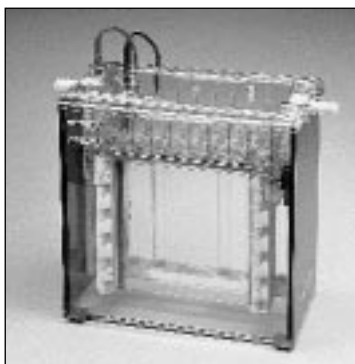


# SE 600 Series

SE 600 and 660 Standard Dual Cooled  
Gel Electrophoresis Units



## User Manual

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# 1 Gel Electrophoresis Unit Function and Description

The SE 600 series vertical slab gel electrophoresis units are intended for electrophoresis under both denaturing or non-denaturing conditions. Up to 28 samples can be compared on a single slab gel.

Applications include protein separations, nucleic acid fractionation, and two-dimensional (2-D) electrophoresis. Both procedures of 2-D electrophoresis can be carried out on the SE 600 with an adapter (ordered separately) if using carrier ampholytes for the first dimension. If using the newly developed immobilized pH gradient (IPG) technology, the first dimension isoelectric focusing of the sample on Immobiline® DryStrip IPG gels is performed on the IPGphor™ Isoelectric Focusing System or the Multiphor® II Flatbed Electrophoresis system. The focused strips are then transferred to the second dimension slab gel for electrophoresis.

All gel plates are 18 cm wide. Table 1 on page 5 lists both models and the corresponding gel plate length (16 or 24 cm). Up to four gels can be run at one time if sandwiches are paired into "club sandwiches." The heat exchanger allows buffer temperature control in the lower chamber.

e	SE 600: 18 × 16 cm SE 660: 18 × 24 cm
Gel size	SE 600: 14 × 16 cm SE 660: 14 × 24 cm
Max. wattage	50 W
Max. voltage	1000 V
Max. amperage	500 mA
Max. temperature	45 °C
Environmental operating conditions	Indoor use: 4–40 °C Humidity up to 80% Altitude up to 2000 m
Installation category	II
Pollution degree	2
Dimensions	width × height × depth SE 600: 32 × 29 × 14 cm (12.5 × 11.5 × 5.5 in.) SE 660: 32 × 37 × 14 cm (12.5 × 14.5 × 5.5 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.

r

## mation

- ▶ 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.



- ▶ 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. Overheating will cause irreparable damage to the unit!

- ▶ Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining, and servicing this product.

## 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.

- ▶ Faire circuler seulement de l'eau ou 50/50 d'eau et d'éthylène glycol dans l'échangeur vertical à circulation 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 pièces 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 afin d'éviter des dommages irréparables à l'instrument.

- ▶ Seulement les accessoires et pièces détachées approuvés ou fournis par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

# 2

## Unpacking and Inventory

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.

**Lower buffer chamber.** The lower buffer chamber is transparent acrylic, which allows visual tracking of electrophoresis progress. The chamber is chemically resistant to common electrophoretic buffers but not to organic solvents or strong acids and alkali. Temperatures above 45 °C may cause the chamber to warp.

**Upper buffer chamber.** The upper buffer chamber is molded polysulfone, which is chemically resistant to common electrophoretic buffers but not to organic solvents or strong acids and alkali. The upper electrode (cathode) runs along the center ridge, and terminates at the banana plug. The upper chamber requires 0.5–0.8 litres of buffer (fill no higher than the top of the plastic ribs).

**Heat exchanger.** The heat exchanger must be installed for every use because it houses the bottom electrode (anode), which runs along the bottom of the frame. When connected to a circulator bath, the heat exchanger regulates the buffer temperature in the lower chamber. Coolant passes through the glass tubes, which are secured with silicone rubber grommets. The heat exchanger connector ports are 13 mm o.d. The heat exchanger is rated to a maximum of 0.8 atmospheres above ambient (12 psig). Connect only to coolant sources under regulated pressure. (Do not connect to the water tap.)

**Safety lid.** The banana plug from the heat exchanger seats into the red jack and the plug from the upper buffer chamber seats into the black jack. The 4-mm shrouded color-coded leads plug into color-coded jacks in the power supply. Always install the safety lid before use!

**Glass plates.** All plates are 18 cm wide, but each model accommodates a specific plate length: The SE 600 takes 16-cm plates, and the SE 660 takes 24-cm plates. Six smooth glass plates are included with each unit. Frosted plates (16 cm length) can be ordered separately for agarose gels. (The rough surface prevents agarose gels from slipping out of the sandwich.) Notched divider plates, ordered separately, pair two gel sandwiches to form a “club sandwich” so that up to four gels can be run at one time.

### Note

The ordering section lists all accessories and replacement parts.

