# Mighty Small II

## SE 250 and SE 260 Mini-Vertical Gel Electrophoresis Units

# User Manual

80-6291-85 SE250&260-IM/Rev D3/7-99

Amersham Biosciences

# 1 Gel Electrophoresis Unit Function and Description

The SE 250 and SE 260 Mighty Small II cooled miniature vertical slab gel units are intended for rapid electrophoresis of protein or nucleic acid samples. Most samples can be run in as little as 45 minutes, and only a minimal amount of sample is required.

The SE 250 holds one or two 10 × 8 cm gel sandwiches. The SE 260, which has a deeper lower buffer chamber, accommodates one or two 10 × 8 cm or 10 × 10.5 cm gel sandwiches. The upper buffer chamber is formed when the notched side of a gel sandwich is sealed against the silicone rubber gasket. The upper buffer chamber core is hollow and can serve as a heat exchanger if cooling is required. (Coolant is circulated through a port on either side of the core.)

**Note**

Use 1.5 mm spacers when casting slab gels for 2-D electrophoresis in order to fit the 1.5 mm tube gels.

Both dimensions of a 2-D procedure can be run on the Mighty Small with accessories that can be ordered separately. Tube gels are first cast in the Hoefer SE 225 Tube Gel Caster and then transferred to the Hoefer SE 220 Mighty Small Tube Gel Adaptor, which attaches to the SE 250 or SE 260 Mighty Small II unit. After running, the tube gels are extruded from the tubes onto a slab gel sandwich for the second dimension separation.

## 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 to use should it become necessary to return the unit.

## Specifications

| | |
|---|---|
| Gel plate size | SE 250: 10 × 8 cm<br>SE 260: 10 × 10.5 cm |
| Approximate gel size | SE 250: 8 × 7 cm<br>SE 260: 8 × 9.5 cm |
| Max. wattage | 12 W |
| Max. voltage | 500 V |
| Max. amperage | 500 mA |
| Max. temperature | 45 °C |
| Environmental operating conditions | Indoor use: 4–40 °C<br>Humidity up to 80%<br>Altitude up to 2000 m |
| Installation category II<br>Pollution degree II | |
| Dimensions (width × height × depth) | SE 250: 16.5 × 16 × 16 cm (6.5 × 6.3 × 6.3 in.)<br>SE 260: 16.5 × 18 × 16 cm (6.5 × 7.1 × 6.3 in.) |
| Product certifications | EN61010–1, UL3101–1, CSA C22.2 1010.1, CE |

**This declaration of conformity is only valid for the instrument when it is:**

- used in laboratory locations,
- used as delivered from Amersham Biosciences except for alterations described in the User Manual, and
- connected to other CE-labeled instruments or products recommended or approved by Amersham Biosciences

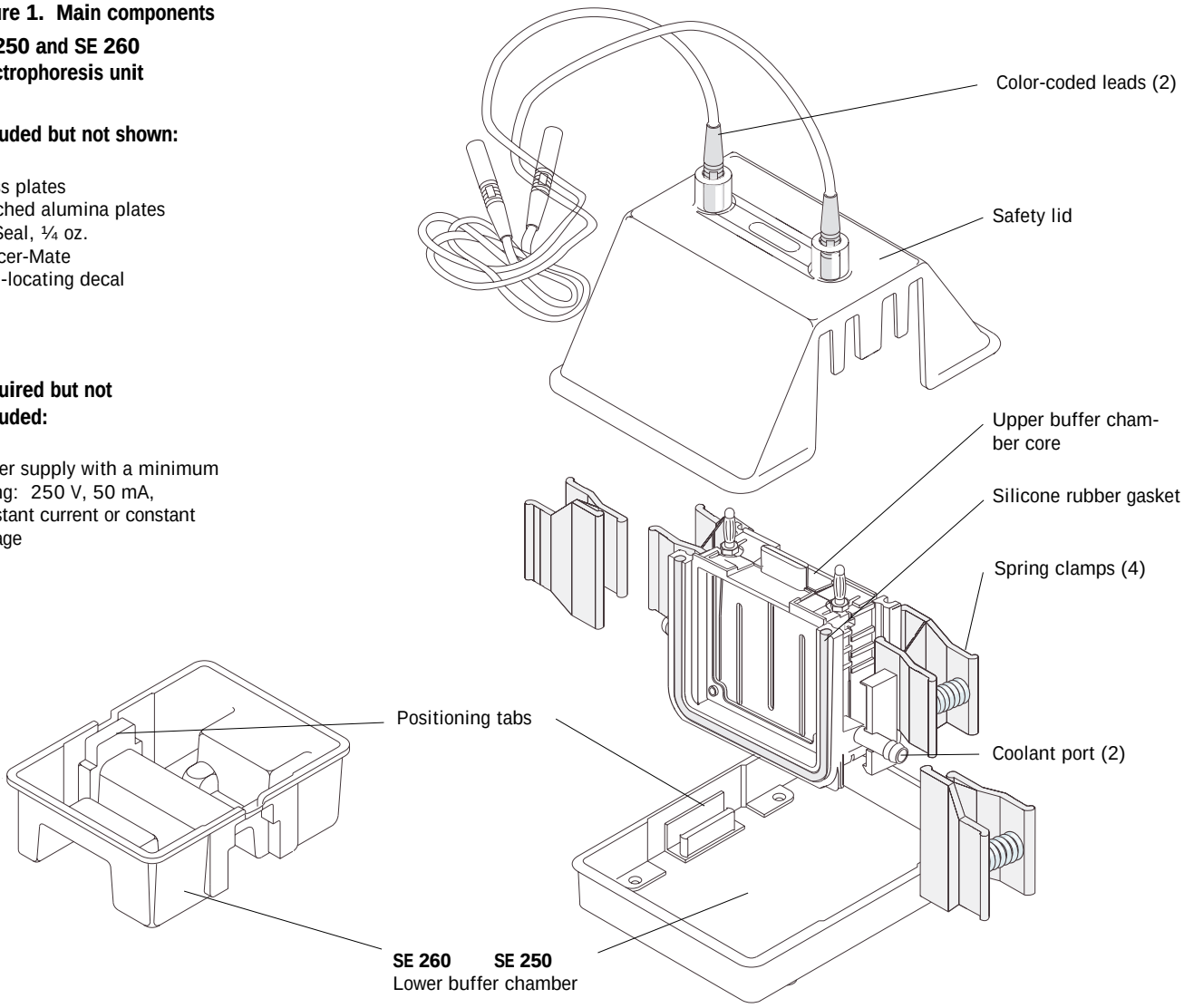
**Figure 1. Main components SE 250 and SE 260 electrophoresis unit**

**Included but not shown:**

Glass plates
Notched alumina plates
GelSeal, ¼ oz.
Spacer-Mate
Well-locating decal

**Required but not included:**

Power supply with a minimum rating: 250 V, 50 mA, constant current or constant voltage



# 2

## Important information

- The safety lid must be in place before connecting the power leads to a power supply.
- Turn all power supply controls off and disconnect the power leads before removing the safety lid.

