Instructions for use

ROTIPHORESE® PROclamp MAXI
Vertical Electrophoresis System

5769.1

ROTIPHORESE® PROclamp MAXI
Unit 20 x 20 cm

With Accessories and Gel Casting Module

For running of up to 4 gels in parallel

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

Warning:
Like all apparatus run by electricity these units are capable of delivering potentially lethal voltage when connected to a power supply. They should be operated only by qualified technically trained personnel. The vertical electrophoresis units from ROTH are designed for long term laboratory use and to obtain reproducible results. Please spend a few moments reading the instruction manual thoroughly.

Please verify that you received the unit completely and without any damage. Any faults or losses have to be reported to ROTH immediately. ROTH can not accept responsibility for goods that were sent back without informing them.

Please take a look at the packing list and check whether all components and accessories are present

Please retain all packaging material until the warranty period has expired.
For further information, please contact us at Phone: ++49721/5606-0

SPECIFICATION

Technical features

- Durable acrylic construction
- All acrylic joints chemically bonded, 100 % leak-proof
- Doubly insulated cables, rated safe up to 1000 volts
- Gold plated electrical connectors, corrosion-free and rated safe up to 1000 volts
- Recessed power connectors, integral with the safety lid
- 0.2 mm diameter platinum electrodes, 99,99 % pure
- User replacable platinum electrodes
- Silicone rubber dovetail seal provides leak-free sealing and is easy to clean or replace
- User-friendly clamping system
- With a wide range of accessories

Environmental Conditions

- This apparatus is intended for indoor use only.
- The unit can be operated safely at an altitude of 2000 m.
- The normal operating temperature range is between 4 °C and 65 °C.
- Maximum relative humidity 80 % for temperatures up to 31°C decreasing linearly to 50 % relative humidity at 40 °C.
- Not for outdoor use.

All Roth products available for delivery have undergone rigorous quality controls.

COMPONENT PARTS

1. Lid and 2 power cables
2. Running module
3. Vertical screw-pins (red + black)
4. Wave-clamps
5. Glass plates
6. Inner buffer chamber (= upper tank)
7. Gasket
8. Detachable cooling coil
9. Outer buffer chamber (= lower tank)
10. Casting module
11. Sealing pad for casting module
12. Comb

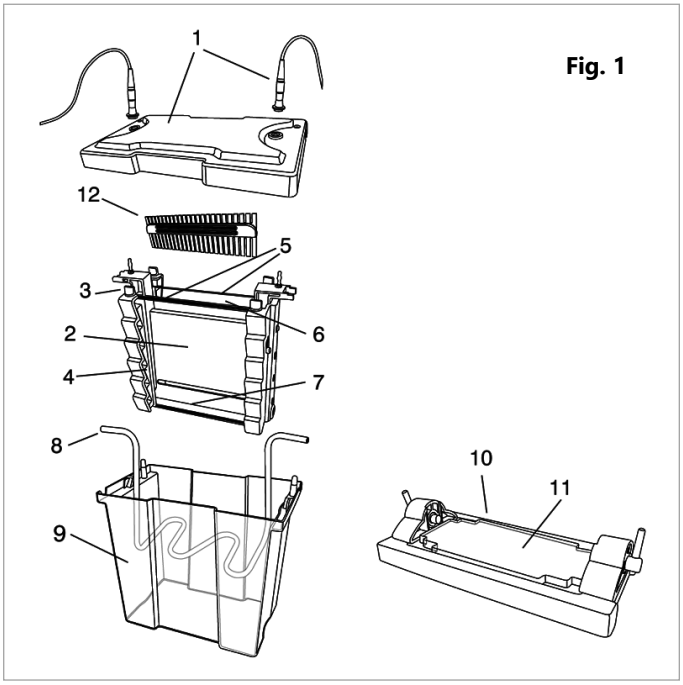


Fig. 1

PACKING LIST

Content	**5769.1**
Tank with lid and cables	1
Running module	1
Detachable cooling coil (inner ø 10 mm)	1
Dummy plate (for run with 1 gel)	1
Yellow wave-clamps (for run with 3-4 gels)	4
Notched glass plates (20 x 20 cm)	2
Glass plates (20 x 20 cm) with 1mm fixed spacers	2
Combs 1mm thick with 24 teeth	2
Casting module	1

ACCESSORIES

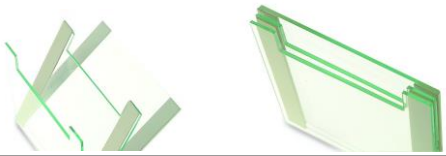
All accessories can be purchased from Carl Roth GmbH + Co. KG. Please use the indicated ordering numbers. Additional reagents and accessories can be found in top L.