**Figure 1. Main components  
SE 600 series**

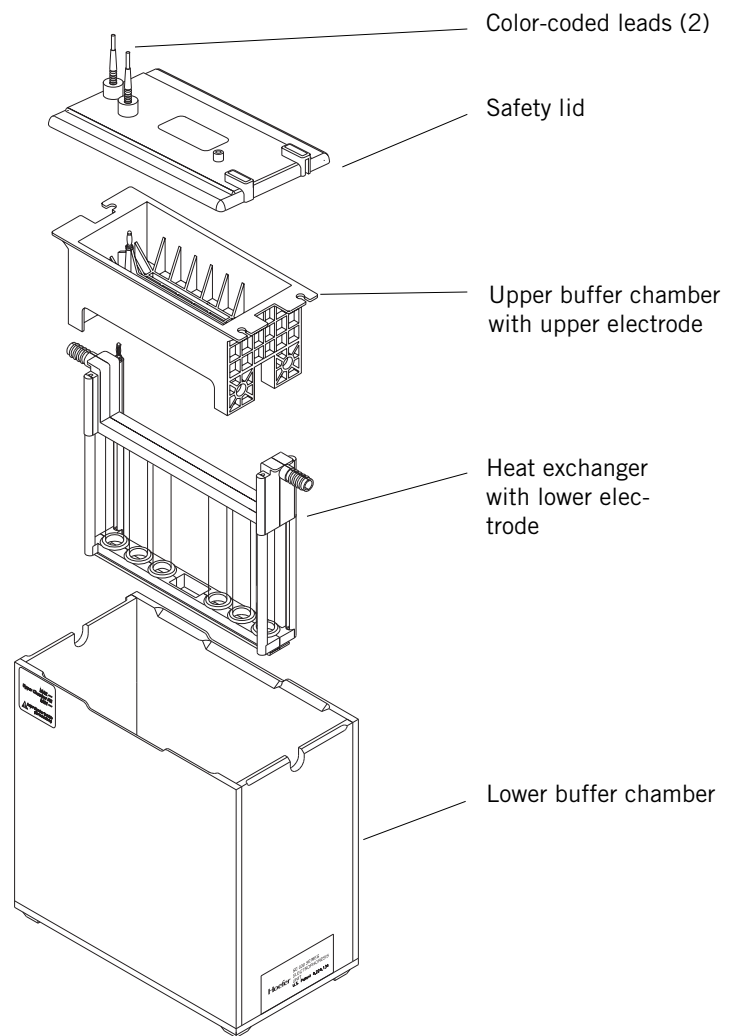
(see Fig. 5 for caster  
components)

Included but not shown:  
GelSeal, 1/4 oz.  
Spacer-Mate  
Well-locating decal  
Glass plates (6)

Complete unit also includes  
spacers (4) and combs (2)

Required but not included:  
Magnetic stirrer  
Power supply with a mini-  
mum rating of 500 V,  
100 mA (constant A or V)

**Optional:**  
Circulator bath



ch. An additional  
the clamp pressure

bar, adjusted with screws, distributes pressure evenly.

**Casting stand.** The casting stand holds assembled gel sandwiches upright for casting gels. Adjustable feet level the caster. A laminated gasket in the bottom of each casting cradle seals the bottom of the sandwich when it is cammed into the stand.

**Cams.** Cams are used twice: first, to secure the assembled sandwich in the casting stand and second, to attach the sandwich to the upper buffer chamber.



**Rubber gaskets.** There are two sets of two gaskets: The solid laminated gaskets fit into the bottom of the casting stand and provide the seal for casting the gel. The slotted gaskets fit under the upper buffer chamber and provide the seal between the upper and lower chambers. The ridges position the gasket to align the slot to expose the gel to the buffer in the upper chamber.

**Spacers.** (May be ordered separately.) Spacers determine the thickness of the gel and are available in three thicknesses (0.75, 1.0, and 1.5 mm) and two widths (1.0 and 2.0 cm).

**Spacer Mate™ assembly template.** Aligns spacers for sandwich assembly.

**Combs.** (May be ordered separately.) Teflon combs are available in sizes that form 10, 12, 15, 20, or 28 wells. Most combs are available in all three thicknesses: 0.75, 1.0, and 1.5 mm. Blank combs form a single large well, and preparative combs include 1 or 2 reference wells in addition to the preparative well.

All blanks, preparative combs, and 10-, 12-, 15- and 20-well combs form wells that are 25 mm deep. The 28-well comb forms wells that are only 15 mm deep so that wells do not collapse when the comb is removed. The sample volume held by each well depends on the gel thickness, well depth and the number of wells per comb. Table 2 lists sample volumes of each well for all combs.

**WonderWedge Plate Separator Tool.** Used to disassemble gel sandwiches and to gauge spacer and comb thickness.

**Two-dimensional electrophoresis.** The SE 600 can be adapted to a 1.5-mm 2-D system with the SE 600 Series Tube Gel Adaptor Kit (ordered separately). Tube gels are first cast and then transferred to the tube gel adaptor, which holds the tubes. After electrophoresis, tube gels are extruded onto slab gels. The second dimension separation is then also run in the same unit. (For details, refer to the Tube Gel Adapter Kit instructions.)

2-D electrophoresis, use spacers the same size as the tube gel to ensure a good fit.

