

Instructions for use



ROTIPHORESE® PROclamp MINI Wide Vertical Electrophoresis System

1395.1

**ROTIPHORESE®
PROclamp MINI Wide unit
20 x 10 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 cannot 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 Tel.: 0721/5606-0.**

SPECIFICATION

Technical features

- ✓ User-friendly injection molded construction, 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.

PACKING LIST

| Content | Pack Qty. |
|--|-----------|
| Tank with lid and 2 power cables | 1 |
| Running module | 1 |
| Dummy plate (for run with 1 gel) | 1 |
| Yellow wave-clamps (for run with 3-4 gels) | 4 |
| 4.0 mm notched glass plates (20 x 10 cm) | 2 |
| 4.0 mm glass plates (20 x 10 cm) with 1 mm fixed spacers | 2 |
| Combs 1 mm thick with 24 teeth | 2 |
| Cooling set | 1 |
| 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 K.

Modules and Accessories

| | Art. No. | Pack Qty. |
|--|---------------|-----------|
| Tank (w/o lid) | 1396.1 | 1 |
| Replacement lid for tank (w/o cables) | 1400.1 | 1 |
| Power cables | 6848.1 | 2 |
| Running module | 1401.1 | 1 |
| Casting module | 5792.1 | 1 |
| Replacement sealing pad for casting module | 5793.1 | 1 |
| Cooling Set | 3512.1 | 1 |
| Replacement platin electrode (Ø 0,2 mm, 650 mm long) | T794.1 | 1 |
| Replacement platin electrode (Ø 0,2 mm, 500 mm long) | 1428.1 | 1 |



Glass Plates and Spacer

| | Thickness (mm) | | Pack Qty. | Art. No. |
|--|----------------|-------|-----------|---------------|
| Standard glass plates (20 x 10 cm) | 4.0 | | 2 | 1404.1 |
| Notched glass plates (20 x 10 cm) | 4.0 | | 2 | 1405.1 |
| Dummy plate (20 x 10 cm) | 10.0 | | 1 | 1421.1 |
| Glass plates (20 x 10 cm) with spacers (0.75 mm) | 4.0 | | 2 | 1422.1 |
| Glass plates (20 x 10 cm) with spacers (1.0 mm) | 4.0 | | 2 | 1423.1 |
| Glass plates (20 x 10 cm) with spacers (1.5 mm) | 4.0 | | 2 | 1424.1 |
| Glass plates (20 x 10 cm) with spacers (2.0 mm) | 4.0 | | 2 | 1425.1 |
| Spacers (1.2 x 10.1 cm) | 0.75 | black | 2 | 3573.1 |
| Spacers (1.2 x 10.1 cm) | 1.0 | white | 2 | 3578.1 |
| Spacers (1.2 x 10.1 cm) | 1.5 | red | 2 | 3579.1 |
| Spacers (1.2 x 10.1 cm) | 2.0 | blue | 2 | 3584.1 |

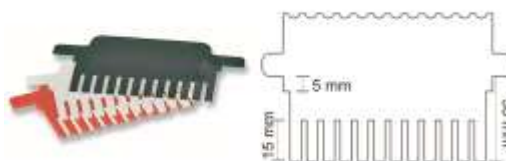
For pouring of double-gels:



| | Thickness (mm) | Pack Qty. | Art. No. |
|---|----------------|-----------|---------------|
| Notched glass plates (20 x 10 cm) w spacers (0.75 mm) | 4.0 | 2 plates | 1426.1 |
| Notched glass plates (20 x 10 cm) w spacers (1.0 mm) | 4.0 | 2 plates | 1427.1 |

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 |

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 (H₂O₂) 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.

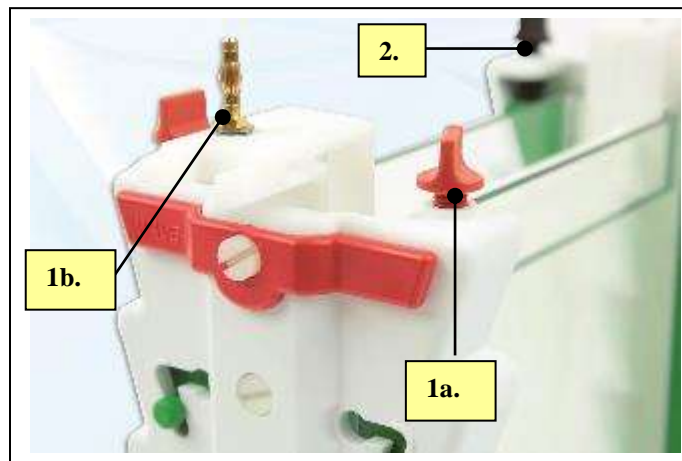
ROTI®Nucleic Acid free (Art. No. HP69) and RNase AWAY™ (Art. No. A998) may also be used. Please consult the instructions for use with acrylic gel tanks.

