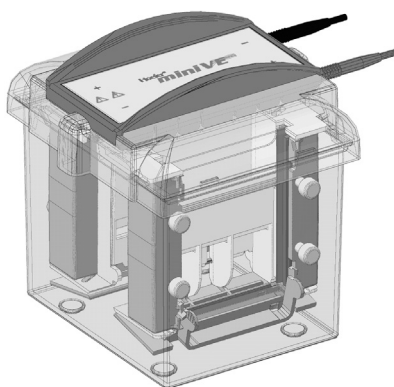


Hoefer SE300 miniVE

Electrophoresis and Electrotransfer Unit





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Important Information – English

- If this equipment is used in a manner not specified by Hoefer, Inc. the protection provided by the equipment may be impaired.
- This instrument is designed for indoor laboratory use only.
- Only accessories and parts approved or supplied by Hoefer, Inc. may be used for operating, maintaining, and servicing this product.
- Only use a power supply that is CE marked or safety certified by a nationally recognized testing laboratory.
- The safety lid must be in place before connecting the power supply leads to a power supply.
- Turn all power supply controls off and disconnect the power leads before removing the safety lid.
- Circulate only water or 50/50 water/ethylene glycol through the heat exchanger if so equipped. Do not connect the heat exchanger to a water tap or any coolant source where the water pressure is unregulated.
- Never introduce antifreeze or any organic solvent into any part of the instrument. Organic solvents will cause irreparable damage to the unit!
- Do not operate with buffer temperatures above the maximum specified technical specifications. Overheating will cause irreparable damage to the unit!

Důležité Informace – Czech

- Pokud by toto zařízení je použito způsobem, který není podle Hoefer, Inc. ochrana poskytovaná na základě zařízení může být narušena.
- Tento nástroj je určen pro vnitřní použití v laboratoři pouze.
- Pouze příslušenství a části schválené, nebo poskytnuté Hoefer, Inc. mohou být použity pro provoz, údržbu, a údržbě tohoto výrobku.
- zdroj napájení používají jen že je opatřen označením CE osvědčena nebo bezpečnost vnitrostátně uznanými zkušebními laboratoř.
- Bezpečnosti lid musí být zavedena před připojením napájecí zdroj napájení vede k.
- Turn veškeré napájení kontroly vypnuto a odpojit před odběrem energie vede bezpečnostní víko.

- Rozeslat pouze voda nebo 50/50 voda/ethylenglykolu prostřednictvím výměník tepla je li to vybavena. Nemají připojení výměník tepla s vodními setřepná nebo jakékoli chladicí kapaliny zdroje, kde tlak vody je neregulováno.
- Nikdy zavést prostředek proti zamrznutí nebo jakákoli organická rozpouštědla do jakékoli části z tohoto nástroje. Rozpuštěním způsobí nenapravitelné poškození jednotka!
- Nejsou provozována s pufru teplotách nad maximální stanovenou technickými specifikacemi. Přehřátí způsobí nenapravitelné poškození jednotka!

Vigtig Information – Danish

- Hvis dette udstyr bruges i en måde ikke specificeret ved Hoefer, Inc. den beskyttelse, som er blevet forsynet af udstyret kan måske svækkes.
- Dette instrument er designet for indendørs laboratoriumbrug bare.
- Bare tilbehør og del godkendte eller forsynede ved Hoefer, Inc. kan måske bruges for drive, funktionsfejl, og betjening dette produkt.
- bruger Bare en strømforsyning, der er CE markerede eller sikkerhed, som er blevet attesteret af en, som nationalt er blevet anerkendt prøve laboratorium.
- Sikkerhedslåget må være på plads før forbinding strømforsyningsblyet til en strømforsyning.
- Drejer alle strømforsyningskontroller af og afbryder kraftblyet før fjerning sikkerhedslåget.
- Cirkulerer bare vand eller 50/50 vand/ethylene glykol gennem varmeveksleren i så fald udrustet. Forbind ikke varmeveksleren til en vandhane eller nogen kølemiddelkilde hvor vandtrykket er unreguleret.
- Introducerer Aldrig antifreeze eller noget organisk opløsningsmiddel ind i nogen del af instrumentet. Organiske opløsningsmidler vil forårsage uboelig skade til enheden!
- Driver ikke med stødpudetemperaturer over maksimummet specificerede tekniske specifikationer. Overhedning vil forårsage uboelig skade til enheden!

Waste Electrical and Electronic Equipment (WEEE)

English



This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

French



Ce symbole indique que les déchets relatifs à l'équipement électrique et électronique ne doivent pas être jetés comme les ordures ménagères non-triées et doivent être collectés séparément. Contactez un représentant agréé du fabricant pour obtenir des informations sur la mise au rebut de votre équipement.

German



Dieses Symbol kennzeichnet elektrische und elektronische Geräte, die nicht mit dem gewöhnlichen, unsortierten Hausmüll entsorgt werden dürfen, sondern separat behandelt werden müssen. Bitte nehmen Sie Kontakt mit einem autorisierten Beauftragten des Herstellers auf, um Informationen hinsichtlich der Entsorgung Ihres Gerätes zu erhalten.

Italian



Questo simbolo indica che i rifiuti derivanti da apparecchiature elettriche ed elettroniche non devono essere smaltiti come rifiuti municipali indifferenziati e devono invece essere raccolti separatamente. Per informazioni relative alle modalità di smantellamento delle apparecchiature fuori uso, contattare un rappresentante autorizzato del fabbricante.

Spanish



Este símbolo indica que el equipo eléctrico y electrónico no debe tirarse con los desechos domésticos y debe tratarse por separado. Contacte con el representante local del fabricante para obtener más información sobre la forma de desechar el equipo.

Swedish



Denna symbol anger att elektriska och elektroniska utrustningar inte får avyttras som osorterat hushållsavfall och måste samlas in separat. Var god kontakta en auktoriserad tillverkarrepresentant för information angående avyttring av utrustningen.