## Informations importantes

- Le couvercle de sécurité doit être en place avant de brancher les prises au générateur.
- Eteindre le générateur et débrancher les prises avant d'enlever le couvercle de sécurité.

---

- Circulate only water or 50/50 water/ethylene glycol through the heat exchanger. Never introduce anti-freeze or any organic solvent into any part of the instrument. Organic solvents will cause irreparable damage to the unit!
- Do not connect the heat exchanger to a water tap or any coolant source where the water pressure is unregulated.
- Do not operate with buffer temperature above 45 °C. All plastic parts are rated for 45 °C continuous duty. Circulate coolant through the heat exchanger during electrophoresis to minimize heating. Additional passive cooling actions include chilling the buffer before use, running the unit in a cold room, or both. Overheating will cause irreparable damage to the unit!
- If running only one gel, block off the unused part of the core with a glass plate. Do not fill this side with buffer.

- Faire circuler seulement de l'eau ou 50/50 d'eau et d'éthylène glycol dans l'échangeur vertical à cirulation d'eau. Ne jamais utiliser d'anti-gel ou tout autre solvant organique avec cet instrument. Les solvants organiques causeraient des dommages irréparables à l'appareil.
- Ne pas connecter l'échangeur vertical à circulation d'eau à un robinet ou quelque source de refroidissement dont la pression n'est pas régulière.
- Ne pas utiliser avec un tampon à une température au dessus de 45 °C. Toutes les piéces en plastique sont prévues pour résister à une température constante de 45 °C. Faire circuler l'eau dans l'échangeur vertical durant l'électrophorèse pour minimiser l'échauffement. L'on peu aussi refroidir le tampon avant l'utilisation et/ou utiliser l'instrument dans une chambre froide. Un surchauffement peut causer des dommages irréparables à l'instrument.
- Pour le coulage d'un seul gel, bloquer la parite de la chambre non utilisée avec une plaque de verre. Ne pas remplir le côté vide avec du tampon.

---

- Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining, and servicing this product.
- Seulement les accessoires et piéces detachées approuvés ou fournis par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

# 3 Operating Instructions

## 3.1

This section covers general operating procedures. A more detailed procedure for SDS protein electrophoresis is provided in Appendix A.

### Prepare the gel sandwich

**Note**

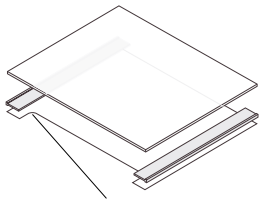
All Hoefer electrophoresis accessories and kits are listed in the the ordering section.

Both precast and self-cast gels can be run in the SE 250 or SE 260 Mighty Small II units. Both units run gels in 10 × 8 cm plates, which can be cast in a Hoefer SE 215, SE 245 or SE 275 Mighty Small Gel Caster. The SE 260 model can also accommodate longer gels in 10 × 10.5 cm plates, which can be cast in the Hoefer SE 235 Mighty Small 4-Gel Caster.

**Note**

Inspect glass plates for chipped edges. Use only unchipped plates to prevent leaking.

Each unit includes notched alumina plates and rectangular glass plates. If casting your own polyacrylamide gels, we recommend using a notched alumina ceramic back plate because it transfers heat 40 times more rapidly than glass. For applications that are not heat sensitive, a notched glass plate is available.




Before loading gels into the electrophoresis unit, the separating gel should already be completely polymerized. Clean away any gel adhering to the alumina back plate. The stacking gel (if applicable) can be cast in place on the electrophoresis unit. Load liquid samples after the gel sandwich is installed.

## 3.2 Prepare the unit

1 **To disassemble a fully assembled unit:** Remove the safety lid by pressing on the handle at the top of the upper buffer chamber core while lifting the lid by the bottom edges. Empty all buffer chambers and remove any gel sandwiches. Then depress both release tabs and lift the upper buffer chamber core.

2 **Rinse the instrument before each use.** Before using the first time, disassemble the unit completely and wash with a dilute solution of a laboratory detergent and thoroughly rinse with water and distilled water.

3 **Check the gasket.** Periodically remove the gray silicone rubber gasket from the core. Inspect for nicks and wear. If the gasket appears to be intact, apply a *light* film of GelSeal, and replace it in the groove. Avoid stretching the gasket by laying it onto the groove and pressing it into place.

4 **Optional cooling.**

**Important:**

Use only water or water and ≤50% ethylene glycol as a coolant. Do not use a commercial antifreeze or any alcohol-based mixture.
Circulating pressure must not exceed 0.8 bar (12 psi) above ambient pressure. Do not connect the cooling core to an unregulated coolant source such as a water tap.

**Note**

If the cooling option is used frequently, it is convenient to attach QuickFit connectors to the tubing. The valves in these fittings prevent coolant spillage.

Connect the cooling core to a circulator bath such as the MultiTemp III. Slide hose clamps (4 total) onto each end of two lengths of 8 mm ($^{5}/_{16}$") vinyl or silicone tubing. Attach one end of each length of tubing to a cooling core port. Attach the free ends of each length of tubing to the circulator bath ports; one to the inlet and the other to the outlet. Secure the connections with the hose clamps.

5 **Install the upper buffer chamber core.** First steady the lower chamber with one hand and then hold the core with the other hand, position it on the positioning tabs, and press, listening for the core to snap into place. (Alternatively, depress both release tabs at either side, position the core on the positioning tabs, press into place, and release the tabs. Check that the core is secure.)