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.

Colour-coded screw pins prevent polarity reversal

1. Red vertical screw pin (1a.) corresponding to positive electrode plug (1b.) colour-coded with **positive** power cable
2. Black vertical screw pin (2.) corresponding to negative electrode plug (not shown) colour-coded with **negative** power cable



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).

NOTE: All glass plates, modules and casting base accessories must be completely dry during set-up. Wet components are more likely to misalign and cause leaks.

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 plates on top. If standard glass plates without bonded spacers are being used, the spacers should be placed along the outer edges of the 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.

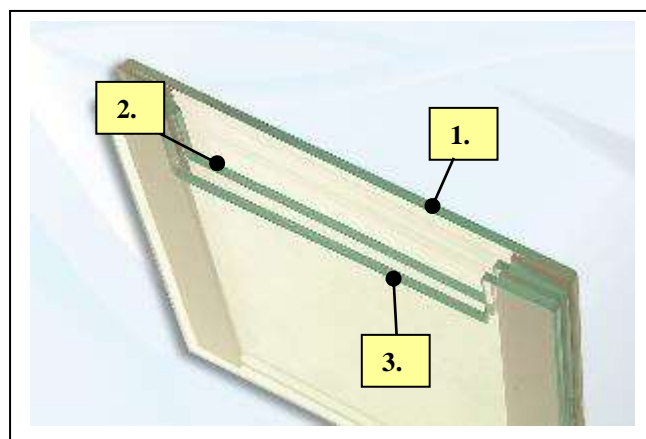
NOTE: The glass plates with bonded spacers have an arrow in the top of the spacers which are slightly longer than the glass plate to indicate the top.

Glass plate set-up for 2-gel sandwich using yellow sliding gel clamps (see page 6):

Plain glass plate (1.) with bonded spacers

Notched glass plate with bonded spacers (2.)

Notched glass plate (3.)



G. Gel Casting

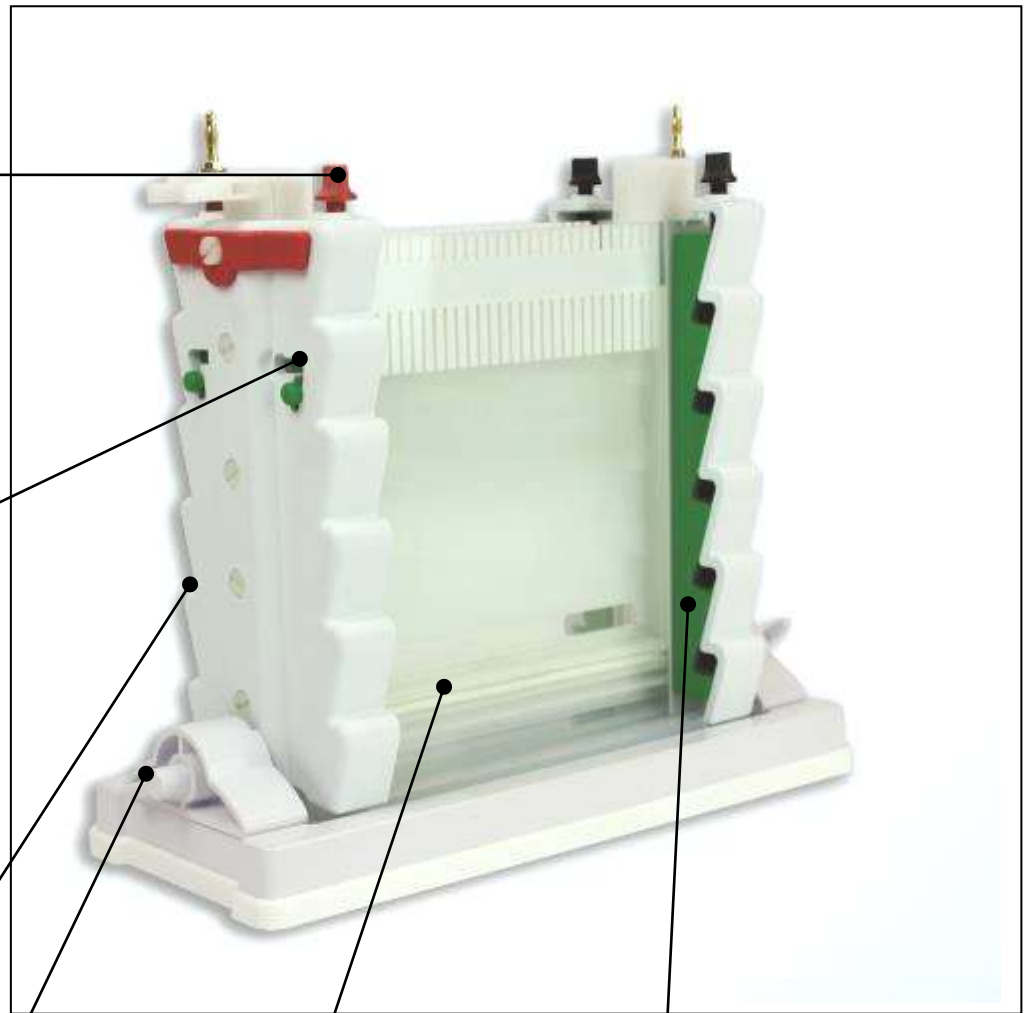
Leak-free Casting Using Vertical Screw-Pin Technology