Note: Minimum power supply ratings: 50 mA, 250 V constant current or constant voltage.

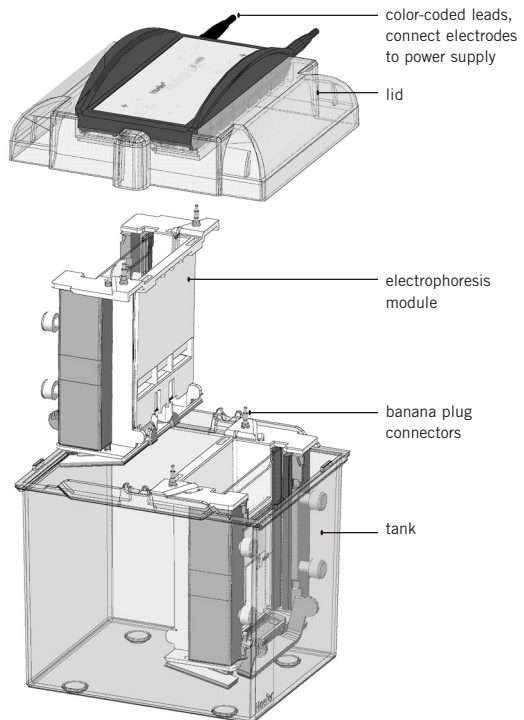
Introduction

The Hoefer® SE300 miniVE vertical electrophoresis system performs vertical gel electrophoresis on mini-format gels. The basic unit includes two electrophoresis modules. Each module holds one gel sandwich, 10 cm wide and up to 10.5 cm long. One gel can be cast in place on each electrophoresis module.

A wide range of accessories, ordered separately (see page 27), lends the miniVE a high degree of versatility. These include:

- a large selection of combs and spacers
- a blot module, to convert the miniVE into a mini blotting unit. (See page 18 for instructions.)

Fig 1. Main components of the Hoefer SE300 miniVE.



Unpacking

- Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived.
- If any part is missing, contact your local sales office. Inspect all components for damage that may have occurred while the unit was in transit. If any part appears damaged, contact the carrier immediately.
- Be sure to keep all packing material for damage claims or for repacking should it become necessary to return the unit.
- Prior to use, wash the tank and module with a dilute solution of non-abrasive laboratory detergent. Thoroughly rinse first with water and then with distilled water.

Specifications

Electrophoresis

| | |
|-------------------|---|
| Gel sandwich size | 10.0 cm wide × 8 to 10.5 cm long |
| Max. tank volume | 1.6 liters with one module in place 1.4 liters with two modules in place |
| Max. voltage | 600 V~ |
| Max. wattage | 25 W per electrophoresis module |

Electrotransfer

| | |
|---|---|
| Max. volume (blot module) | 350 ml per module |
| Max. tank volume (for passive cooling) | 1.7 liters with one module in place 1.2 liters with two modules in place |
| Max. wattage | 15 W per blot module |
| Max. current | 400 mA |

SE300 miniVE specifications

| | |
|---|---|
| Max. operating temp. | 75 °C |
| Chemical compatibility | For use only with dilute aqueous solutions between pH 2 and pH 12. Not compatible with organic solvents or concentrated alcohols, acids, bases, and oxidizing agents. |
| Environmental operating conditions | Indoor use: 4–40 °C Humidity: up to 80% Altitude: up to 2000 m Installation category: II Pollution degree: 2 |
| Dimensions (w × d × h) | 19.2 × 17.2 × 18.8 cm (7.6 × 6.8 × 7.4 in.) |
| Weight (tank, lid, and two gel modules) | 1.2 kg (2.65 lbs) |
| *Product certifications | EN61010–1, UL61010A-1, CSA C22.2 1010.1, CE Certified |

*This declaration of conformity and the warranty are only valid when the instrument is used: in laboratory locations, within the conditions specified in the user manual, as delivered from Hoefer, Inc. except for alterations described in the user manual, and connected to other CE labeled instruments or products recommended or approved by Hoefer, Inc.

The Electrophoresis module

This section describes the use of the electrophoresis module. For instructions on using the blot module, see page 18.

The electrophoresis module accepts both self-cast and precast gels 8 cm wide, from 8–10.5 cm long, and 0.75–1.5 mm thick. For instructions on using the module with precast gels, see page 10.

Preparing the module

To position the module to accept the gel sandwich, each of the three hinged sealing elements must be opened.

1

Release the sealing plate by applying gentle inward pressure to both tabs as indicated by the arrows (Fig 2).

2

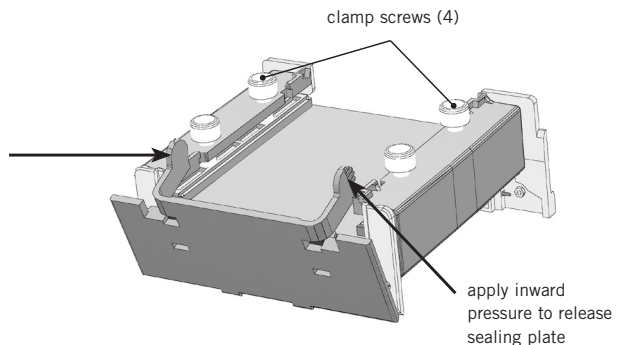
Holding the tabs, move the plate into the fully open position.

3

Loosen all four screws 4–5 turns in the counter-clockwise direction. Do not attempt to remove the screws from the clamps.

Note: The sealing plate has three positions: closed, or sealed for casting; half open, for electrophoresis; and fully open, for placing the gel sandwich.

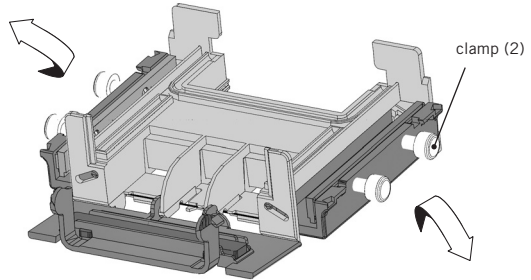
Fig 2. Module in the closed position.