Modules and Accessories

	Art. No.
Tank (w/o lid and cooling coil)	5807.1
Replacement lid for tank	5809.1
Running module	5786.1
Casting module	5792.1
Replacement sealing pad for casting module	5793.1
Detachable cooling coil (inner ø 10 mm)	5812.1
Replacement platinum electrode (Ø 0,2 mm, 650 mm long)	T794.1
Replacement platinum electrode (Ø 0,2 mm, 500 mm long)	1428.1

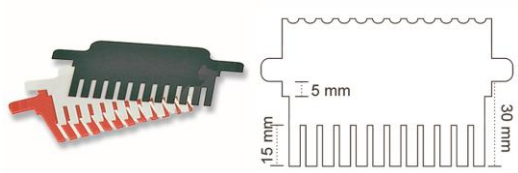
Glass Plates and Spacer

	Thickness (mm)		Pack Qty.	Art. No.
Standard glass plates (20 x 20 cm)	4.0		2 plates	5870.1
Notched glass plates (20 x 20 cm)	4.0		2 plates	5871.1
Dummy plate (20 x 20 cm)	10.0		1 plate	5875.1
Glass plates (20 x 20 cm) with spacers (0.75 mm)	4.0		2 plates	5877.1
Glass plates (20 x 20 cm) with spacers (1.0 mm))	4.0		2 plates	5878.1
Glass plates (20 x 20 cm) with spacers (1.5 mm)	4.0		2 plates	5893.1
Glass plates (20 x 20 cm) with spacers (2.0 mm)	4.0		2 plates	5897.1
Spacers (1.9 x 20 cm)	0.75	black	2 spacers	5908.1
Spacers (1.9 x 20 cm)	1.0	white	2 spacers	5917.1
Spacers (1.9 x 20 cm)	1.5	red	2 spacers	5921.1
Spacers (1.9 x 20 cm)	2.0	blue	2 spacers	5922.1



For pouring of double-gels:

	Thickness (mm)	Pack Qty.	Art. No.
Notched glass plates (20 x 20 cm) w spacers (0.75 mm)	4.0	2 plates	5904.1
Notched glass plates (20 x 20 cm) w spacers (1.0 mm)	4.0	2 plates	5906.1
Notched glass plates (20 x 20 cm) w spacers (1.5 mm)	4.0	2 plates	6011.1
Notched glass plates (20 x 20 cm) w spacers (2.0 mm)	4.0	2 plates	6020.1



Combs

Pack Qty.: 1 comb, respectively

Wells	**1 + 1****	**5**	**10**	**18***	**24**	**30**	**36***	**48**
Thickness	Art. No.	Art. No.	Art. No.	Art. No.	Art. No.	Art. No.	Art. No.	Art. No.
0.75 mm	5925.1**	5928.1	5934.1	5936.1*	5940.1	5941.1	5944.1*	5947.1
1.0 mm	5949.1**	5952.1	5953.1	5955.1*	5957.1	5959.1	5960.1*	5961.1
1.5 mm	5962.1**	5964.1	5967.1	5968.1*	5969.1	5970.1	5971.1*	5972.1
2.0 mm	5974.1**	5977.1	5981.1	5983.1*	5984.1	5999.1	6005.1*	6007.1

* Compatible with multi-channel pipettor ** Combs for preparative gels

Max. sample volume per well								
Wells	**1 + 1****	**5**	**10**	**18***	**24**	**30**	**36***	**48**
0.75 mm	1100 µl	160 µl	80 µl	40 µl	30 µl	25 µl	20 µl	15 µl
1.0 mm	1500 µl	200 µl	100 µl	50 µl	40 µl	35 µl	25 µl	20 µl
1.5 mm	2200 µl	320 µl	160 µl	80 µl	60 µl	50 µl	40 µl	30 µl
2.0 mm	3000 µl	400 µl	200 µl	100 µl	80 µl	70 µl	50 µl	40 µl