Vertical screw-pins, colour-coded to prevent polarity reversal, push gel clamps out of the resting slots to secure glass plates firmly within the running module.

Resting slots allow the gel clamps to sit conveniently out of the way, to aid hindrance-free loading of the glass plate sandwiches into the running module.

Ergonomic 'wave' design of the running module provides convenient finger grips for easy handling.

Cam pins lock the running module onto the ultra-soft silicone mat within the casting base to provide leak-free seal.



Flat, level gasket prevents current leakage from inner buffer chamber.

Sliding gel clamps available in two thicknesses and colours to accommodate single- (**green**) and double-gel (**yellow**) glass plate sandwiches.

Detailed set up of the system:

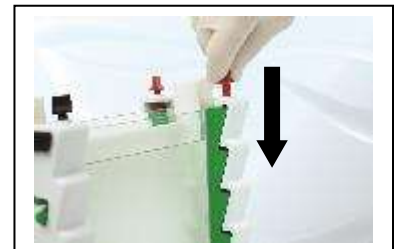
1. Assemble each gel cassette on a flat level surface, by placing the plain glass plate down with the spacers facing upwards followed by the notched glass plate.



2. Loosen the vertical screw-pins in the running module to release the locking mechanism, allowing the gel clamps to sit in the resting slots.



3. Insert a gel cassette into each side of the inner buffer chamber in the running module, and begin tightening the vertical screw-pins.



4. Continue to tighten the screw-pins until the gel clamps glide out of the resting slots and fix firmly against the glass plates pushing them upright.



5. Check the bottom of the glass plates to ensure that they are flush together, and place the running module on the casting base; make sure that the cams are facing downwards.



6. Insert cams and turn until tightened, drawing the running module onto the casting to form a leak-proof seal.



7. Pour in the gel solution, insert the combs and allow the wells to polymerise. Gel pouring is best performed using a 25 ml or 50 ml pipette.



8. Transfer the running module to the gel tank. Fill the inner and outer buffer chambers before loading samples.



9. Replace the lid, connect to the power supply and run.



Converting the System from a 2- to 4-gel Configuration

The thicker green sliding gel clamps are recommended to secure up to 2 gels (i.e. 1 gel either side of the running module) for gels up to a maximum thickness of 2mm. For 4 gels (i.e. 2 gels either side of the running module: made using 1 plain glass plate and 1 notched glass plate, both with bonded spacers, and 1 notched plate without spacers), the thinner YELLOW sliding gel clamps **must** be used.

To convert the system from a 2- to a 4-gel configuration please adhere to the following instructions:

1. To replace the green sliding gel clamps, begin by unscrewing the colour-coded vertical screw pins. There should be no glass plates within the running module, in the side(s) being unscrewed. Once the screw pins are unscrewed sufficiently the green clamp should sit in the resting slot as shown.



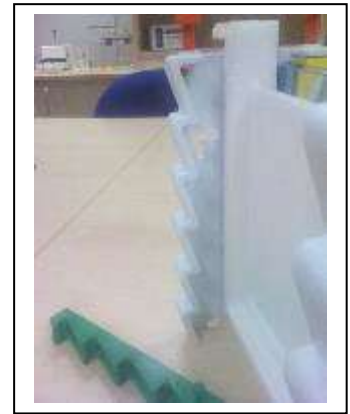
2. Gently push the green sliding clamp horizontally towards the core of running module until it can move no further.