4

To open the module, swing the clamps outward.

Fig 3. Module in the open position.



5

Lay the module flat on a work surface.

Preparing self-cast gels

One single gel can be cast on the module. To cast several gels, use a 4-gel caster such as the Hoefer SE235 caster (see Ordering information on page 27).

Assemble the gel sandwich

1

Prepare the module, as described on page 4.

2

Choose one notched plate, one rectangular glass plate, and two spacers. Use only unchipped plates to prevent leaking.

3

Assemble the gel sandwich with the notch at the top of the sandwich and the spacer ridges align along the glass plate edges on the sides of the sandwich (Fig 4).

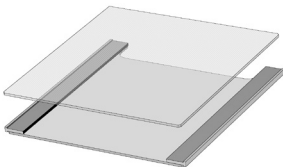


Fig 4. Gel sandwich assembly.

Important! Proper alignment is essential to prevent leakage.

Note: Once the sandwich is carefully aligned, hold the flat sides firmly between your thumb and fingers, near the notch.

Fig 5. Placing the sandwich in the module.

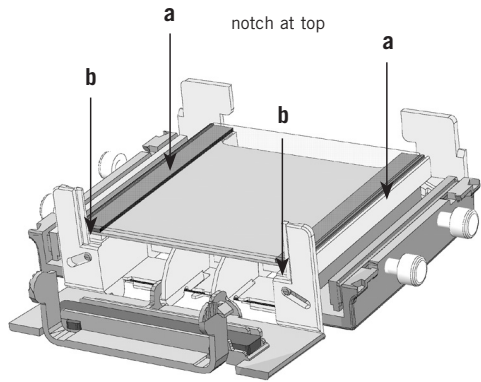
Place and seal the sandwich on the module

1

Take care to “square” the three sealing sides of the sandwich. Hold the sandwich like a deck of cards and gently tap the bottom against a flat surface.

2

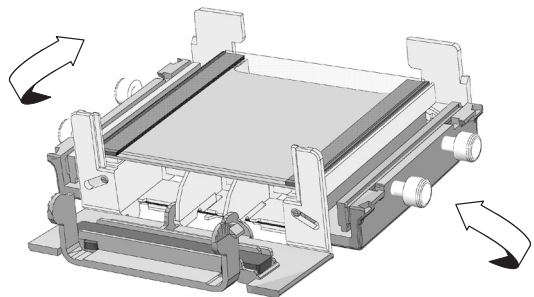
Notched plate side down, lay the sandwich on the module (Fig 5). Fit the gel sandwich within the guides at both sides (a) and against the guide feet at the bottom (b).



3

While gently holding the sandwich against the module, swing one clamp into position over the spacer, taking care not to bump the sandwich out of alignment (Fig 6).

Fig 6. Positioning the clamps.



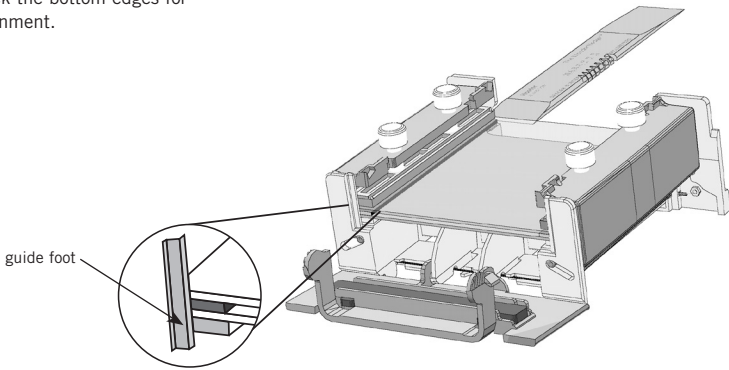
4

Important! Check the alignment of the bottom edge of the sandwich against the guide feet (Fig 7).

Turn each screw (alternating to keep the pressure even) until the clamps are loosely secured and will allow the spacers to be adjusted, if necessary. Repeat on the other side.

If the spacers and glass plates are not perfectly aligned against the stops, use the stiff end of the Hoefer Wonder Wedge to press against the edges of the spacer and glass plates and position them flush against the guide foot.

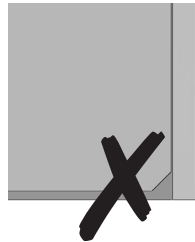
Fig 7. Check the bottom edges for proper alignment.



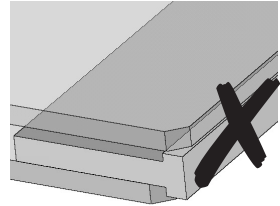
5

Complete clamping by tightening each screw firmly, hand tight. Do not overtighten, as the plates may crack. Check the spacer alignment.

Fig 8. Misalignments cause leaks.



The spacer must not protrude out of the sandwich (or be recessed into it).

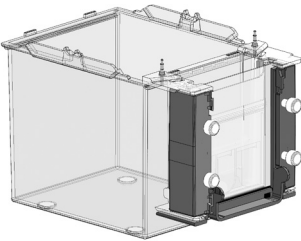


The glass plate should not be resting on the head of the spacer "T".

Fig 9. Assembled module, with tabs engaged in top notch.

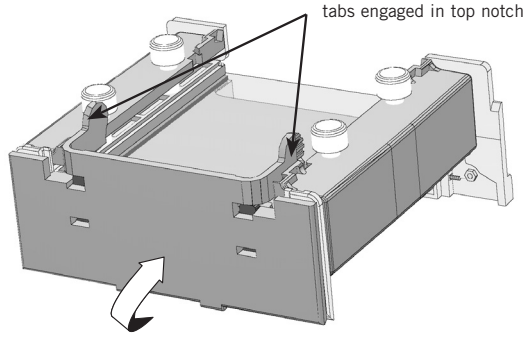
Note: To test for alignment, pass a corner of the Wonder Wedge across the bottom edge of the spacers and glass plates. If an edge “catches,” realign. Check both sides.