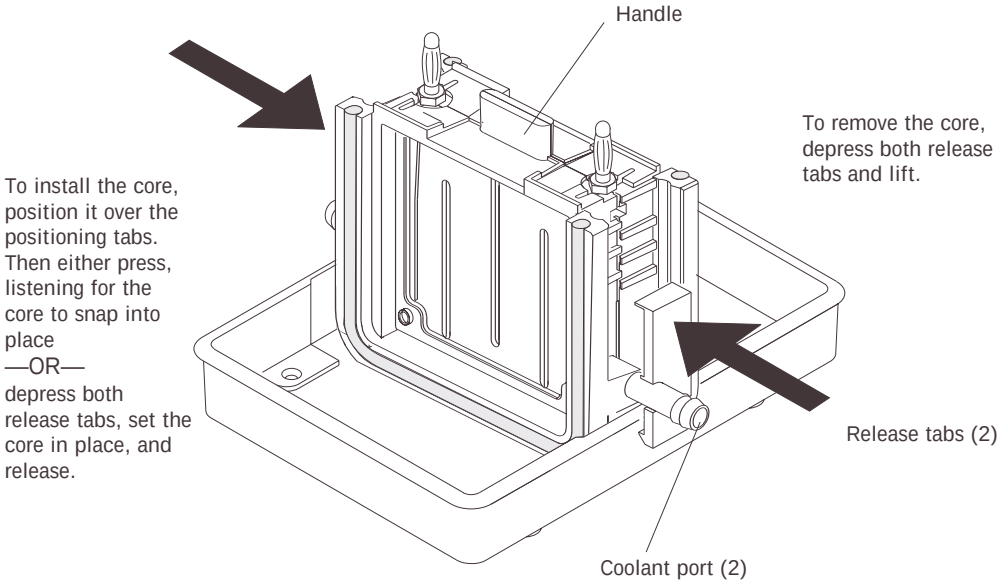



**Figure 2. Core installation and removal**

## 3.3 Place the gel sandwich

### SE 250

1 Rinse the sandwich top with distilled water to remove the overlay and drain.

2 **Self-cast or pre-cast 10 × 8 cm plates.** Orient the sandwich so that the notched alumina plate faces the gasket and the notches are at the top. Set the bottom of the sandwich on the bottom of the lower buffer chamber and the center the plate so that the gasket seals both sides. (Fig. 3a)

**Figures 3a–c Gel sandwich installation.**

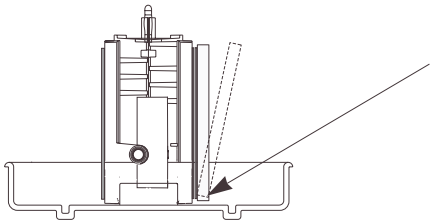


Fig 3a.
A 10 × 8 cm gel sandwich fits flush with the bottom of the upper buffer chamber core.

### SE 260

1 Rinse away the overlay with distilled water and drain any excess water.

2 If installing a self-cast or precast **10 × 8 cm** gel sandwich, align the bottom of the plate with the bottom of the core. (Fig. 3b) The bottom of the notched plate must cover the silicone rubber gasket.

If installing a self-cast or precast **10 × 10.5 cm** gel sandwich, orient the sandwich so that the notched plate faces the gasket, notches at the top. Set the bottom of the sandwich on the supporting ledges in the bottom of the lower chamber and center the plate so that the gasket seals both sides. (Fig. 3c)

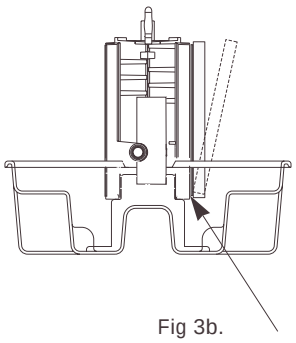


Fig 3b.
A 10 × 8 cm gel sandwich fits flush with the bottom of the upper buffer chamber core.

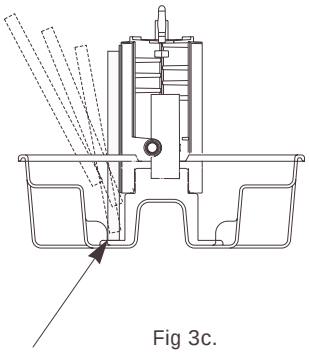


Fig 3c.
A 10 × 10.5 cm gel sandwich fits against the bottom of the lower buffer chamber.

## Clamp the sandwich in place

1. Lightly press the sandwich against the gasket and secure it to the core with one spring clamp on each side. Position the jaw so that the shorter rounded jaw edge fits into the core groove and the longer edge fits on the glass plate. (Proper positioning is important to achieve a seal and to minimize glass breakage.) Slide the clamps down to the stop.

2. Repeat step 1 for the second sandwich, or, if running only one gel, clamp a blank cassette or a plain glass plate on the unused side of the core to prevent a possible short circuit with the unused electrode. (Do not fill this chamber with buffer if no gel sandwich is in place.)