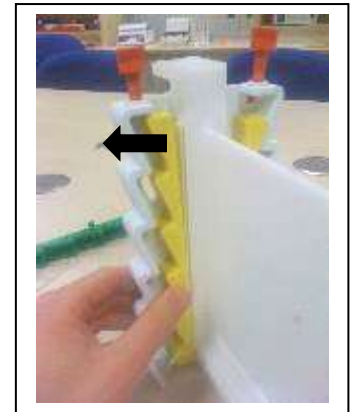
3. Push the green sliding gel clamp out of the running module by gently pressing the protruding pin as shown.



4. Once the green sliding gel clamp is removed the running module is ready to accept the thinner yellow sliding gel clamps for 2-gel sandwiches, either side of the running module, to convert the system to a 4-gel configuration.



5. Insert the yellow sliding gel clamp into the hole closest to the core of the running module. Once inserted gently withdraw the sliding clamp outwards away from the core of the running module as shown.



6. Withdraw the sliding gel clamps so that they sit suspended in the resting slots as shown. Repeat steps 1-6 to replace the remaining green sliding gel clamps. Once complete the running module is ready for 4-gel assembly.

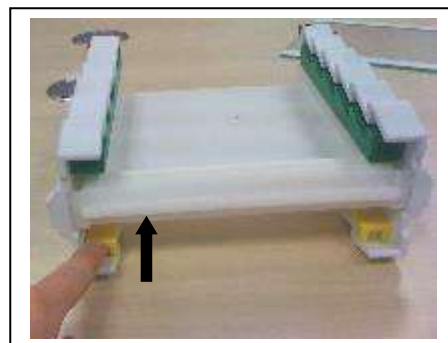


4-gel Assembly

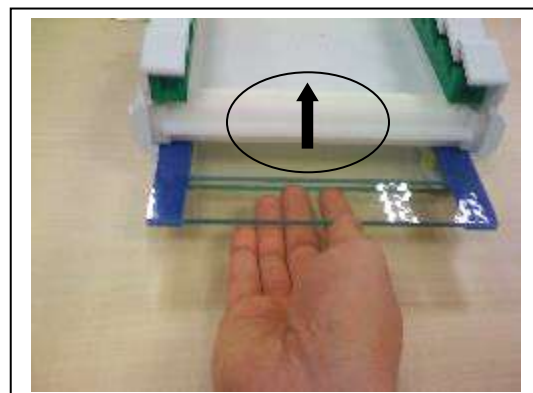
Once converted into the 4-gel configuration using the yellow sliding gel clamps the system is ready to accept and run a maximum of four gels ranging in thickness from 1 mm to 2 mm.

- **1-mm-thick 2-gel sandwiches** may be inserted into the running module from the top as shown in Figures 1-9 in the detailed set up of the system section on pages 7-8.
- However, for **1.5- and 2-mm-thick 2-gel sandwiches**, it may be necessary to load the system by positioning the running module on its side in order to overcome any resistance that may be posed by the gasket. The **instructions below** illustrate how best to undertake this:

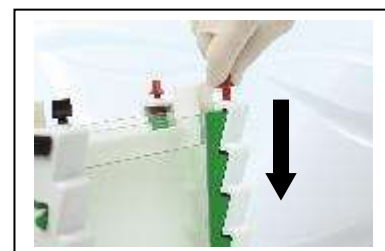
1. With the running module upright in its normal position, unscrew the screw pins sufficiently so that that the sliding gel clamps sit suspended within the resting slots. Turn the running module on its side and push the sliding clamps until they lie completely flat within the running module parallel with the bench surface.



2. Form a **2-gel sandwich** on an even bench surface, as described on page 5. Push the 2-gel sandwich with the notched plate uppermost into the running module. This notched plate will be closest to the core of the running module when it is upright. The uppermost notched glass plate may encounter some resistance from the gasket. This may be overcome by pushing the plain and notched glass beneath it into the running module (**encircled**).



3. Once the resistance is overcome push the uppermost notched glass plate into the running module. Restore the module to its normal upright position and begin securing the glass plates for vertical electrophoresis, as described in steps 3-9 of the **detailed set up of the system** on pages 7-8.