Fig 10. Hang the module on the narrow side of the tank to pour the gel.



6

Lock the sealing plate into the closed position by engaging each tab in its topmost notch (Fig 9).



7

Hang the module from the narrow side of the tank or stand it on the benchtop to cast the gel (Fig 10).

When hanging the module on the tank, either fill the tank or hang the second module on the other side as a counterbalance.

Pouring the resolving gel

1

Prepare the monomer solution.

2

Pipet the solution into the sandwich slowly so that it flows along a spacer, taking care not to trap any air pockets.

No stacking gel

Fill the solution to the desired level and then insert a comb, at a slight angle, into the sandwich, taking care not to trap air under the teeth.

Warning! Acrylamide is a neurotoxin. Always wear gloves and observe all laboratory safety procedures.

Note: Approximately 10 ml of monomer solution is required to cast one 1-mm thick gel.

A 1-cm stacking gel below the wells

Fill to 3 cm below the top of the rectangular glass plate. Overlay each gel with a thin layer of water-saturated *n*-butanol, water, or diluted gel buffer to prevent exposing the monomer solution to oxygen. Use a glass syringe fitted with a 22-gauge needle to apply 100 μ l of the overlay solution slowly to one side of the sandwich, near the spacer. Allow the solution to flow across the surface unaided.

After polymerization

①

Allow a minimum of one hour for the gel to polymerize.

②

If a comb is in place, remove it by carefully pulling on the comb while gently rocking it back and forth to break the vacuum. Rinse the wells with electrophoresis buffer to remove any unpolymerized acrylamide.

If an overlay was applied, rinse the sandwich several times with double-distilled water to remove it. Invert the module to drain.

To ensure seamless contact between the resolving and stacking gels, remove residual liquid by blotting one corner of the gel with a lint-free tissue.

Casting the stacking gel

①

Prepare the stacking gel monomer solution.

②

Deaerate the stacking gel monomer solution, add catalyst and initiator and then pour.

Use a pipette to deliver the solution into one corner of the plate, taking care not to trap any bubbles.

Note: If the gel has wells, skip to “Final assembly” on page 11.

Tip: To calculate the volume, measure the distance in centimeters, from the top of the resolving gel to the notch in the glass plate. This should be at least 2 cm. Multiply this distance by the gel width (8 cm) and the gel thickness (cm) for the required volume (ml).

Note: To aid in sample loading, mark the well locations with a laboratory marking pen.

3

Insert a comb (at a slight angle to prevent trapping air) into the sandwich, allowing the comb sides to rest on the spacers.

4

Allow a minimum of one hour for the gel to polymerize.

Working with precast gels

1

Prepare the electrophoresis module as described in “Preparing the module” on page 4. Follow the manufacturer’s instructions to prepare the gel for electrophoresis. This may involve removing tape or breaking off the sealing edge from the bottom of the cassette.

2

Remove the comb and rinse the wells with electrophoresis buffer to remove any unpolymerized acrylamide.

3

If the gel is ready for electrophoresis, move the sealing plate into the “half open” position. Apply gentle pressure to both tabs and lock them into the lower notch.

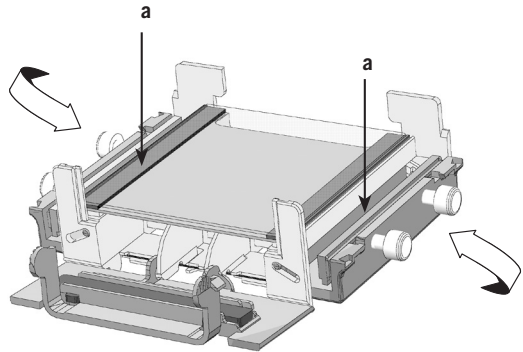
4

Position the cassette on the module. Orient the cassette so that the notched side is against the gasket, and the wells are at the top of the module. Center the cassette within the guiderail at both sides of the module (a) (Fig 11).

5

Secure the cassette. Swing each clamp into position over the sides of the cassette. Tighten each screw, alternating to apply even pressure until the cassette is secure. The gasket around the upper buffer chamber should be fully compressed to provide a seal, but the screws should not be tightened to the point that pressure stresses the cassette.

Fig 11. Securing the cassette.



6

Check that both gel surfaces will contact buffer.
Check that the bottom gel-contact slot is exposed.

7

Move the sealing plate into the “half open” position to prepare for electrophoresis. Apply gentle inward pressure to both tabs and lock them into the lower notch.

Final assembly

1

Make sure the sealing plate is in the “half-open” position. The arrow in Fig 12 indicates the correct position.

2

Lower each module into the tank, seating it in the locating slots.

The module seats properly in only one orientation— with the banana plugs toward the center of the tank and the gel facing outward.

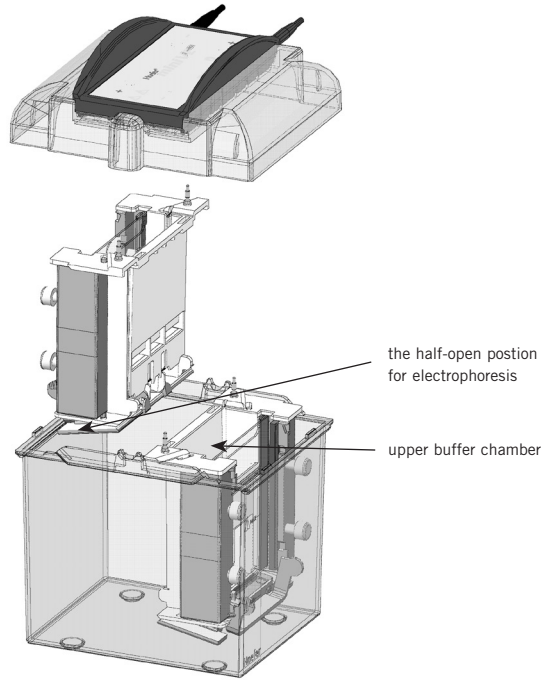
3

Add the appropriate amount of electrophoresis buffer to the tank.