First dimension isoelectric focusing of Immobiline DryStrip IPG strips require the IPGphor Isoelectric Focusing System or the Multiphor II Electrophoresis System. Details of each procedure are described in the respective protocol guide (see the ordering information section). The SE 600 is especially suited to perform the second dimension on thicker gels, which are required to handle high sample loads.

## 3

## Operating Instructions

Gel casting and electrophoresis procedures follow. Assembly of both models is identical. Included are instructions for polyacrylamide gels (used with continuous or discontinuous buffer systems), gradient gels, and agarose gels. See Appendix A for recipes and Appendix B for a bibliography.

### 3.1 Prepare the gel sandwich

Glass plates, spacers, and clamp sets are sized so that the assembled sandwich can be easily aligned to create the seal required first to cast the gel and then to run it. For best results, **take extra care to align all components when assembling sandwiches.** Table 1 summarizes gel casting options.

#### SE 600

Both precast gels and self-cast gels can be used. To self-cast multiple gels, kits can be ordered separately: the SE 615 Multiple Gel Caster Kit holds up to 10 sandwiches, and the SE 675 Gel Caster Kit holds up to four sandwiches. (See the accompanying gel caster user manual for complete instructions.)

#### SE 660

The 24-cm gels required for the SE 660 must be self-cast. The Dual Gel Caster (included) holds two gel sandwiches.

**Table 1**

Model	no. of gels	gel plate size (cm)	available gels and casters
SE 600	1–4*	18 × 16	Dual Gel Caster, Gel Caster Kit <sup>§</sup> Multiple Gel Caster Kit <sup>†</sup> Commercially available gels
SE 660	1–4*	18 × 24	Dual Gel Caster

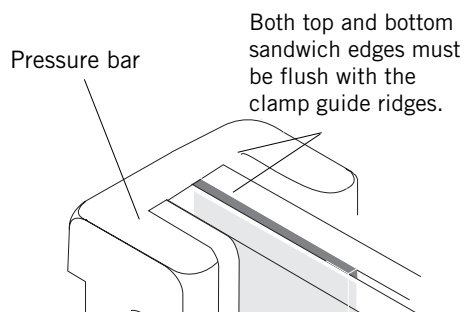
\*Two accessory notched divider plates are required to run 2 extra gels

<sup>§</sup>Casts up to 4 gels

<sup>†</sup>Casts up to 10 gels

### 3.11 Construct the gel sandwich and insert into caster

- 1 Prepare the caster and clamps.** Place the spirit level into the caster center and adjust the leveling feet. Loosen all clamp screws and make space for the sandwich by sliding the pressure plates toward the screws.
- 2 Construct each gel sandwich.** (Agarose gels: also see section 3.14.) For each sandwich, choose two perfectly clean unchipped glass plates and two spacers. Lay one plate on a flat surface, lay the Spacer Mate assembly template onto the plate (wide side at the top of the plate), place a spacer along each edge, and lay the second glass plate on top.
- 3 Secure the sandwich with clamps.** Slide one clamp at a time along the sandwich sides. Finger tighten one screw on each clamp, set the sandwich upright on a flat surface, and loosen the screw to align the stack. Take great care in aligning to ensure a seal. Finger tighten all screws. Remove the Spacer Mate.

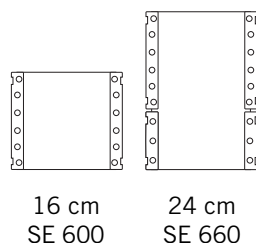


**Tip:** Use the casting cradle to hold the sandwich during alignment. Remove the laminated gasket from the cradle and, instead of setting the sandwich upright on a flat surface, set it into the casting cradle.

**Figure 2. Sandwich assembly**  
Inspect glass plates for nicks. Use only unchipped plates to prevent leaking.

**Figure 3. Required number of clamps**

	Clamp size	
	8 cm	16 cm
SE 600		2
SE 660	2	2

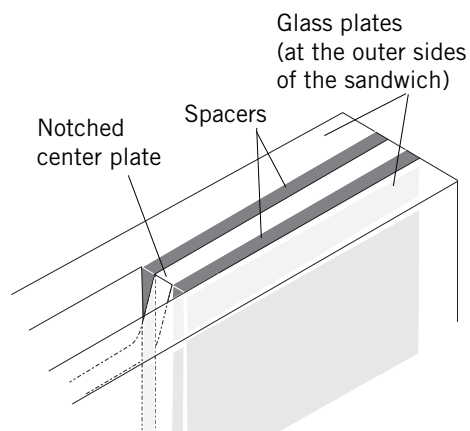


Long sandwiches require two clamp assemblies on each side. When assembling 24-cm sandwiches, align each end separately. That is, align one end, finger-tighten the screws, turn the sandwich 180° and align the other end. In each case allow the clamp to slide down and align perfectly with the top (or bottom) edge of the glass plates.

#### Club sandwich

A 16- or 24-cm long, notched-center divider plate (ordered separately) pairs two sandwiches to double the number of gels that can be cast and run.

Assemble a club sandwich in the same manner as a regular sandwich, except before laying the top glass plate, lay the divider plate and a second set of spacers. Place the notch so that it will be at the top of the gels. It is essential that the spacers and plates align perfectly in order to create a seal. For 24-cm long plates, position the 8-cm clamp along the side at the bottom of the sandwich farthest from the notch.