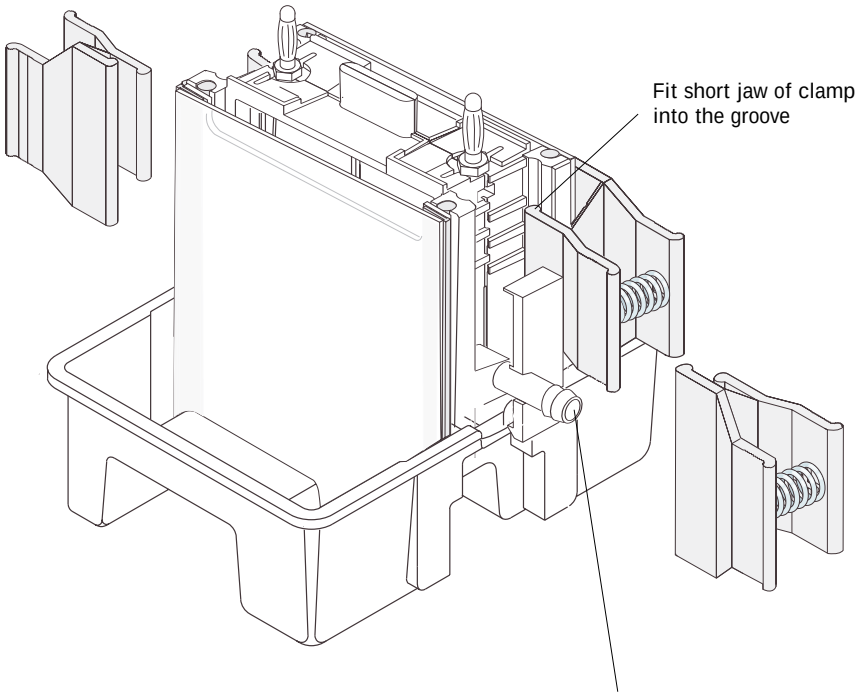



**Figure 4. Securing the gel sandwich onto the upper buffer chamber core**
Each sandwich requires two clamps. The rounded edge of the short jaw on the clamp fits into the groove behind the gasket, and the long jaw presses on the glass plate over the spacer.

## 3.4 Sample preparation and loading

**1** If wells are already in place, skip to step 2.

**If applicable, cast the stacking gel in the unit.**

**Notes**

Stacking gel resolution is optimal when poured just before electrophoresis.

Before pouring, move the clamps to the top of the gel sandwich to prevent leaking.

Calculate the stacking gel monomer solution volume: measure the distance, in cm, from the top of the resolving gel to the notch in the alumina plate. (This should be at least 2 cm—more if the sample depth in the well is unusually high.) Multiply this distance by the gel width (8.3 cm) and the gel thickness (cm). This product is the required volume in ml.

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

Overlay each gel with a thin layer of water-saturated n-butanol, water, or diluted gel buffer to prevent gel exposure to oxygen. *Slowly* deliver the overlay solution from a glass syringe fitted with a 22-gauge needle. Apply the solution near the spacer at the side of the sandwich and allow it to flow across the surface unaided. Allow a minimum of one hour for the gel to polymerize.

**2** **Prepare the sample.** Increase liquid sample density with 10% glycerol or sucrose. Add a tracking dye such as phenol red or bromophenol blue.

**Note**

For dilute samples, use 6X treatment buffer as described in Appendix A.

For SDS protein gels, use 2X treatment buffer to denature both liquid and dry samples in a test tube. **To liquid protein solutions**, add an equal volume of 2X buffer. **To dry protein samples**, add equal volumes of buffer and $ddH_2O$ to achieve the desired concentration. Heat the tube in boiling water for 90 seconds, then chill it in ice until ready to use. Treated samples can be stored frozen for future runs. (Store at -40 °C to -80 °C.)

**3** To aid in loading samples, wet the well-locating decal and apply it to the front of the glass plate so that the appropriate edge outlines the sample wells. Note: The side wells for standards of a preparative comb correspond to the outer-most wells formed by the 10-well comb.

4 Fill the sample wells and each upper buffer chamber that will be used with running buffer. One upper buffer chamber holds approximately 75 mL.

5 Underlay the sample into the wells using a fine-tipped microsyringe. The width of the wells depends on the number of wells per comb. If the comb has fewer wells, they are wider, and require more volume to raise the level 1 mm, as shown in the following table.

**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.

**Volume of sample (µL) per 1 mm 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 |
| 15 | 2.2 | 2.9 | 4.4 |
| 18 | | 2.9 | |

## 3.5 Final assembly

1 Fill the lower buffer chamber with running buffer. The SE 250 lower buffer chamber holds about 150 ml and the SE 260 holds about 250 mL. Check that the lower electrode (running along the bottom of the the upper buffer chamber core) is completely submerged.

**Note:** If using Novex gels, check that the gel-contact slot is exposed (the colored plastic tape must be removed.)

2 Place the safety lid on the unit.

3 Plug the color-coded leads into the jacks of an approved power supply such as the EPS 301 or EPS 2A200. The red lead plugs into the red output jack, and the black lead plugs into the black output jack.

**Important**

Do not use antifreeze or any alcohol-based mixture, as these will irreparably damage the core.

4 **Optional cooling:** Begin circulating cold water or a chilled 50/50 water/ethylene glycol solution.

## 3.6 Running the Gel

Precast gels are run under the same current and voltage conditions as self-cast gels. Gels may be run at either constant current or constant voltage. A constant current setting is traditionally used with a discontinuous buffer system so that the rate of electrophoretic migration remains unchanged throughout the run. Under these conditions, voltage increases as the run proceeds. A lower current setting is recommended for higher resolution.

**Important**

After initial monitoring, do not leave the unit unattended for more than 45 minutes before checking the progress of the the bands and the buffer level.

It takes about one hour to run two 7 cm x 0.75 mm Laemmli gels at 40 mA (20 mA per gel, constant current). Check band progress after 5 minutes, and again after half an hour, keeping an eye on the position of the tracking dye. The run is complete when the tracking dye reaches the bottom of the gel. Watch the buffer level and, if necessary, replenish it before it falls below the level of the notched plate. (A small volume of buffer may leak past a chipped plate or nicked gasket, or it may wick out through the gel.)

Refer to the Appendix for buffer recipes and electrophoretic conditions.

### After the run

**Important**

Always disconnect the high voltage leads from the power supply before removing the lid from the unit.

1. Once the tracking dye reaches the bottom of the gel, turn off the power supply, disconnect the leads, and remove the safety lid.
2. If coolant is circulating, stop the flow and disconnect the fittings or tubing.
3. Pour out the buffer by inverting the core assembly, then remove both clamps, and lift away gel sandwich(es) from the upper buffer chamber core.
4. Gently loosen and then slide away both spacers. Slip an extra spacer or a Hoefer WonderWedge into the *bottom* edge (to prevent breaking the ears of the notched plates) and separate the plates. The gel usually adheres to the alumina plate. Carefully lift the gel from the plate and lay it into a tray of stain or fixative.