Add 1.2–1.6 liters of buffer to the tank when only one module is in place, and 1.1–1.4 liters when two modules are in place.

Fig 12. Preparing for electrophoresis.

Tip: As an aid in loading samples, mark the well location with a laboratory marking pen or use locating decal. The locating decal only works with miniVE combs and not precast gels.



The minimum and maximum levels are marked. Verify that the lower electrode, which is approximately 2 cm from the bottom of the module, is completely submerged. To prevent buffer from entering the upper buffer chamber, verify that the buffer level is not above the maximum level.

4

Add the appropriate amount of electrophoresis buffer to the upper buffer chamber.

Fill the upper buffer chamber to a level 3–5 mm above the notched plate. This requires approximately 100 ml.

5

Prepare and apply the sample.

Increase liquid sample density with 10% glycerol or sucrose. Add a tracking dye, such as bromophenol blue.

Note: The amount of protein sample added to each well depends on both the sensitivity of the staining method and the distribution of protein among separate bands. With Coomassie™ Blue, it is possible to detect 1 µg in a single band; with the more sensitive silver stains, it is possible to detect as little as 10 ng.

Underlay the sample into the wells using a micropipet or fine-tipped microsyringe. Table 1 shows the volume of sample required for different numbers of wells and comb thicknesses.

Table 1:
Well capacities: volume of sample (µl) per 1mm depth

| no. of wells | comb thickness (mm) | | |
|--------------|---------------------|------|------|
| | 0.75 | 1.0 | 1.5 |
| 5 | 9.5 | 12.7 | 19.1 |
| 9 | — | 5.8 | — |
| 10 | 3.6 | 4.8 | 7.2 |
| 12 | — | 4.75 | — |
| 15 | 2.2 | 2.9 | 4.4 |
| 18 | — | 2.9 | — |

Electrical connections

1

Position the safety lid over the unit and seat the lid so the banana plugs engage the jacks in the lid. The lid is symmetrical and fits in either orientation (Fig 13).

2

Plug the color-coded leads into the jacks of an approved power supply (red to red, black to black). The minimum power supply rating is 250 V, 50 mA, constant current or constant voltage. (Recommended power supply: PS300B.)

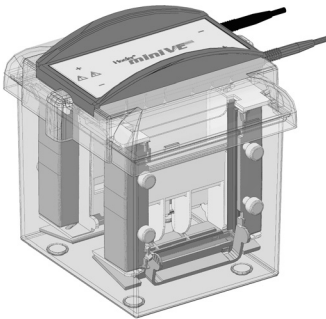


Fig 13. Fully assembled miniVE with electrophoresis module.

Electrophoresis

For optimal resolution, start electrophoresis immediately after sample loading.

Gels may be run at either constant current or constant voltage. For Laemmli SDS separations, the recommended voltage range is 100–250 V and should not exceed 300 V. If running gels at constant current, the current should be 10–20 mA per gel, depending on gel thickness (10 mA for 0.75 mm, 15 mA for 1.5 mm).

Check progress after 5 minutes, and again after half an hour, monitoring the position of the tracking dye. The run is complete when the tracking dye reaches the bottom of the gel.

After electrophoresis

1

Turn off the power supply and disconnect the leads.

2

Remove the safety lid and lift out the module(s).

3

Release each gel sandwich or cassette from the module.

Move the sealing plate to the fully open position by pressing inward on both tabs and guiding the plate to open out. Then loosen all four screws 4–5 turns in the counterclockwise direction. Swing the clamps outward.

4

Remove the gel from the sandwich or cassette.

Gently loosen and then slide away both spacers. Slip an extra spacer or the Hoefer Wonder Wedge into the bottom edge to prevent breaking the “ears” of the notched plates and separate the plates.

If using precast gels, follow gel manufacturer’s instructions.

5

Carefully lift the gel from the plate and lay it into a tray containing stain, fixative, or transfer buffer.

6

Clean the unit as described in “Care and maintenance” below.

Care and maintenance

- Do not autoclave or heat any part above 75°C.
- Do not immerse the safety lid in any liquid.
- Do not use organic solvents, strong or oxidizing cleaning solutions, abrasives, or strong acids or bases on any part of the instrument.

1

Immediately after each use, rinse the tank and modules with water and then rinse thoroughly with distilled water. Handle the module with care to prevent damage to the banana plugs. Allow to air dry.

2

Wipe the lid with a damp cloth. If necessary, briefly rinse the underside of the lid with water.

3

Clean glass plates and spacers with a dilute solution of a laboratory detergent such as RBS-35™, then rinse thoroughly with tap and distilled water. Glass plates can be treated with, but not stored in, acid cleaning solutions.

4

Wipe plates with isopropanol to remove any Gel Seal residue.

Electrophoresis Troubleshooting

| problem | solution |
|---|---|
| Smile effect on the buffer front | <i>To reduce the running temperature:</i> <ul style="list-style-type: none">• Fill the tank to the maximum (marked) buffer level.• Prechill the buffer.• Decrease the current or voltage setting. (10 mA per 0.75 mm gel, 15 mA per 1.5 mm thick gel.)• Run the gel in the cold room. |
| Protein streaks vertically | <ul style="list-style-type: none">• Centrifuge or filter sample before loading to remove particulates.• Dialyze or desalt the sample. |
| Unusually slow (or fast) run | <i>Adjust the solutions:</i> <ul style="list-style-type: none">• Check recipes, gel concentrations, solutions, and dilutions. (For instance, do not use Tris-HCl instead of Tris.)• If the required pH of a solution is exceeded, do not back-titrate. Prepare fresh buffer.• Dispose of older acrylamide solutions and use only stock of the highest quality.• Only use freshly deionized urea. <i>Adjust the voltage or current settings:</i> <ul style="list-style-type: none">• To increase or decrease the migration rate, adjust the voltage or current by 25–50%. |
| Bands are skewed or distorted | <i>Check gel preparation and polymerization:</i> <ul style="list-style-type: none">• Degas the stacking gel solution and avoid trapping air bubbles under the comb teeth.• Overlay the running gel with water-saturated <i>n</i>-butanol before polymerization begins to avoid forming an uneven gel surface. <i>Check sample preparation:</i> <ul style="list-style-type: none">• Dialyze or desalt the sample.• Centrifuge or filter sample before loading to remove particulates. |

problem**solution**

Stained sample collects:

Near the buffer front:

- Protein is not sufficiently restricted by the resolving gel; increase the % T.