**Figure 4. Club sandwich assembly**  
Only thinner gels can be paired; no spacers thicker than 1.5 mm can be used.

- 5 Remove the sandwich and inspect the bottom to make sure that edges are aligned flush in order to ensure a complete seal. Adjust if necessary.

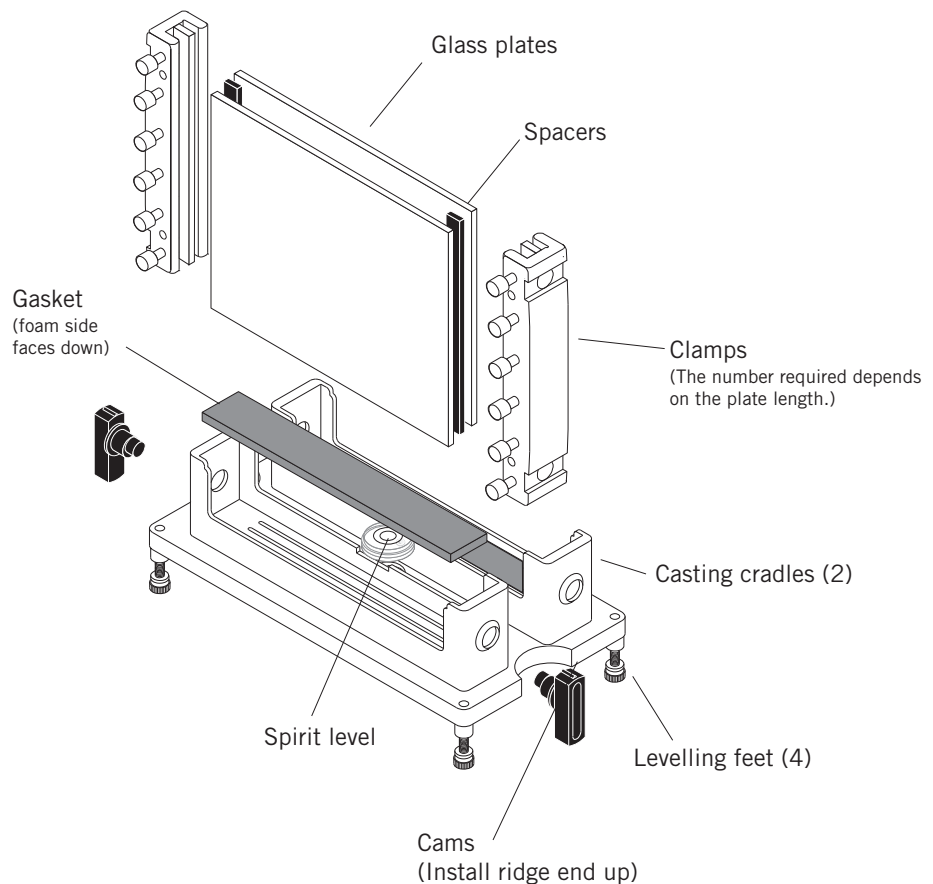
**Optional:** Apply a light film of GelSeal **only** on the bottom corner surfaces created by the spacers and plates if your sandwiches tend to leak.

**Note**

Do not use silicone grease or petroleum jelly to seal the sandwich. These substances are difficult to remove and ultimately cause artifacts.

- 6 Place the laminated gasket into the casting cradle (See Figure 5) with the *foam side down*. Place the clamp assembly in the casting cradle, screw side facing out. For 24-cm plates, place the sandwich so that the longer clamp is at the top.
- 7 Insert a cam into the hole on each side of the casting tray with the ridge (short end) pointing up. Seal the gel sandwich by turning both cams as far as needed, usually 90° to 150°, up to 180°. The camming action presses the plates into the gasket and thereby seals the bottom of the sandwich. The seal is complete once the glass edge appears darker and nearly transparent against the gasket. Do not cam past this point.

**Figure 5.**  
Caster components and set up



**Note**

When turning the cams, it is easier to keep the caster balanced if you turn both toward the center of the caster.

## 3.12 Acrylamide gels

- 1 Prepare the monomer solution and pour the gel.** Prepare the required amount of monomer solution. De-aerate and add the initiator and catalyst just prior to pouring the gel. Pipet the solution into one corner of the sandwich, taking care not to introduce any air bubbles. See below for the appropriate solution level according to the application.

No stacking gel (Continuous system) Fill solution to just below the top of the upper plate edge. If bubbles are trapped, remove with a pipet or syringe. Introduce a comb (at a slight angle) into each sandwich, taking care not to trap air bubbles under the teeth.

Club sandwich Pipet the solution into both sandwiches, filling each to the same level below the notched edge.

Stacking gel Fill solution to 3–4 cm below the top of the glass plate. This height allows 1 cm of stacking gel below the wells. Pour the gel and apply an overlay (see step 2). After the gel is set, prepare the stacking gel as described below.