H. Gel Preparation

1. 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, but make sure only to warm the aliquot needed for the gel(s). Avoid exposure to heat and sun.
2. Separation of proteins. For a 12 % gel, size 20 x 10 cm, prepare a total of 60 ml mixture in a clean glass: 24 ml of 30 % acrylamide stock solution, 15 ml Tris (1.5 M, pH 8.8), 600 µl SDS (10 %), distilled water to make up 60 ml. Add the following directly before casting: 600 µl freshly prepared ammonium persulfate (10 %), 24 µl TEMED.
Separation of DNA. For a 12 % gel, size 20 x 10 cm, prepare a total of 60 ml mixture in a clean glass: 24 ml of 30 % acrylamide stock solution, 12 ml 5 x TBE, distilled water to make up 60 ml. Add the following directly before casting: 420 µl freshly prepared ammonium persulfate (10 %), 24 µl TEMED. Mix carefully avoiding the formation of air bubbles.
3. Test a small amount in an extra vessel before casting the gel. Polymerization should take place within 5 – 10 mins. If this is not the case, adapt the conditions by either increasing or decreasing the TEMED amount. Do not cast gels in direct sunlight.
4. If you are only working with one gel, please insert a dummy plate on the other side of the running module.
5. The gel casting can be carried out directly in the gel casting module. Pour the mixture slowly between the glass plates. Avoid the formation of air bubbles.
6. If you wish to cast a two-phase gel (stacking gel and resolution gel), first cast the resolution gel up to approx. 2 cm below the edge of the indentation in the notched plate. Remove any air bubbles from the gel with a comb and then carefully cover the resolution gel 3-5 mm high with isopropanol. Polymerization is improved by excluding all air.
7. Following polymerization of the resolution 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 separation gel with distilled water and remove aqueous remnants.
8. Optional: Prepare and pour the stacking gel, carefully avoiding the formation of air bubbles. Stacking gels are usually composed of 5 % acrylamide. Prepare a total of 10 ml mixture in a clean glass: 1.6 ml of 30 % acrylamide stock solution, 1.2 ml µl Tris (1 M, pH 6.8), 100 µl SDS (10 %), distilled water to make up 10 ml. Add the following directly before casting: 100 µl freshly prepared ammonium persulfate (10 %), 10 µl TEMED.
9. Insert a comb between the gel plates before the gel polymerizes, and allow the acrylamide solution to complete polymerize.
Attention: Push the comb slowly! Avoid any splashing of the acrylamide! Wear eye protection for this step!
10. The gel can be used as soon as the stacking gel is polymerized. Remove the pins from the gel casting device and take out the running module including the cast gels.

For gel runs: Do **NOT** open the clamps of the running module, but straightforward place the assembly into the tank.

For storage: Carefully open the clamps. Wrap the slightly damp gel in cling foil and store it at 4 °C for 2 days in maximum.

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

Total Gel Volume

1 mm thick gels:

| | |
|---|---------|
| Single – one gel, one dummy plate | 17.5 ml |
| Double – two gels | 35.0 ml |
| Four – four gels, using a triple plate sandwich | 70.0 ml |

For different thicknesses of gel multiply the above amounts by spacer thickness.

Buffer Volume

| | Inner Tank | Outer Tank | Cooling potential | Buffer Volume |
|------------------------|---------------------------|--|--|--|
| Minimum | Filled to above the wells | Filled to just flood the bottom of the glass | At minimum - may affect resolution | Inner Tank: 300 ml Outer Tank: 500 ml |
| Maximum | Filled to above the wells | Filled to maximum fill line | High – good resolution | Inner Tank: 300 ml Outer Tank: 2800 ml (2 gels) Outer Tank: 2300 ml (4 gels) |
| Using cool pack | Filled to above the wells | Filled to maximum fill line | At maximum – further enhanced resolution | Inner Tank: 300 ml Outer Tank: 2300 ml (2 gels) Outer Tank: 1800 ml (4 gels) |

Running buffer for protein gels: Tris-glycine-buffer: (25 mM Tris-base, 250 mM glycine (pH 8.3), 0.1 % SDS)
Running buffer for DNA-gels: 1 x TBE.

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 conditions must be determined empirically.

If desired, fit the cooling pack(s) into the end of the tank. These should be pre-frozen and fitted with the longest side positioned sideways with the end(s) of the tank and pressed into the recess. Or these can be fitted down the front of the tank. If the cool set is used, lower buffer volume has to be increased to 1000 ml in minimum.