# 4 Care and Maintenance

- **Do not autoclave or heat any part above 45 °C.**
- **Do not use organic solvents, abrasives, strong cleaning solutions, or strong acids or bases to clean the chambers.**

Immediately after each use, rinse the unit with water and then rinse thoroughly with distilled water. Handle the upper buffer chamber core with care to prevent damage to the banana plug. Allow to air dry.

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

# 5 Troubleshooting

## Smile effect on the buffer front

*To reduce the running temperature:*

- ✓ Circulate coolant through the upper buffer chamber core.
- ✓ 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

- ✓ Centrifuge or filter sample before loading to remove particulates.
- ✓ Dialyze or desalt the sample.

## Unusually slow (or fast) run

*Adjust the solutions*

- ✓ 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.

*Adjust the voltage or current settings*

- ✓ To increase or decrease the migration rate, adjust the voltage or current by 25–50%.

## Bands are skewed or distorted

*Check gel preparation and polymerization*

- ✓ Degas the stacking gel solution and avoid trapping air bubbles under the comb teeth.
- ✓ Overlay the running gel with water-saturated n-butanol before polymerization begins to avoid forming an uneven gel surface.

*Check sample preparation*

- ✓ Dialyze or desalt the sample.
- ✓ Centrifuge or filter sample before loading to remove particulates.

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 °C 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.

Appendix

# A Running Laemmli System Gels on SE 250 and SE 260 Mini-Vertical Units

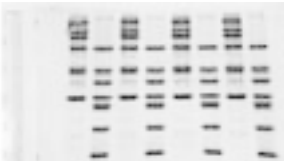
The following Laemmli system is slightly modified for use with the Mini-Vertical units. The Laemmli system is the most common electrophoresis protocol for SDS-denatured proteins. The leading ion in this discontinuous buffer system is chloride and the trailing ion is glycine. Accordingly, the resolving gel and the stacking gel contain Tris-Cl buffers (of different concentration and pH), and the electrophoresis buffer contains Tris-glycine. All buffers contain 0.1% SDS.

Polyacrylamide gel composition is indicated by two different percentages:

$$\%\,T = \text{total acrylamide} = \frac{g\,(\text{acryl} + \text{bis})}{100\ mL} \times 100$$

$$\%\,C = \text{crosslinker} = \frac{g\,(\text{bis})}{g\,(\text{acryl} + \text{bis})} \times 100$$

The total percent of acrylamide (% T) in the separating gel, which can range from 5 to 20%, determines the pore size. Commonly, the amount of crosslinker used (% C) is 2.6%. In the following example system, the resolving gel composition is 10% T, 2.6% C, which results in a medium pore size. The stacking gel composition is 4% T, 2.6% C. The % T in the stacking gel is lower because a larger pore size is required.



**SE 250 results:**

Lane 1: SDS-6H, high MW standard mixture, Sigma
Lane 2: SDS-7 Dalton Mark VII-L™, Sigma
(10 µl per lane)

**Gel**

12% SDS PAGE
Stained with Coomassie Blue

**Running conditions**

20 mA, one hour

| | Final concentrations | | |
|---|---|---|---|
| | **Separating gel** | **Stacking gel** | **Electrophoresis buffer** |
| Acrylamide conc. | 10% T*, 2.6% C | 4% T, 2.6% C | |
| Tris-Cl | 0.375 M | 0.125 M | |
| Tris-Glycine | | | 0.025 M Tris base<br>0.192 M glycine |
| pH | 8.8 | 6.8 | ~8.3 |
| SDS | 0.1% | 0.1% | 0.1% |
| Ammonium persulfate (APS) | 0.05% w/v | 0.05–0.1% w/v | |
| TEMED† | 0.05% v/v | 0.05–0.1% v/v | |

*To achieve any other desired final concentration, adjust the acrylamide stock and water volumes. Volumes for different concentrations are listed on p. 18.

†Tetramethylethylenediamine

**Important!** Refer to the material safety data sheet (MSDS) accompanying each chemical for detailed handling and safety information.

## Solutions

**Note: Filter solutions 1–4 through a 0.45 µm filter.**

**Caution**

Acrylamide is a neurotoxin. Always wear gloves while handling in any form and wear a mask while weighing the powder. Never mouth pipette the solution.

**1 Acrylamide stock solution**
*(30.8% T 2.6% C Bis, 200 mL)*

| | | |
|---|---|---|
| Acrylamide (FW 71.08) | 30% w/v | 60 g |
| Bis* (FW 154.2) | 0.8% w/v | 1.6 g |
| Deionized $H_2O$ | | to 200.0 mL |

Store at 4 °C away from light.

*N,N' Methylenebisacrylamide

**2 1.5 M TrisCl, pH 8.8**
*(4X Resolving gel buffer, 200 mL)*

| | | |
|---|---|---|
| Tris (FW 121.1) | 1.5 M | 36.3 g |
| 4 N HCl | | to pH 8.8 |
| Deionized $H_2O$ | | to 200 mL |

**3 0.5 M TrisCl, pH 6.8**
*(4X Stacking gel buffer, 50 mL)*

| | | |
|---|---|---|
| Tris (FW 121.1) | 0.5 M | 3.0 g |
| 4 N HCl | | to pH 6.8 |
| Deionized $H_2O$ | | to 50 mL |

**4 10% SDS solution**
*(50 mL)*

| | | |
|---|---|---|
| Sodium dodecylsulfate (SDS) (FW 288.4) | 0.35 M | 5.0 g |
| Deionized $H_2O$ | | to 50 mL |

**5 10% APS**
*(Initiator, 1 mL)*

| | | |
|---|---|---|
| Ammonium persulfate (APS) (FW 228.2) | 0.44 mM | 0.1 g |
| Deionized $H_2O$ | | to 1.0 mL |

Fresh APS "crackles" when water is added. If yours does not, replace it with fresh stock. Prepare just prior to use.

**6 0.375 M TrisCl, 0.1% SDS, pH 8.8**

*(Resolving gel overlay, 100 mL)*

| | | |
|---|---|---|
| 1.5 M Tris-Cl, pH 8.8 (Solution #2) | 0.375 M | 25.0 mL |
| 10% SDS (Solution #4) | 3.5 mM | 1.0 mL |
| Deionized $H_2O$ | | to 100.0 mL |

**—OR—**

**Water-saturated *n*-butanol**

Shake *n*-butanol and deionized $H_2O$ in a separatory funnel. Remove the aqueous (lower) phase. Repeat this procedure several times. Use the upper phase.