2-D electrophoresis (Discontinuous system) Fill solution to about 1 cm below the top of the glass plate to allow 4 to 5 mm for the IPG strip or tube gel and an agarose seal. (A stacking gel will require extra space). Seal the IPG strip or tube gel in place with agarose dissolved in running buffer. Take care to avoid trapping any air bubbles between the first and second dimension gels.

- 2** 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.
- 3** Allow the gel to polymerize for a minimum of one hour.

### Stacking gel preparation

Pour the stacking gel while the sandwich is still in the gel caster. Stacking gel resolution is optimal when poured just before electrophoresis.

- 1** Remove the overlay by rinsing the top of the gel several times with distilled water. Invert the caster to drain. To ensure a seamless contact between the resolving and stacking gels, remove residual liquid by blotting one corner with a lint-free tissue.
- 2** Calculate the stacking gel monomer solution volume.
- 3** Prepare the stacking gel monomer solution, deaerate it, and add catalyst and initiator. Pour the stacking gel onto the resolving gel with a disposable or Pasteur pipette to a level about 2 mm from the top of the plate.
- 4** Introduce a comb (at a slight angle) into the sandwich, taking care not to trap air under the teeth. Allow a minimum of one hour for the gel to polymerize.

#### Note

Appendix A lists recipes for the Laemmli gel system.

### 3.13 Gradient gels

Both linear and exponential gradient gels can be poured in the dual gel caster. We recommend using a SG Series Gradient Maker. Gradient gels are poured from the top of the caster with a cannula if using the provided dual gel caster or from the bottom if using a Multiple Gel Caster (see instructions accompanying the caster). A stacking gel is then poured over the gradient gel.

**Figure 6.**  
**Pouring a gradient gel**

Alternatively, the gel solution may be introduced into the gel sandwich through a pipet tip at a rate that maintains a continuous stream.

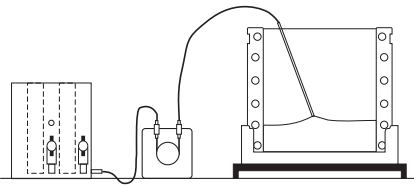
**Note**

Gradient gels poured in the SE 615 or SE 675 Multiple Gel Caster are introduced through the bottom.

**Note**

When pouring an exponential gradient gel, position a plunger above the liquid to hold the volume in the mixing chamber constant.

#### Pouring a linear gradient gel

- 1 Assemble sandwich(es) into the dual gel casters as described in section 3.11.
- 2 **Set up the monomer solution flow path.** Run a length of Tygon tubing through a peristaltic pump. Attach one end of the tubing to the gradient maker outlet port and the other end to an 8-inch cannula. (The OD of the cannula must be less than the spacer thickness.) Place the cannula so that it rests at the bottom of the sandwich, midway between the spacers.
 
- 3 **Prepare the monomer solution.** Calculate the volume of monomer solution needed. Divide the total volume in half and prepare this volume of both the higher and lower % acrylamide solutions. (Optional: Add 15% sucrose or 25% glycerol, final conc., to the higher % solution to improve layering.)
- 4 Pour the "light" solution into the reservoir chamber (the chamber furthest from the inlet). Open the stopcock long enough to displace air between the chambers and then close. Pour the "heavy" solution into the mixing chamber and place a stirring bar into this chamber. Place the gradient maker onto a magnetic stirrer and begin stirring at a rate that does not introduce bubbles in the solution.
- 5 **Mix the gradient and pump the solution into the sandwich.** While the solution is stirring, begin pumping from the mixing chamber and open the stopcock to the reservoir chamber. Raise the cannula as liquid enters the sandwich, keeping the tip at the gel surface. Prepare more gels as required.
- 6 **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.
- 7 **Allow the gels to polymerize** for a minimum of one hour. After polymerization, pour off the overlay and rinse the gel surface several times with distilled water.
- 8 **Prepare the stacking gel** monomer solution, pour the stacking gel and introduce a comb (at a slight angle) into the sandwich, taking care not to trap air under the teeth. Allow a minimum of one hour for the gel to polymerize.

## 3.14

### Assembling the gel sandwich

Follow the general assembly instructions with the following exception:

#### Frosted plate code numbers

SE 600	80-6179-37
SE 660	not available

**Use one (or two) frosted glass plates instead of smooth glass plates.** Use one frosted and one clear plate if blotting the gel after electrophoresis. Place the membrane against the smooth (clear glass) side of the gel. Or, use two frosted plates for more secure retention of the gel between the plate while handling.

Assemble the sandwich so that the frosted side faces inward. Orient the sandwich so that the only frosted edge is at the bottom. (The smooth side edges ensure a better seal between the spacers and the plate, and the smooth top allows easy removal of the comb.)

### Casting agarose gels

Follow casting instructions with the following exceptions:

- 1** Warm a 25-ml pipet, the casting stand and the assembled gel sandwiches in an oven set to 45 °C. This assures that the agarose stays liquefied while being handled.
- 2** Melt the agarose and cool to 75 °C before transferring it with the warmed pipet into the warmed sandwiches.
- 3** Insert a comb in each sandwich to a depth of **no more than 1 cm**. Deeper wells are easily damaged when removing the comb. Allow the agarose to gel. (The adjustable comb back aids in placing combs.)