FILLING VOLUMES

Volume of gel solution for 1 mm thick gels

1 gel, 1 dummy plate	35 ml
2 gels	70 ml
4 gels	140 ml

Buffer volume

	Upper tank	**Lower tank**	**Cooling potential**	**Buffer vol.**
Minimum	Filled to above the wells	Filled to just flood the bottom of the glass plates.	At minimum	Upper tank: 640 ml Lower tank: 1000 ml
Maximum	Filled to above the wells	Filled to maximum fill line	High	Upper tank: 640 ml Lower tank: 5400 ml (max.) for 2 gels 4800 ml (max) for 4 gels
Using cooling coil	Filled to above the wells	Filled to maximum fill line	At maximum	

OPERATING DATA

For 1 mm thick, 12 % gels

Table 1

Voltage (V)		Current (mA)	
maximum	recommended	maximum	recommended
350 V (2-4 gels)	92 -120 V (collecting gel) 120 – 180 V (separating gel)	30 mA (1 gel) 60 mA (2 gels)	35 mA (2-4 gels)

USING THE VERTICAL GEL ELECTROPHORESIS UNITS

A. Safety Precautions

Please read the entire instruction manual thoroughly 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 exceed the maximum operating voltage or current (see table 1).

Acrylamide is a volatile, concentrated neurotoxin which is suspected to be carcinogenic. Please always wear protective clothing when working with acrylamide and follow and observe the working instructions / directions for disposal carefully. Polymerized gels contain residue of unpolymerized monomer.
Please wear always protective gloves while working.

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.

B. General Care and Maintenance

Clean the apparatus with hand warm water and a mild detergent only. Often, a thorough rinse with distilled water is all that is required. Dry components with clean tissues prior to use, e.g. ROTH tissues (ref. 0087.2)

Important: Acrylic plastic is not resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 25 %) and acids (over 25 %), they will cause „crazing “of the plastic and should not be used for cleaning. Do not use abrasive creams or scourers. The units should never come into contact with the following cleaning agents; these will cause irreversible and accumulative damage: Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol, Alkalis.

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. If any leakage is seen do not attempt to repair or use the apparatus, but notify Carl Roth GmbH & Co. KG immediately (+49/0721/5606-510).

The replacement platinum electrodes are partially shrouded for protection. However, when cleaning the main tank do not use cleaning brushes in the electrode area.

Ensure that the connectors are clean and dry before usage or storage.

C. RNase Decontamination

Clean the units with a mild detergent as described above.
Wash with 3% hydrogen peroxide (H_2O_2) for 10 minutes.
Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using. ROTI®Nucleic Acid free (Art. No. HP69) and RNAse AWAY(TM) (Art. No. A998) may also be used. Please consult the instructions for use with acrylic gel tanks.

D. Fitting Electrode Cables.

1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the unit. Note if the lid is not removed, fitting the cables may result in un-tightening of the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

E. Preparing the gel plates

1. Wash the glass plates, spacer and combs with a mild detergent (e.g. washing-up liquid). Do not use a scouring agent. For gels which require an extremely clean surface (e.g. large or very thin gels, silver staining) you can follow up by washing the glass plates with ethanol, acetone and ethanol again.
2. If required, the glass plates can be hydrosiliconed by vaporizing them with di-Methyldichloro-hydrosilicon for easier separation of plate from gel after gel run.
3. We recommend that you only touch the glass plates with gloves (finger prints can be removed with acetone).

F. Assembly of glass plates

Lay the clean glass plates with the bonded spacers upwards on a flat, clean base. Then place the likewise clean, notched glass plates on top. If standard glass plates without bonded spacers are being used, the spacers should be placed along the outer edges of the short sides of the plates and notched plates laid on top. Be sure to arrange the glass plates in such a way that the mattfinished sides will be at the lower edge of the gel.
NOTE: The glass plates with bonded spacers have an arrow in the top of the spacers which are slightly longer than the glass plate to indicate the top.