**7 2X Sample treatment buffer**

*(0.125 M TrisCl, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol, pH 6.8, 10 mL)*

For most samples, use 1 part sample per 1 part 2X SDS treatment buffer.

| | | |
|---|---|---|
| 0.5 M Tris-Cl, pH 6.8 (Solution #3) | 0.125 M | 2.5 mL |
| 10% SDS, 0.35 M (Solution #4) | 0.14 M | 4.0 mL |
| Glycerol (FW 92.09) | 20% v/v | 2.0 mL |
| 2-mercaptoethanol (FW 78.13) | 2% v/v | 0.2 mL |
| [–OR– Dithiothreitol (DTT) (FW 154.2) | 0.2 mM | 0.31 g] |
| Bromphenol Blue (FW 691.9) | 0.03 mM | 0.2 mg |
| Deionized $H_2O$ | | to 10.0 mL |

Divide into 1.0 mL aliquots and store at -40 °C to -80 °C.

**—OR—**

**6X Sample treatment buffer**

*(0.35 M TrisCl, 10% SDS, 30% glycerol, 9.3% DTT, pH 6.8, ~10 mL)*

For dilute samples, use 5 parts sample to 1 part 6X SDS treatment buffer.

| | | |
|---|---|---|
| 0.5 M Tris-Cl, pH 6.8 (Solution #3) | 0.35 M | 7.0 mL |
| SDS (FW 288.4) | 0.35 M | 1.0 g |
| Glycerol (FW 92.09) | 30% v/v | 3.0 mL |
| DTT (FW 154.2) | 0.6 M | 0.93 g |
| Bromphenol Blue (FW 691.9) | 0.175 mM | 1.2 mg |

Divide into 1.0 mL aliquots and store at -70 °C.

**8 0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3**

*(Electrophoresis buffer, 4.0 liters)*

Fill both the upper and lower buffer chambers with the electrophoresis buffer.

| | | |
|---|---|---|
| Tris (FW 121.1) | 0.025 M | 12.1 g |
| Glycine (FW 75.07) | 0.192 M | 57.6 g |
| SDS (FW 288.4) | 3.5 mM | 4.0 g |
| Deionized $H_2O$ | | to 4.0 liters |

The pH of this buffer is approximately 8.3. Do not adjust pH. Up to 20 liters can be prepared and stored for up to 2 months.

**9 Coomassie Stain Protocol** (see below for the more sensitive silver stain protocol)

**Coomassie stain solution**
*(0.025% Coomassie Blue R-250, 40% Methanol, 7% Acetic acid, 2 liters)*

| | | |
|---|---|---|
| Coomassie Blue R-250 (FW 826) | 0.3 mM | 0.5 g |
| Methanol (Stir until dissolved) | 40% v/v | 800.0 mL |
| Glacial acetic acid (99%) | 7% v/v | 140.0 mL |
| Deionized $H_2O$ | | to 2.0 liters |

**Destaining solution I**
*(40% methanol, 7% acetic acid, 1 liter)*

| | | |
|---|---|---|
| Methanol | 40% v/v | 400.0 mL |
| Glacial acetic acid (99%) | 7% v/v | 70.0 mL |
| Deionized $H_2O$ | | to 1.0 liter |

**Destaining solution II**
*(7% acetic acid, 5% methanol)*

| | | |
|---|---|---|
| Methanol | 5% v/v | 50.0 mL |
| Glacial acetic acid (99%) | 7% v/v | 70.0 mL |
| Deionized $H_2O$ | | to 1.0 liter |

**Silver Stain Protocol**
*(adapted from Morrissey, 1981)*

Note: Because this is a highly sensitive staining method, it is important to wear gloves when handling gels and to use clean containers. To reduce the background, use only high-purity reagents and remove all buffer from the gels during the fixing and destaining steps.

Note: Gentle agitation is recommended throughout this procedure.

**Caution**
Glutaraldehyde should only be handled in a fume hood.

A Stain the gel as usual with Coomassie Blue. Destain the gel with several changes of Destain II
—OR—
Fix the gel in 100 mL Destain I for 30 minutes. Place the gel in 100–200 mL Destain II for 30 minutes, discard destain, refill, and wash with Destain II a second 30 minutes.

B Transfer the gel to 100 mL 10% glutaraldehyde for 30 minutes.

C Decant the glutaraldehyde and rinse the gel with several changes of deionized water over a period of two hours.
—OR—
Soak the gel in 500 mL of deionized water overnight. Rinse the gel with several changes of deionized water over 30–60 minutes.

**Note**
The Hoefer Processor Plus used with Amersham Biosciences staining kits simplifies and automates staining protocols.

D Place the gel in 100–200 mL of 5 µg/mL DTT in deionized water for 30 minutes.

E Pour off the DTT solution but do not rinse the gel. Add 100 mL of silver nitrate solution (0.1% in deionized water) directly to the gel. Shake gently for 30 minutes and then rinse the gel for 1–2 seconds with deionized water.

F Add 50 mL of developer solution (50 µl of 37% formaldehyde in 100 mL of 3% sodium carbonate), quickly swirl the gel, and pour off developer. Repeat once more.

Add 100 mL of developer and agitate until the bands are visible. Be sure to stop the development before the background becomes significant by neutralizing the solution with 5 mL of 2.3 M citric acid. Alternatively, pour off developer and add 100 mL Destain II. Note: Some bleaching may occur if using Destain II as a stop solution.

G Wash the gel in 2–3 changes of deionized water. Keep the gel in Destain II or dry it for long-term storage.

## Gel recipes

The Laemmli gel recipes are for 30 mL of a single concentration solution. Tabulated are ingredients and volumes for relatively large pore gels (7.5–10% T range) as well as smaller pore gels (12.5 to 15% T range). A 4% stacking gel is common. The linear gradient recipe is for 100 mL of solution. The total volume needed depends on the number of gels cast and the gel thickness; adjust as necessary. All gels are crosslinked with 2.6% C.