### Loading the sample

Follow the loading instructions in section 3.15. Take great care to not damage the wells when removing the combs. Combs may slide out more readily if distilled water or buffer is added to the well area. “Walk” the comb out by gently rocking the comb (from spacer to spacer) and lifting it straight up.

### 3.15 Sample preparation and loading

The sample can be loaded either while the sandwich is in the caster or after the upper buffer chamber is attached.

#### Note

With Coomassie Blue, it is possible to detect 1  $\mu\text{g}$  in a single band. With the more sensitive silver stains, it is possible to detect as little as 10 ng.

The amount of sample loaded depends on the thickness of the gel, the sensitivity of the detection method used, and the amount of sample expected in each band. In a continuous buffer system, the protein sample should be relatively concentrated because no stacking gel is used. In a discontinuous buffer system, the zone into which each molecular species migrates is sharpened by the stacking gel so the sample need not be as concentrated.

- 1 Prepare the wells.** Remove the comb by gently rocking it side to side and then lifting it straight up to avoid damaging the well walls. Carefully rinse each well with distilled water to remove unpolymerized acrylamide and then drain by inverting the gel sandwich (or caster). Fill each well with electrophoresis buffer.
- 2 Prepare the sample.** Increase liquid sample density with 10% glycerol or sucrose. Add a tracking dye such as phenol red, bromphenol blue, or pyronin y.

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 2X sample buffer and ddH<sub>2</sub>O to achieve the desired concentration.

- 3** Heat the tube in boiling water for 90 seconds, then allow to cool to room temperature. Treated samples can be stored at -40 to -80 °C for future runs.

Heat membrane proteins to 60 °C for 20 minutes. Store unused sample at 4 °C.

- 4** Underlay the sample into the wells using a fine-tipped microsyringe or gel loading pipet tip.

#### Note

Once the sample is in the wells, take care to not jar the sandwiches so that the samples are not disturbed.

**Table 2**  
Sample volume for each comb size

No. of wells	Volume of sample ( $\mu\text{l}$ ) per 1 mm depth		
	Comb thickness (mm)		
	0.75	1.0	1.5
10	6.2	8.3	12.4
12	5.8		11.5
15	4.3	5.7	8.6
20	3.1	4.1	6.2
28	2.1	2.7	4.1



## 3.2 Final assembly

### Upper buffer chamber

- 1 Rinse both buffer chambers with water and distilled water thoroughly before each use. **Note:** Before using the first time, disassemble the unit and wash with a dilute solution of a laboratory detergent and rinse thoroughly first with water and then distilled water.

Clean away any gel adhering to the exterior of the gel sandwiches.

- 2 **If running only one gel:** Block the second upper buffer chamber slot by installing the acrylic buffer dam included with the unit. Fit clamps onto the dam, taking care to align the clamp ends and dam edges. Install the "dummy" gel, screws facing out, in the second cradle in the dual gel caster.
- 3 **Attach the gel sandwich to the upper buffer chamber.**

Turn the upper buffer chamber upside down and place a slotted gasket into both sandwich holder recesses. Both the slot in the gasket and the slot in the recess must align. Grooves along each slot help keep the gasket in place.

Release the sandwiches from the caster by removing all bottom cams (if present). Lower the upper buffer chamber onto the gel sandwiches in the casting stand. Install the cams, ridge pointing down, into the buffer chamber cam holes. Cam the sandwich in place by simultaneously turning one cam clockwise and the other counterclockwise a **full 180°**.

**Note:** Do not force the cams. If encountering unusual resistance, disassemble and inspect clamp and glass alignment along the top of the sandwich. Align and reinstall.

- 4 Use a pipet to carefully fill each slot above the sample wells with buffer in order to minimize disturbing the samples. Then pour 100 ml of buffer into the chamber, directing the buffer stream toward the side wall. Check that no buffer is leaking.

#### Note

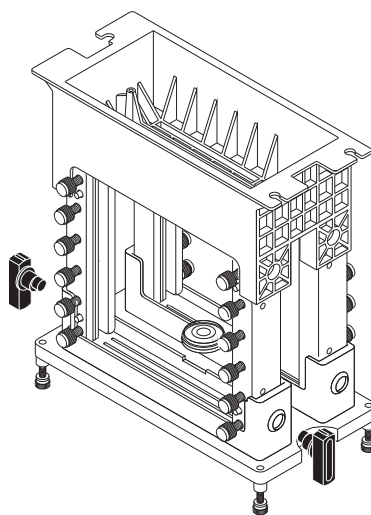
To help hold the gasket against the upper buffer chamber, dab a small amount of GelSeal at each end of the gasket **only** and then install.

#### Important

A smooth fit between the sandwich and gasket is essential to a good seal.

#### Figure 7. Attaching gel sandwiches to the upper buffer chamber

If the assembly leaks, take it to a sink and partially release the cams to allow buffer to drain out of the upper chamber. Disassemble, check alignment of all sandwich components, and adjust if necessary.