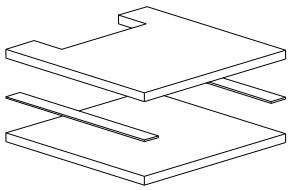


Fig. 2 Assembly of glass plates

For doubling gel capacity from 2 to 4 use triple glass plate sandwiches allowing to cast 2 gels in parallel. You need two notched glass plates – one with and the other without bonded spacers – as well as a plain glass plate with bonded spacers
(Fig. 3). The method is convenient for gels ranging in thickness from 1 mm to 2 mm.

G. Preparing the Running Module

The running module contains green wave-clamps sitting in wave-shaped resting slots. When tightening the vertical screw-pins (colour-coded black and red, respectively) the clamps glide out of the resting slots and fix firmly against the glass plates (Fig. 4). In this way they impart even pressure transfer onto the sealing edges of the plates, ensuring complete sealing.

For converting the unit from a 2 to 4-gel configuration replace the green clamps by the thinner yellow ones (part of the set). Every clamp has a protruding pin with which it is tied to the running module. The pin is sitting in a rectangular guide rail in the resting slot (Fig. 5). When the vertical srew-pin is unscrewed, the clamp-pin is positioned in the angle of the guide rail. When the srew-pin is tightened, the clamp-pin slides down the vertical rail. For replacing the clamps, the srew-pins must always be loosened. Gently push the green clamp horizontally towards the core of the running module until it can move no further. Remove the clamp by pressing the protruding clamp-pin. For inserting the yellow clamps put the appropriate clamp-pin into the horizontal rail and withdraw the clamp outwards.

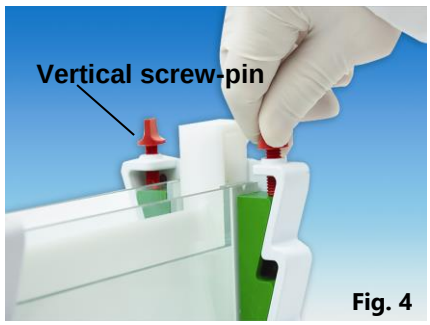


Fig. 4

1. Position the running module on a flat surface. Do **NOT** at this stage insert the running module into the casting base.
2. Loosen the vertical srew-pins so that the wave-clamps sit suspended within the resting slots. Place the assembled glass plates between clamps and core of the running module. Tighten the srew-pins. When only one gel is being run, the dummy plate must be used in the second position and fully tightened. At this stage, check that the bottom edges of the spacers and glass plates are perfectly aligned.
   For inserting the triple glass plate sandwiches – especially when 1.5 mm and 2 mm gels are used – we recommend to turn the running module on its side in order to overcome any resistance that may be posed by the gasket. The side to be loaded is lying downwards. Push the triple glass plate sandwich with the notched plate uppermost into the gap between clamps below and core of the running module incl. gasket above. Restore the running module to its normal upright position, check the bottom edges (s. above) and tighten the vertical screw-pins.
3. Position the running module in the casting base such that the cam pins have handles pointing downwards and are located in the insert holes. The top of the gel running module may need to be pushed down very slightly to locate the cam pins.
4. With the cam pin handles facing directly downwards, turn the cam pins fully through 180^0 or until the insert has tightened onto the silicone mat. It is best to turn the cams in opposite directions to each other. Do **NOT** overturn as this will cause the glass plates to push upwards and the assembly will be more likely to leak. The unit is now ready for gel preparation and pouring.
5. Always reverse the silicone mat after casting to avoid indentations from persisting. Never leave the casting up-stand with glass plates tightened into the casting base for long periods of time as this will also cause indentations in the silicone mat.