**Laemmli gel**
(per 30 mL separating gel solution, 5 mL stacking gel solution)

| | Separating gel | | | | Stacking gel |
|---|---|---|---|---|---|
| | **7.5%** | **10%** | **12.5%** | **15%** | **4%** |
| Acrylamide stock (Soln. #1) | 7.5 mL | 10.0 mL | 12.5 mL | 15.0 mL | 0.67 mL |
| 1.5 M TrisCl, pH 8.8 (Soln. #2) | 7.5 mL | 7.5 mL | 7.5 mL | 7.5 mL | |
| 0.5 M TrisCl, pH 6.8 (Soln. #3) | | | | | 1.25 mL |
| 10% SDS (Soln. #4) | 0.3 mL | 0.3 mL | 0.3 mL | 0.3 mL | 0.05 mL |
| Deionized $H_2O$ | 14.6 mL | 12.1 mL | 9.6 mL | 7.1 mL | 3.00 mL |
| 10% APS (Soln. #5) | 150 µL | 150 µL | 150 µL | 150 µL | 25 µL |
| TEMED | 10 µL | 10 µL | 10 µL | 10 µL | 2.5 µL |
| Final Volume | 30.0 mL | 30.0 mL | 30.0 mL | 30.0 mL | 5.0 mL |

For linear gradient gels, use equal volumes of low % and high % acrylamide solutions. Less APS is added to extend polymerization time, and less still is added to the higher %T solution to allow polymerization to occur from the top down. In our experience with the concentrations in the 10–20% gradient example below, ten gel sandwiches can be poured in a multiple gel caster at a flow rate of 5–10 mL/min.

**Linear gradient gel**
(per 100 mL of solution)

| | **10% T** | **20% T** |
|---|---|---|
| Acrylamide stock (Solution #1) | 33.30 mL | 66.70 mL |
| Sucrose | — | 15.00 g |
| 1.5 M TrisCl, pH 8.8 (Solution #2) | 25.00 mL | 25.00 mL |
| 10% SDS (Solution #4) | 1.00 mL | 1.00 mL |
| Deionized $H_2O$ | to 100.00 mL | to 100.00 mL |
| 10% APS (Solution #5) | 0.300 mL | 0.060 mL |
| TEMED | 0.036 mL | 0.036 mL |

Appendix B

# Bibliography

Adams, L.D. and Gallagher, S.R., Two-Dimensional Gel Electrophoresis Using the O'Farrell System. *Current Protocols in Molecular Biology*, 10.4.1–10.4.13 (1992).

Gallagher, S.R., and Smith, J.A., Electrophoretic separation of proteins. *Current Protocols in Molecular Biology*. (F.A. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, eds.) 10.2.1–10.2.21 (1991).

Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T. *Nature*. **227**, 680–685 (1970).

Matsudaira, P.T. and Burgess, D.R., SDS microslab linear gradient polyacrylamide gel electrophoresis. *Anal. Biochem*. **87**, 386–396 (1978).

Reisfeld, R.A., *et al.* Acidic buffer system for resolution of cationic proteins. *Nature*. **195**, 281 (1962).

Sasse, J., and Gallagher, S.R., Staining proteins in gels. *Current Protocols in Molecular Biology*. (F.A. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, eds.) 10.6.1–10.6.8 (1991).

Towbin, H., *et al.* Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose: procedure and some applications. *Proc. Natl. Acad. Sci.* USA. **76**, 4350–4353 (1979).

Weber, K., and Osborn, M., The reliability of molecular weight determinators by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem*. **224**, 4406–4412 (1969).

# Customer Service Information

## Technical Service and Repair

Amersham Biosciences offers complete technical support for all our products. If you have any questions about how to use this product, or would like to arrange to repair it, please call or fax your local Amersham Biosciences .

**Important:** Request a copy of the Amersham Biosciences "Health and Safety Declaration" Form before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

## Ordering information

| Product | Qty. | Code No. |
|---|---|---|
| **Mini-Gel System** | | |
| For 10 x 8 cm gels | | |
| SE 250 Mighty Small II<br>Mini-Vertical Unit for 2 slab gels, Basic<br>Includes: 10 glass plates, 2 alumina notched plates, well-locating decal, Spacer-Mate assembly template,<br>(Order combs, spacers, and gel caster separately.) | 1 | 80-6147-26 |
| SE 250 Mighty Small II<br>Vertical Unit for 2 slab gels, Complete<br>Includes: Basic unit, SE 245 Dual Gel Caster,<br>2 each 0.75 mm thick 10-well combs and 0.75 mm thick spacer sets. | 1 | 80-6147-45 |
| For 10 x 10.5 cm gels | | |
| SE 260 Mighty Small II<br>Vertical Unit for pre-cast gels, Basic<br>(Order combs, spacers, plates and gel caster separately.) | 1 | 80-6149-73 |
| SE 260 Mighty Small II<br>Mini-Vertical Unit for 2 slab gels, Complete<br>Includes: basic unit, 10 glass plates, 2 alumina notched plates, well-locating decal, Spacer-Mate assembly template,<br>SE 245 Dual Gel Caster, 2 each 0.75-mm thick 10-well combs and 0.75-mm thick spacer sets. | 1 | 80-6149-35 |
| **Dual 2-D System For 10 x 8 cm gels** | | |
| SE 250 Mighty Small II<br>Mini-Vertical System for 2-D electrophoresis<br>Includes: 10 glass plates, 2 alumina notched plates, well-locating decal, Spacer-Mate assembly template, two 1.5-mm thick preparative combs with single-reference well, and SE 220 Tube Gel Adaptor Kit. | 1 | 80-6147-64 |
| **Electrophoresis Unit Replacement Parts** | | |
| Foam gasket, 4.5 mm x 61 cm | 1 | 80-6137-19 |
| Upper buffer chamber for SE 250 and SE 260 | 1 | 80-6148-40 |
| Lower buffer chamber for SE 250 | 1 | 80-6148-59 |
| Deep lower buffer chamber for SE 260 | 1 | 80-6148-78 |
| Precast gel kit for SE 250 (Deep lower buffer chamber plus 4 clamps) | 1 | 80-6148-97 |
| Lid with cables for SE 250 and SE 260 | 1 | 80-6149-16 |
| Wonder Wedge plate separation tool | 1 | 80-6127-88 |
| High voltage safety lead set | 1 | 80-6177-09 |
| GelSeal (0.25 oz) | 1 | 80-6421-43 |
| Red clamps, for SE 250, SE 260 and gel casters | 4 | 80-6147-83 |

| Product | Qty. | Code No. |
|---|---|---|
| **Glass and Alumina Plates** | | |
| **10 x 8 cm, for SE 250** | | |
| Notched alumina plate | 1 | 80-6136-24 |
| Notched alumina plates | 10 | 80-6136-43 |
| Rectangular glass plates | 10 | 80-6136-81 |
| **10 x 10.5 cm, for SE 260** | | |
| Notched alumina plates | 5 | 80-6150-68 |
| Rectangular glass plates | 5 | 80-6150-87 |