**A** Remove cams from the lower cam holes. Place the upper chamber onto the sandwiches and then insert the cams into the upper cam holes, ridge (short end) pointing down.

**B** The final cam position (not shown) must be vertical so that the assembly fits into the lower buffer chamber.

### Important assembly notes

- IEF runs: the buffer level in the lower buffer chamber must never reach the upper buffer chamber; allow at least 2 cm clearance.
- Do not fill the upper or lower chamber above the recommended levels illustrated on the next page. Avoid buffer contact with the electrode posts.
- Pour buffer slowly and away from the slots in the upper buffer chamber to avoid disturbing the samples.
- Use only water or 50/50 water/ethylene glycol as coolant. Never use a commercial antifreeze or any alcohol-based mixture or irreparable damage to the heat exchanger will result.
- Do not connect the heat exchanger to a water tap or any other source where the water pressure is unregulated.

### Lower buffer chamber

- 1 Place a magnetic spin bar into the lower chamber and place the unit on a magnetic stirrer. Partially fill the lower chamber.
- 2 Lower the heat exchanger into the lower chamber, fitting the ports into the notches in the rim. (The heat exchanger must be in place for every run because the lower electrode is integrated into the heat exchanger.) If no cooling is required, skip to step 3.

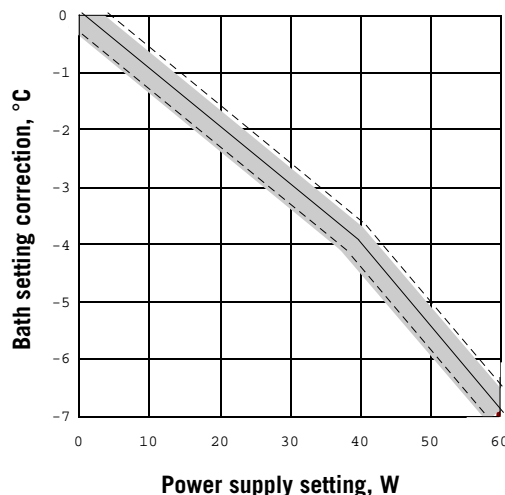
Connect the heat exchanger to a thermostatic circulator such as the MultiTemp III. Slide hose clamps (4 total) onto each end of two lengths of 10–12 mm i.d. ( $\frac{3}{8}$ – $\frac{1}{2}$ " ) vinyl or silicone tubing. Attach one end of each length of tubing to a heat exchanger port. Attach the free 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.

Use the chart below to estimate a starting point for the circulator bath temperature setting. Adjust as necessary for variables such as ambient temperature, changes in power output, and circulator bath efficiency. If accurate temperature control is critical, measure the temperature and adjust as necessary. Optional - Prechill the buffer.

venient to attach Quick-fit connectors to the tubing. The valves in these fittings prevent coolant spillage.

### Approximate circulator bath temperature setting

Set the circulator bath temperature setting lower than the desired run temperature by the amount indicated on the graph.



### Example

Run parameters  
200 V, 0.05 A (50 mA)

- 1 Calculate W if your power supply does not display power directly:  
 $W = V \times A$   
 $10 \text{ W} = 200 \text{ V} \times .05 \text{ A}$
- 2 Interpolate the number of degrees to subtract from the desired run temperature.  
10 W intersects the graph at about -1 °C.  
If the desired temperature is 23 °C, set the bath to  $23 - 1 = 22$  °C.  
If the desired temperature is 4 °C, set the bath to  $4 - 1 = 3$  °C.

- 3 Fit the upper buffer chamber assembly into the lower buffer chamber. Use a steady hand to avoid disturbing the samples: Grasp the assembly in the casting stand by the upper buffer chamber and carefully lower it into the lower buffer chamber.
- 4 Inspect the installation and check the buffer levels.

**Upper chamber.** The electrode along the upper chamber ridge must be submerged about 1 cm. This level requires 450– 600 ml buffer: just enough to cover the upper chamber ribs, but not high enough to contact the banana plug.

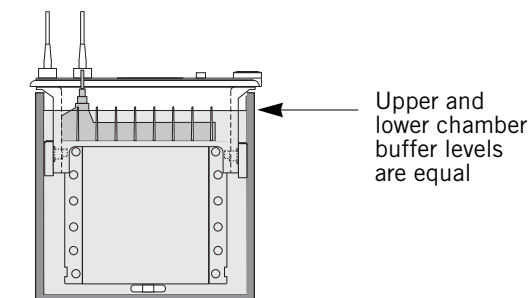
**Lower chamber.** The lower buffer level depends on running conditions and the buffer system as described below.