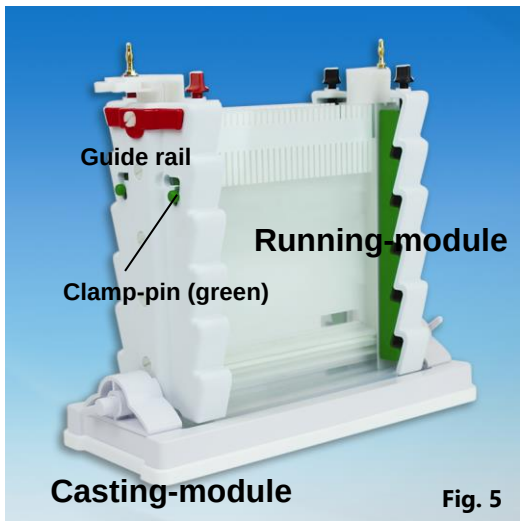


Fig. 5

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H. Casting the gel

For reproducible results and for your safety, we recommend using acrylamide stock solutions (e.g. ROTIPHORESE®Gel 30, ROTIPHORESE®Gel 40; further acrylamide stock solutions can be found in the main Roth catalogue). Acrylamide solutions should be stored in a cool, dark place (refrigerator). For casting the gels, the solutions should have room temperature. Avoid exposure to heat and sun.

1. Separation of proteins: for a 12 % gel, size 20 x 20 cm, prepare a total of 100 ml mixture in a clean glass: 40 ml of 30 % acrylamide stock solution, 25 ml Tris (1.5 M, pH 8.8), 1 ml SDS (10%), distilled water to make up 100 ml. Add the following directly before casting: 1 ml freshly prepared ammonium persulfate (10%), 40 µl TEMED.
   Separation of DNA: for a 12% gel, size 20 x 20 cm, prepare a total of 100 ml mixture in a clean glass: 40 ml of 30 % acrylamide stock solution, 20 ml 5 x TBE, distilled water to make up 100 ml. Add the following directly before casting: 700 µl freshly prepared ammonium persulfate (10%), 40 µl TEMED. Mix carefully avoiding the formation of air bubbles.

2. Test a small amount in an extra vessel before casting the gel. Polymerization should take place within 5 – 10 min. If this is not the case, adapt the conditions by either increasing or decreasing the TEMED amount. Do not cast gels in direct sunlight.
3. The gel casting can be carried out directly in the gel caster. Pour in the mixture slowly between the glass plates. Avoid the formation of air bubbles.
4. If you wish to cast a two-phase gel (collecting gel and separating gel), first cast the separating gel to app. 3 cm under the edge of the indentation in the notched plate. Please ensure that there are no air bubbles in the gel and then carefully cover the separating gel 3-5 mm high with isopropanol. Better polymerization can be achieved by excluding all air.
5. Following polymerization of the separating gel pour off the isopropanol. Absorb remnants of isopropanol with kim wipes, while carefully avoiding to touch the gel surface. Rinse the upper rim of the separating gel with destilled water and remove aqueous remnants.
6. Prepare and pour the collecting gel, carefully avoiding the formation of air bubbles. Separating gels are usually composed of 5 % acrylamide. Prepare a total of 20 ml mixture in a clean glass: 3.3 ml of 30 % acrylamide stock solution, 2.5 µl Tris (1 M, pH 6.8), 200 µl SDS (10 %), distilled water to make up 20 ml. Add the following directly before casting: 200 µl freshly prepared ammonium persulfate (10 %), 20 µl TEMED.
7. Insert a comb between the gel plates before gel polymerizes and allow the acrylamide solution to complete polymerizing. **Attention:** Push the comb slowly! Avoid any splashing of the acrylamide! We recommend wearing eye protection for this step.
   The gel can be used as soon as the collecting gel has polymerized. Remove the screws from the gel casting device and take out the running module with the cast gels.
   For gel runs: Do **NOT** open the screws on the running module, but place it with the gel into the tank.
   For storage: Carefully open the screws, releasing the pressure gradually and evenly. Wrap the slightly damp gel in cling foil and store it at 4 °C for maximum 2 days.

I. Gel and buffer volumes / Conditions for the gel run

If you are only working with one gel, please insert a dummy plate on the other side of the running module. Pour min. 1 l running buffer into the outer (lower) and app. 640 ml running buffer into the inner (upper) buffer tank.
Running buffer f. protein gels: Tris-glycine-buffer: 25 mM Tris-base, 250 mM glycine (pH 8.3), 0.1% SDS
Running buffer f. DNA gels: 1 x TBE buffer
Recommendations for working conditions are given in Table 1, the conditions vary, however, depending on the gel amount, its composition, and the cross-linking grade of polyacrylamide. The required current increases proportionally to the gel amount or thickness, provided it's not limited by the voltage. For example, two gels require twice the amount of current compared to one gel at the same voltage. By increasing the gel concentration, the electric resistance is also increased and as a result the velocity of migration is reduced. Higher voltages can be fed, however, please take care that the gel is not overheated. Conductivity of gels with non-dissociated buffer systems varies enormously and the conditions must be determined empirically.