Near the top of the gel when the buffer front has reached the bottom:

- The gel pore size is too small. Decrease the % T of the resolving gel.
- The protein has precipitated. Heat the sample at a lower temperature (70 °C or less) for 1–2 minutes.

Poor band resolution

- Use only the highest quality reagents.
- Conduct the separation at a lower current or voltage setting.
- Dialyze or desalt the sample.
- Reduce the sample volume or concentration.
- Only use freshly deionized urea.
- Improve dissociation of subunits by heating sample in SDS sample buffer 1–2 minutes at 100 °C.
- Add more mercaptoethanol or dithiothreitol; check sample treatment.
- Only use gels that were recently prepared.
- Check pH values of the separating and stacking gel solutions. Do not back-titrate buffers.

Sample preparation:

- Heat samples for no more than 1–2 minutes at 100 °C. Store on ice after heating.
- Store sample on ice before it is denatured.
- Add protease inhibitors if necessary to prevent proteolytic degradation of sample.
- Store samples to be frozen in aliquots to prevent repeated freezing and thawing. (Store at -40 to -80 °C.)

Bromophenol blue doesn't sharpen into a concentrated zone in the stacking gel

- Pour a taller stacking gel. (For best results, allow a stacking gel height of 2.5 times the height of the sample in the well.)
- Dispose of outdated acrylamide solutions and use only the highest grade of acrylamide.
- When preparing samples, avoid using solutions with a high sodium or potassium concentration.

The blot module

The Hoefer miniVE Blot Module, which can be ordered separately, performs electrotransfers on mini-format gels. Each module holds up to two gels, 8.2 cm wide and up to 10.4 cm long. One or two modules can be run at the same time.

Assembly

1

Prior to use, wash the tank and blot module with a dilute solution of non-abrasive laboratory detergent. Thoroughly rinse with water and distilled water.

2

Separate out two of the four strands of gaskets included with each module.

3

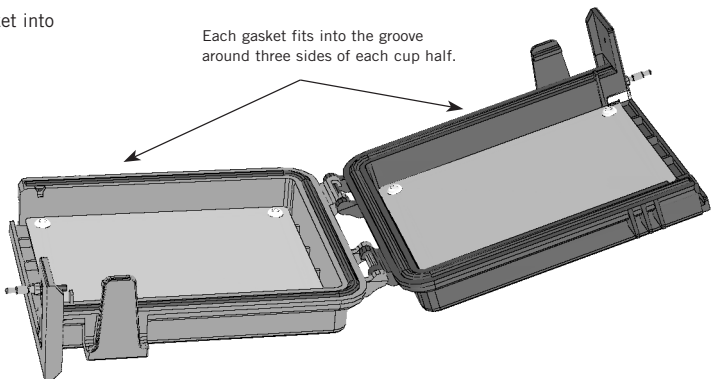
Open the module by releasing both tabs.

4

Lay a gasket along the entire groove around three sides of each cup half.

Avoid stretching or twisting the gasket; the length should just fit. Gently press into place.

Fig 14. Install a gasket into each cup half.



Preparation

Optional: Passive cooling

Chill approximately 2 liters of deionized water to 4 °C. (Filling the tank with chilled water serves as a heat sink during electrotransfer.)

Prepare transfer buffer

Stack assembly requires approximately 250 ml transfer buffer and an additional 300–350 ml buffer is required to fill each module. The recipe for Towbin buffer is listed below. The Bibliography on page 26 lists sources for other buffers.

Towbin buffer

(25 mM Tris, 192 mM glycine, 0–20% (v/v) methanol, pH 8.3, 1 liter)

| | | |
|--------------------|---------------------|--------|
| Tris (FW 121.1) | 25 mM | 3.0 g |
| Glycine (FW 75.07) | 192 mM | 14.4 g |
| SDS* (FW 288.4) | up to 0.1% (3.5 mM) | 1.0 g |

*Optional: Adding SDS can improve transfer efficiency.

1

Dissolve in 750 ml distilled water.

2

Add methanol as required.

Depending on the membrane type selected, adding methanol can improve the transfer results. Because buffers containing methanol may deteriorate if stored for long periods, add methanol just prior to transfer.

3

Bring to 1 liter with distilled water. Do not adjust the pH, which should be between 8.2 and 8.4.

Optional: Chill before use.

Prepare the transfer stack

Transfer the sample as soon as possible after electrophoresis to minimize sample diffusion within the gel. Electrophoretic transfer can be performed on as many as four mini gels at one time, if two gels are placed in each of two modules.

The transfer stack consists of the gel and membrane, filter paper, and three packing sponges. The gel determines the size of the membrane and filter paper.

1

For each gel, cut the membrane and two pieces of filter paper the same size as the gel, but no larger than 8.5×10.5 cm.

2

Equilibrate the gel in transfer buffer for 10 minutes.

Equilibration allows the gel to swell or shrink before it contacts the transfer membrane and removes excess buffer salts and detergents from the gel. Longer equilibration may result in diffuse bands.

3

Pre-wet nitrocellulose or nylon membranes in distilled water, taking care not to trap air bubbles.

Dip one end of the membrane into the buffer and slowly submerge it, allowing it to wet by capillary action.

