™ TG 16 % Tris-Glycine	43216
SERVAGel™ TG 4-12 % Tris-Glycine	43232
SERVAGel™ TG 4-20 % Tris-Glycine	43230
SERVAGel™ TG 8-16 % Tris-Glycine	43231
SERVAGel™ Neutral pH 7.4	43220
SERVAGel™ Neutral pH 7.4 Gradient	43221
SERVAGel™ SDS PAGE Starter Kit	43200
SERVAGel™ Native PAGE Starter Kit	43201
SERVA Tris-Glycine/SDS sample buffer (2x)	42527
SERVA Tris-Glycine/SDS electrophoresis buffer (10x)	42529
SERVA Tris-Glycine native sample buffer (2x)	42528
SERVA Tris-Glycine native electrophoresis buffer (10x)	42530
SERVA Tris-Tricine/SDS sample buffer (2x)	42551
SERVA Tris-Tricine/SDS electrophoresis buffer (10x)	42552
SERVA Tris-Tricine/SDS electrophoresis buffer (20x)	42560
SERVA Tris-MOPS/SDS electrophoresis buffer (20x)	42561
Glycine	23390
Tris	37190
Sodium dodecylsulfate (SDS)	20760
2-Mercaptoethanol	28625
Dithiothreitol (DTT)	20710
Dithioerythritol (DTE)	20697
SERVA Blue G	35050
SERVA Blue R	35051
Bromophenol Blue-Na-salt	15375

A comprehensive range of SERVA products for electrophoresis is available listed in the Main Catalogue available from **SERVA Electrophoresis**. Please inquire.