If you want to cool the gel during the run insert the cooling coil into the tank before positioning the running module. Connect it to convenient tubing allowing water supply and drain. The inner diameter of the coil is 10 mm.

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.

J. Sample application

1. Carefully remove the sample comb and immediately rinse out the slots with a syringe or plastic Pasteur pipette filled with running buffer.
   No forerun is necessary when using denatured SDS gels. When using native protein gels or DNA gels, allow these to run first for app. 30 min in the unit before applying the samples.
   Table 2 shows the protein amounts which can successfully be applied.

Table 2

Gel wells	Single band	Numerous bands	Sample volume
1 mm x 5 mm	1,25 – 7,5 µg	35-75 µg	<60 µl
1,5 mm x 5 mm	1,25 -12,5 µg	65-125 µg	<95 µl

2. Mix the protein samples with 1/3 volume ROTI®Load 4 x (SDS-running buffer) or resuspend the pellet in app. 30 µl 1 x ROTI®Load. Heat the sample for 3 min to 100 °C or for 5 min to 80 °C. Centrifuge for 5 min at 12000 g. DNA is mixed with 1/5 volume 6 x sample buffer (e.g. 6 x ROTI®Load DNA) or pelleted DNA is resuspended in 1 x running buffer (e.g. 1 x ROTI®Load DNA).
3. Apply the samples using a pipettor with gel loading tip. Avoid removing any samples from the sediment at the bottom of the tube. The pipettor tip should be held 1-2 mm above the base of the recess during sample application to minimize dilution of sample and to apply a thick layer of sample. Fill the unused slots with the same volume of sample 1 x buffer to receive constant electric resistance over the total gel. Close the safety lid tightly to ensure that the electric connectors have good contact.
4. Couple the electrophoresis chamber to the mains receiver and connect the latter to the current. Set the mains receiver at the required values (see table 1) according to the manufacturer's instructions.

K. At the End of the Run

1. Turn the power supply settings to zero, turn off the mains supply and **disconnect the power leads.**
2. Turn off the water supply if the unit is being cooled.
3. Remove the safety lid by pressing against the tank from the top and take out the lid by the handles.
4. Remove the running module from the tank and pour the buffer from the top buffer chamber. Now you can loosen the screws carefully and then remove the gel(s).
5. Separate the glass plates with a broad, stiff blade beginning at the bottom of the gel.
6. Carefully transfer the gel to a staining chamber and stain it with Giemsa (e.g. ROTI®Blue A152) or via silver staining. It can also be transferred onto a membrane for blotting. DNA-gels can be stained with ethidium bromide.
7. After removing the gel, clean the plates thoroughly and rinse in distilled water. A clean sheet of foam rubber placed at the bottom of the sink serves as a usual support and minimises the risk of glass plate damage.
8. Drain the lower buffer chamber using a vaccum pump or pour off the buffer carefully.
9. Rinse the chambers with distilled water, then dry the electrode connectors with tissue. Do not use organic solvents. Ensure that the connectors are clean and dry before usage or storage.

L. Additional Items and Reagents

Acetone, >99.5%, for synthesis	5025
Ammonium persulfate (APS)	9592
Clamps	e.g. 0566
Ethanol 70%, DAB	7301
Ethidium bromide	7870
Ethidium bromide solution 1%	2218
Filter papers	4926
Isopropanol	6752
Levelling table	N854
ROTI®Blue 5x colloidal Coomassie staining	A152
ROTI®GelStain (Alternative to ethidium bromide)	3865
ROTI®GelStain Red (Alternative to ethidium bromide)	0984
ROTI®Load 1, 4x (reducing)	K929
ROTI®Load 2, 4x (non-reducing)	K930
ROTI®Load 3 (LDS), 4x (non-reducing)	3359
ROTI®Load DNA 1x (with glycerol)	0100
ROTI®Load DNA 6x (glycerol / ficoll)	X904 / X905