Pre-wet PVDF or other hydrophobic membranes in methanol.

After pre-wetting, soak all membrane types in transfer buffer for 2–5 minutes.

4

Wet the two pieces of filter paper in transfer buffer.

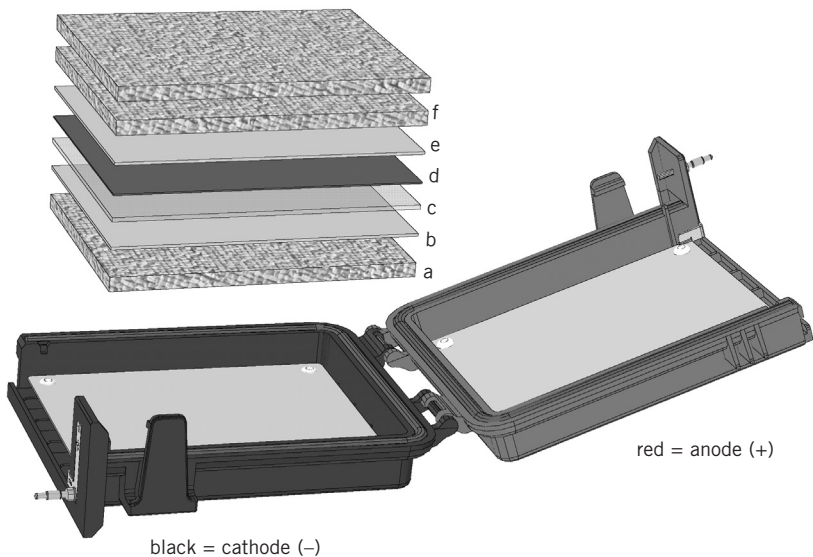
Important! Try to place the gel correctly the first time. Proteins may begin to transfer immediately. Once transfer begins, moving the gel will distort results or cause “shadow bands” on the blot.

5

Assemble the transfer stack so that molecules will migrate to the membrane (Fig 15).

For negatively charged macromolecules—such as proteins run in an SDS gel and nucleic acids— assemble the transfer stack on the black (cathode) side. Proteins will transfer towards the red (anode) side.

Fig 15. Assembling the transfer stack.



Note: For best results, avoid trapping air bubbles as each layer is applied. Always establish full contact along one side and maintain contact as the layer is lowered into position.

- a. Center a packing sponge on the black cathode side.
- b. Lay one piece of wet filter paper on the sponge.
- c. Position the equilibrated gel on the filter paper. Wet the gel surface with a few drops of transfer buffer.
- d. Lay the membrane on the gel. Do not reposition the membrane once it contacts the gel. Use a glass rod to roll out any air bubbles.
- e. Lay one piece of wet filter paper on the membrane.
- f. Lay two packing sponges on the filter paper. A second transfer stack, if added, is placed between these two sponges. Repeat steps b–e.

6

Check the position of the transfer stack.

The transfer stack should be centered on the electrode plate. No layer should be pinched when the module is closed.

7

Fold the empty half of the cup over the stack and press the halves together to snap the module closed.

The transfer stack should be held firmly in place when the cup is closed. Replace old and compressed sponges, if needed, to fill the cup.

Final assembly

1

Slowly pour 300–350 ml of transfer buffer into the top of the module, to allow air to be displaced by the buffer as it fills the cup. Tap the blotting cup lightly to dislodge any air bubbles in the packing sponges.

2

Position the module(s) in the tank with the banana plugs toward the center, the red side facing outward.

3

Add deionized water to the tank — 1.7 liters for one module and 1.2 liters for two modules.

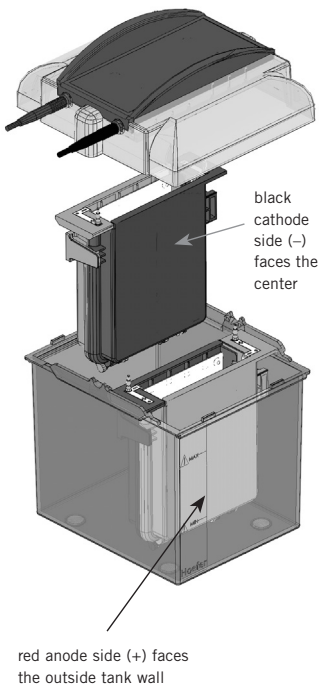
To avoid rapid evaporation, buffer temperature should not exceed 75 °C. Passive cooling is recommended if the transfer will be longer than one hour, if biological activity must be retained, or if transferring nucleic acids. Chill deionized water to ~4 °C before adding to the tank.

4

Place the safety lid on the tank. Either orientation fits and is correct.

Plug the color-coded leads into the jacks of an approved power supply, such as the PS300B or PS200HC: red to red, black to black.

Fig 16. Final assembly.



Important! Buffer conductivity increases with increasing temperature, providing a positive feedback that results in rapid heating. We recommend programming the power supply to hold the current setting constant to avoid possible overheating, especially if no passive cooling is in place. If the only programming option is to hold the voltage setting constant, monitor and adjust the voltage to maintain the current at or below 400 mA.

Electrotransfer

Electrophoretic transfer conditions for blotting proteins in Towbin buffer: 25 V for 1–2 hours, 300–400 mA.

After electrotransfer

1

Turn off the power supply and disconnect the leads.

2

Remove the safety lid.

3

Lift out each module and drain it by inverting it over a sink. Avoid wetting the banana plugs with buffer.

4

Open the module. Remove the gels and membranes. Save the packing sponges. Discard the blotting paper.

5

Label each membrane and indicate the sample side. Lift the membrane(s) with blunt forceps and allow to air dry.

6

Rinse the unit immediately after use.

Blotter care and maintenance

- Do not autoclave or heat any part above 75°C.
- Do not use organic solvents, strong or oxidizing cleaning solutions, abrasives, or strong acids or bases on any part of the instrument.
- Immediately after each use, rinse the unit with water and then rinse thoroughly with distilled water. Handle the module with care to prevent damage to the electrode plugs. Allow to air dry.