Please note: Never fit the cool packs underneath the module in the bottom of the tank as this will prevent the flow of current through the gel and cause slow runs and over-heating.

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.

K. Sample application

- Carefully remove the sample comb and immediately rinse the slots with a syringe or plastic Pasteur pipette filled with running buffer. No forerun is necessary when running denatured SDS mini gels. When running native protein gels or DNA gels, allow these to run first for app. 30 min in the unit before applying samples. The table below shows the protein amounts, which can be applied and successfully separated.
- Mix the protein samples with 1/3 volume ROTI®Load 4 x (SDS-running buffer) or resuspend the pellet in app. 20 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), pelleted DNA is resuspended in 1 x sample buffer (e.g. 1 x ROTI®Load DNA).

| Gel wells | Single band | Numerous bands | Sample volume |
|---------------|-------------|----------------|---------------|
| 1 mm x 4 mm | 1-6 µg | 30-60 µg | <40 µl |
| 1.5 mm x 4 mm | 1-10 µg | 50-100 µg | <60 µl |

- Apply the samples using a pipettor with gel loading tip. Avoid 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 1 x sample buffer to receive constant electric resistance over the gel. Close the safety lid tightly to ensure that the electric connectors are in good contact.
- Link the electrophoresis chamber with the Power Supply and connect the latter to the current. Set the Power Supply at the required values according to the gel run.

Recommended values for 1 mm thick, 12 % gels:

| | Voltage (V) | Current (mA) |
|----------|--------------------|---------------------|
| 2-4 gels | 90 – 225 V | 20 – 45 mA |

L. At the End of the Run

1. Turn the power supply settings to zero, turn off the Power Supply and **disconnect the power leads**.
2. Remove the safety lid by pressing against the tank from the top and take off the lid.
3. Remove the running module from the tank and pour the buffer from the upper buffer chamber. Loosen the clamps carefully and remove the gel(s).
4. Separate the glass plates with a broad, stiff blade beginning at the bottom of the gel.
5. Carefully transfer the gel to a staining chamber and stain with Giemsa (e.g. ROTI®Blue A152) or via silver staining. Alternatively transfer the gel onto a membrane for blotting. DNA-gels can be stained with ethidium bromide.
6. 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.
7. Drain the lower buffer chamber by using a vacuum pump or pour off the buffer carefully.
8. Rinse the chambers with distilled water. Then dry the unit according to the instructions given in B. Ensure that the connectors are clean and dry before usage or storage.

M. Additional Items and Reagents

| | |
|---|------------------|
| ROTIPHORESE®-Gel 30 or 40 | 3029 oder 3030 |
| ROTIPHORESE® 10x SDS PAGE ready-to-use running buffer | 3060 |
| Ammonium persulphate APS | 9592 |
| TEMED | 2367 |
| 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 (with glycerol / ficoll) | X904 / X905 |
| ROTI®Load DNastain SYBR®Green 1-3, 6x (with fluorescent dye SYBR®Green) | 1CN5, 1CN6, 1CN7 |
| ROTI®Mark SMALL-KOMBI <i>ready-to-use</i> (not pre-dyed) | 1LCN |
| ROTI®Mark ALL BLUE (pre-dyed) | 2242 |
| ROTI®Mark TRICOLOR (pre-dyed) | 8271 |
| ROTI®Mark TRICOLOR XTRA (pre-dyed) | 2244 |
| ROTI®Blue, Coomassie protein staining solution | A152 |
| ROTI®Blue quick, rapid staining solution | 4829 |
| Silver staining ROTI®Black P (Protein) | L533 |
| Silver staining ROTI®Black N (DNA) | N769 |
| Ethidium bromide | 7870 |
| Ethidium bromide solution 1% | 2218 |
| ROTI®GelStain (repl. for ethidium bromide) | 3865 |
| ROTI®GelStain Red (repl. for ethidium bromide) | 0984 |
| Acetone, >99.5%, for synthesis | 5025 |
| Isopropanol | 6752 |
| Ethanol 70%, DAB | 7301 |
| Sodium azide | K305 |
| Levelling table | N854 |
| Filter papers for gel blotting | 4926 |

N. 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 the clamps and the pins of the casting module have been tightened evenly.
- Apply some vaseline to the spacers before assembling the gel plates.
- Seal the bottom 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 pouring the gels, the bottom end of the plate assembly 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 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. Do not 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 will not 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.

Electrophoresis Unit ROTIPHORESE® PROclamp MINI Wide

1395.1

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