ROTI®Load DNAstain 1-3 SYBR® Green 6x (with glycerol / fluorescent dye SYBR® Green)	1CN5, 1CN6, 1CN7
ROTIlabo®-clips	0827
ROTI®Mark BI-PINK	8269
ROTI®Mark ALL BLUE	2242
ROTI®Mark TRICOLOR	8271
ROTI®Mark TRICOLOR XTRA	2244
ROTIPHORESE®-Blue R Coomassie staining	3074
ROTIPHORESE®Gel 30 or 40	3029 or 3030
ROTIPHORESE® 10 x SDS PAGE ready-to-use running buffer	3060
Silver staining ROTI®Black N (DNA)	N769
Silver staining ROTI®Black P (Protein)	L533
Sodium azide	K305
TEMED	2367

M. Trouble shooting and tips

The gel leaks during casting

- Please ensure that the glass plates and spacers are clean and there are no dirt particles sticking to them.
- Take care that the gel plates are flush to the lower edge of the running module after clamping.
- Please also make sure that all screws have been tightened evenly.
- Smear some vaseline on the spacers before assembling the gel plates.
- Seal the bottom edge of the gel with agarose after fixing the gel plates into the running module and gel casting device. For this prepare 1% agarose in 375 mM Tris, pH 8.8 (protein gels) or 1 x TBE (DNA-gels) by melting until no more streaks are visible. Tilt the gel casting device with the plates and allow some of the hot agarose to run down inside on one side of the gel. Place the gel casting device upright so that the agarose can spread on the bottom and form a sealing. You can fill the agarose to a height of app. 5 mm and then cast the polyacrylamide gel a few minutes later. The agarose needn't be removed prior to the run, but remains between the plates during the run.
- Before fixing the gel plates, the bottom end can be taped with packing tape. Fasten the plates together after assembling them with strong clamps and stick a strip of broad packing tape lengthwise to the lower edge of the glass plates to close the slit. The tape should overlap a few centimeters on both sides. After folding and sticking the tape onto the glass plates it should be pressed down firmly. The glass plates can now be fixed for casting the gel into the running module and gel casting device. After the gel has polymerized, the packing tape must be removed before the gel can be fixed again and prepared for the run.

Air bubbles in the gel during casting

- Either remove the bubbles immediately with a thin spacer or tilt the gel slightly and knock the air bubbles down to the edge.

The gel doesn't polymerize entirely

- Can occur due to low temperatures, too low amounts of TEMED, too old (degraded) TEMED, too old APS or too low acrylamide concentration. Use fresh solutions, particularly freshly prepared APS. Keep all other solutions in the refrigerator. Degas the gel solution before use.

Gel doesn't run / no air bubbles on the electrodes

- Check all connections, contact pnotcheds and switches. Take care that the level of the upper buffer covers the indentation of the notched plate.

Glass plates are cracked after the run or break during the run

- The gel was subjected to too much tension. Don't tighten the screws so firmly. Take care that the pressure on all screws is increased slowly and evenly. Reduce the voltage during the run. The gel won't get quite so hot.

Gel edge pulled up to top, “Smiling”

- Gel temperature was not distributed evenly. Reduce the voltage during the run, increase the cooling. Be sure to fill empty wells with sample buffer.

Vertical smears in the bands

- Possibly there were dirt particles in the gel mix. Use Roth acrylamide stock solutions, making sure that the gel solution is prepared in clean glass containers. The solution can be filtered and degassed before adding APS and TEMED.
- The sample was not centrifuged before applying or sediment was also applied.
- Too much protein was applied. Dilute the sample.
- Reduce the voltage during the run.

Bands are horizontally smudged

- Is caused by diffusion of the sample prior to the run. Apply probes more quickly and then carry out the run immediately.

Bands are diffuse

- Too large protein amounts for too small wells, or gel is too thin. Apply less protein.
- Too high a voltage reduces the running time, but results in a poorer separation of proteins. Reduce the voltage during the run.
- With DNA-gels: Use the same mixture 5 x TBE to prepare the gel and the running buffer. Slight variations in the concentration can impair separation considerably.

ROTIPHORESE® PROclamp MAXI Unit	20 x 20 cm	**5769.1**

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