Blotter troubleshooting

| problem | possible cause | solution |
|------------------------------|-------------------------------------|---|
| Incomplete transfer | Blank areas on the membrane | Remove all trapped air bubbles in the transfer stack; take especially great care during stack assembly to prevent air bubbles from forming as each layer is placed. Check electrode continuity. Use a lower ionic strength buffer. |
| | Molecules do not migrate out of gel | Increase the field strength. Increase the transfer period. (Try doubling it.) Do not expose the gel to staining or fixing agents before transfer. Use a thinner gel. Reduce the gel acrylamide concentration. For proteins, use 3.5 mM SDS (0.1%) in the transfer buffer. Decrease the methanol in the protein transfer buffer or reduce the amount to a minimum. Typically 10% methanol is required for good binding to nitrocellulose membranes. Increase the length of time DNA blots are dehydrated. Check the buffer pH. Most buffers should not be titrated; make fresh buffer. For native gels, increase the net charge on the protein by changing to a transfer buffer with a different pH. Lower pH (<6–7) increases the positive charge on proteins; higher pH (>6–7) increases the negative charge on proteins. |
| Diffuse band patterns | | Conduct the electrotransfer immediately after electrophoretic separation. Shorten or eliminate the equilibration step before electrotransfer, or conduct equilibration in the cold room. If the transfer buffer contains methanol ($\geq 10\%$), equilibrate the gel for 30 minutes to allow it to shrink fully. <i>Note: Gel shrinkage may slow the migration of large molecules out of the gel.</i> Take care that the gel does not shift once it contacts the membrane. Check that any preferred binding surface of the membrane faces the gel. |

| problem | possible cause | solution |
|----------------------------|-----------------------|---|
| Inefficient binding | Chemical parameters | <p>Fix or crosslink the mole to the requirements of the nucleic acid, protein, or membrane type.</p> <p>Prepare protein transfer buffer without SDS. SDS can improve transfer efficiency but reduces binding.</p> <p>Verify the optimal amount of methanol required for the membrane type and check the buffer solution. Add 10–20% methanol to the transfer buffer to enhance binding to nitrocellulose.</p> |
| | Membrane parameters | <p>Wear gloves when handling membranes.</p> <p>Store membranes properly. Protect them from temperature extremes and direct sunlight.</p> <p>If proteins pass through the selected membrane, try a different type or one with a smaller pore size (0.10–0.20 μm).</p> <p>If different proteins may be migrating in opposite directions, place a membrane on both sides of the gel.</p> <p>If the sample load may be exceeding the capacity of the binding surface area, apply two membranes. If “blow through” occurs, reduce the sample load.</p> |

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Ordering information

| product | quantity | code no. |
|--|----------|---------------|
| Hoefer SE300 miniVE, complete Includes Basic unit, 3 rectangular glass plates, 3 notched plates, 2 each 1.0 mm thick, 10 well combs and 1.0 mm thick spacer sets. | 1 | SE300-10A-1.0 |
| Blot Module Includes 3 Dacron™ packing sponges (¼" thick), 25 sheets of blotter paper. | 1 | SE302 |

Accessories

| | | |
|--|--------|---------------|
| Glass plates, 10 × 10.5 cm | 5/pk | SE262P-5 |
| Notched glass plates, 10 × 10.5 cm | 5/pk | SE262GN-5 |
| Spacers, 0.75 mm thick | pair | SE2619T-2-.75 |
| Spacers, 1.0 mm thick | pair | SE2619T-2-1.0 |
| Spacers, 1.5 mm thick | pair | SE2619T-2-1.5 |
| Comb; 5 well, 0.75 mm thick | 1 | SE211A-5-.75 |
| Comb; 5 well, 1.0 mm thick | 1 | SE211A-5-1.0 |
| Comb; 5 well, 1.5 mm thick | 1 | SE211A-5-1.5 |
| Comb; 9 well, 1.0 mm thick (microtiter) | 1 | SE211A-9-1.0 |
| Comb; 10 well, 0.75 mm thick | 1 | SE211A-10-.75 |
| Comb; 10 well, 1.0 mm thick | 1 | SE211A-10-1.0 |
| Comb; 10 well, 1.5 mm thick | 1 | SE211A-10-1.5 |
| Comb; 12 well, 1.0 mm thick | 1 | SE211A-12-1.0 |
| Comb; 15 well, 0.75 mm thick | 1 | SE211A-15-.75 |
| Comb; 15 well, 1.0 mm thick | 1 | SE211A-15-1.0 |
| Comb; 15 well, 1.5 mm thick | 1 | SE211A-15-1.5 |
| Comb; 18 well, 1.0 mm thick (microtiter) | 1 | SE211A-18-1.0 |
| Comb; prep/ref., 0.75 mm thick | 1 | SE211A-R-.75 |
| Comb; prep/ref., 1.0 mm thick | 1 | SE211A-R-1.0 |
| Comb; prep/ref., 1.5 mm thick | 1 | SE211A-R-1.5 |
| Gasket cord | 100 cm | FH2208 |

| product | quantity | code no. |
|--|-----------------|-----------------|
| Blotting accessories | | |
| Sponge, Dacron packing | 3/pk | SE3005 |
| Blotting paper, 9–10.5 cm | 50/pk | TE26 |
| Gel casters | | |
| Hoefer SE235 4-Gel Caster, 2 to 4 gels, 10 × 10.5 cm | 1 | SE235 |
| Power supplies | | |
| PS200HC Power Supply, 200 V, 2000 mA, 200 W | 1 | PS200HC |
| PS300B Power Supply, 300 V, 500 mA, 90 W | 1 | PS300B |
| Gel drying system | | |
| Hoefer Easy Breeze™ Air Gel Dryer, 115 V | 1 | SE1200-115V |
| Hoefer Easy Breeze Air Gel Dryer, 230 V | 1 | SE1200-230V |



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