## Spacers

| For SE 250 Thick (mm) | Length (cm) | Qty. | Code No. |
|---|---|---|---|
| 0.75 | 8 | 2 | 80-6137-95 |
| 1.00 | 8 | 2 | 80-6138-14 |
| 1.50 | 8 | 2 | 80-6138-33 |
| **For SE 260** | | | |
| 0.75 | 10.5 | 2 | 80-6149-92 |
| 1.00 | 10.5 | 2 | 80-6150-11 |
| 1.50 | 10.5 | 2 | 80-6150-30 |

## Teflon Combs

| Well No. | Well Thickness (mm) | Well Width (mm) | Qty. | Code No. |
|---|---|---|---|---|
| 5 | 0.75 | 13.0 | 1 | 80-6140-23 |
| 5 | 1.00 | 13.0 | 1 | 80-6140-42 |
| 5 | 1.50 | 13.0 | 1 | 80-6140-61 |
| 9[a] | 1.00 | 5.8 | 1 | 80-6140-80 |
| 10 | 0.75 | 4.8 | 1 | 80-6138-71 |
| 10 | 1.00 | 4.8 | 1 | 80-6138-90 |
| 10 | 1.50 | 4.8 | 1 | 80-6139-09 |
| 15 | 0.75 | 2.9 | 1 | 80-6139-47 |
| 15 | 1.00 | 2.9 | 1 | 80-6139-66 |
| 15 | 1.50 | 2.9 | 1 | 80-6139-85 |
| 18[a] | 1.00 | 2.9 | 1 | 80-6140-04 |
| 1/1[b] | 0.75 | 68/5 | 1 | 80-6141-56 |
| 1/1[b] | 1.00 | 68/5 | 1 | 80-6141-75 |
| 1/1[b] | 1.50 | 68/5 | 1 | 80-6141-94 |

*a Microtiter spacing, b Preparative/reference well*

| Product | Qty. | Code No. |
|---|---|---|
| **Gel Casters** | | |
| **For 1 or 2 gels, 10x8, -10.5, -12 cm** | | |
| SE 245 Mighty Small Dual Gel Caster | 1 | 80-6146-50 |
| **For 5 to 10 gels, 10x8 cm** | | |
| SE 215 Mighty Small Multiple Gel Caster, Complete<br>Includes: 20 rectangular glass plates, 10 notched alumina plates, 100 sheets of wax paper, space saver plate, 5 filler sheets, set of filler plugs and Spacer-Mate. (Order combs and spacers separately.) | 1 | 80-6142-51 |
| **For 2 to 4 gels, 10x8 cm** | | |
| SE 275 Mighty Small 4-Gel Caster, Complete<br>Includes: 10 rectangular glass plates, 4 notched alumina plates, 100 sheets of wax paper, space-saver plate, 5 filler sheets, Spacer-Mate and filler plugs. (Order combs and spacers separately.) | 1 | 80-6151-06 |
| **For 2 to 4 gels, 10x10.5 cm** | | |
| SE 235 Mighty Small 4-Gel Caster, Complete<br>Includes: 4 notched alumina plates, 5 rectangular glass plates, 100 sheets wax paper, space saver plate, 5 filler sheets, set of filler plugs and Spacer-Mate assembly template.(Order combs and spacers separately.) | 1 | 80-6146-12 |
| **Tube Gel Adaptor & Accessories** | | |
| SE 220 Mighty Small Tube Gel Adaptor Kit, for 6 tube gels, 1.5 mm i.d. x 7.5 cm<br>Includes: 12 gel tubes, SE 225 tube gel caster, 12 O-rings, 24 stoppers and tube gel extractor. | 1 | 80-6144-03 |
| Stoppers for SE 220 | 24 | 80-6036-49 |
| Gel tubes, 1.5 mm i.d. x 7.5 cm long | 24 | 80-6144-22 |
| SE 225 Tube gel caster Includes: 12 tubes 1.5 mm i.d. x 7.5 cm | 1 | 80-6144-98 |
| Outer tube casting chamber | 5 | 80-6145-17 |
| Casting cup with support rod | 1 | 80-6145-36 |
| **Power Supplies** | | |
| Hoefer EPS 2A200 | 1 | 80-6406-99 |
| EPS 301 | 1 | 18-1130-01 |
| EPS 601 | 1 | 18-1130-02 |
| **Miscellaneous** | | |
| SE 100 PlateMate washing and storage unit | 1 | 80-6116-29 |
| TE 22 Transphor Tank Unit | 1 | 80-6204-26 |
| Hoefer Processor Plus | | |
| For Gel Staining | 1 | 80-6444-80 |
| For Membrane Processing | 1 | 80-6444-23 |
| MultiTemp III Themostatic Circulator | | |
| 115 VAC | 1 | 18-1102-77 |
| 230 VAC | 1 | 18-1102-78 |
| QuickFit connector, female, 5/16” | 1 | 80-6115-34 |
| QuickFit connector, male, 3/8” | 1 | 80-6115-53 |

# Notes

# Notes

Amersham Biosciences UK Limited Amersham Place Little Chalfont Bucks HP7 9NA England
Amersham Biosciences AB SE-751 84 Uppsala Sweden
Amersham Biosciences Inc 800 Centennial Avenue PO Box 1327 Piscataway NJ 08855 USA
Amersham Biosciences Europe GmbH Postfach 5480 9 D-79021 Freiburg

Printed in the USA