**Figure 8.**  
**Buffer chamber levels.**

- ▶ Low voltage runs
- ▶ Systems using the same buffer in both the upper and lower chambers

Approximate volumes required:

SE 600	4.5 liters
SE 660	7.0 liters

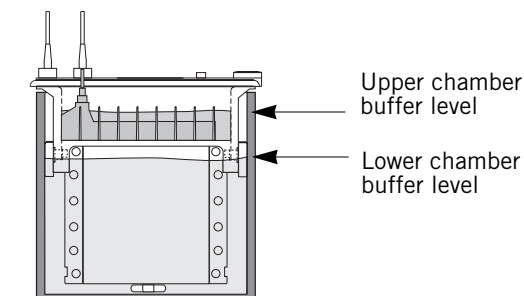


—or—

- ▶ High voltage runs
- ▶ Isoelectric focusing
- ▶ Systems using different buffers in the upper and lower chambers

Approximate volumes required:

SE 600	4.0 liters
SE 660	6.0 liters



The buffer level should cover most of the gel sandwich (to allow the dissipation of heat) but must allow at least 2 cm clearance below the upper buffer chamber.

- 5 Place the safety lid on the unit.
- 6 Plug the color-coded leads into the jacks of an approved power supply, such as the EPS 601. Plug the red lead into the red output jack and the black lead into the black output jack. In most systems, the red lead, which is connected to the bottom electrode, is the anode (+), and the black lead, connected to the top electrode, is the cathode (–).

### 3.3 Separating the sample

**Note**

All SE 600 series models use 18-cm wide plates. The gel thickness determines the cross section (and current requirement) The length of the plate determines the running time.

**Electrophoresis parameters for discontinuous polyacrylamide gels**

Gels may be run at either constant current or constant voltage settings. 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. The optimal current level must be determined empirically; the main factors that must be balanced include the gel concentration and migration speed, and the resulting Joule heating and band distortion. Table 3 on the next page lists starting point guidelines and adjustments for gel thickness, number of gels, and migration rate.

**Current**

Current acts on the total cross-sectional area of all the gels, and in terms of a circuit, the gels are considered to run in parallel. Therefore, any current setting for one gel must be multiplied by the number of gels run. For a gel 1.5 mm thick, we suggest a starting point current setting of 25 mA. (Two 1.5 mm gels = 50 mA.)

**Note:** Cooling may be required to control Joule heating.

**Voltage**

The starting voltage for a 1.5 mm slab gel connected to a power supply set to 25 mA is usually 80 to 90 V (using the SE 600 with a Laemmli discontinuous buffer system for SDS gels). The final voltage is typically 250 to 400 V, depending on the length of the gel. (See Table 3.)

**Time**

A run is complete when the tracking dye reaches the bottom of the gel. In a 16-cm gel (SE 600), a 1.5-mm thick Laemmli SDS gel, run at 25 mA/gel without cooling, usually requires 5 hours. Longer gels require proportionally more time; separations in 24-cm gels (SE 660) require 8 hours.

**Electrophoresis parameters for agarose and DNA/acrylamide gels**

Agarose gels are usually run at a constant voltage setting, and since agarose buffer systems are continuous, both current and voltage readings remain constant throughout the run. Running conditions are expressed in units of V/cm. This allows electrophoresis results from both vertical and horizontal units to be compared. Published running conditions vary widely, but voltages in the range of 1 to 3 V/cm are common for overnight runs. In the SE 600, we have run gels at up to 12.5 V/cm. (Cooling is required at this voltage level.)

**Record each run**

Keep a record of the current or voltage setting, number and thickness of gels, buffer system, and the starting and final current or voltage readings for each run so that results can be compared. Inconsistent results for the same system and settings indicate potential problems such as leaking current, incorrect buffer concentrations, high salt concentrations or inconsistent chemical quality.

**Table 3.**

<b>Laemmli buffer system starting point guidelines</b>		
Gel thickness*	1.5 mm	
Current per gel <sup>†</sup>	25 mA constant current	
Starting voltage <sup>§</sup>	80–90 V	
<b>Model</b>	<b>gel length (cm)</b>	<b>final voltage (V)</b>
SE 600	16	200–250
SE 660	24	275–325
<p>*Thicker or thinner gels require proportionally more or less current. For example, a 0.75-mm gel, which is half as thick as a 1.5-mm gel, requires half as much current, or 12.5 mA.</p> <p><sup>†</sup>The current must be multiplied by the number of gels. For instance, if two club sandwiches are installed, the 4 gels require 4 times as much current. The current can be increased for faster runs if active cooling is used, and it can be decreased for slower overnight runs.</p> <p><sup>§</sup>At 25 mA per gel.</p>		

**Caution**

After initial monitoring, do not leave the unit unattended for more than 1 hour before checking the progress of the bands and the buffer level.

Check band progress after 5 minutes, and again after an hour, keeping an eye on the migration rate 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 as required to keep the top electrode submerged. (A small volume of buffer may leak past a nicked plate or gasket, or buffer may pass through the gel.)

Refer to Appendix A for buffer recipes and electrophoretic conditions.

### 3.4 After electrophoresis

- 1 Once the tracking dye reaches the bottom of the gel, turn off the power supply, disconnect the leads and remove the safety lid, using finger leverage between the lid and the top of the heat exchanger. (Lift straight up to avoid bending the banana plugs.)
- 2 If coolant is circulating, stop the flow and disconnect the fittings or tubing.
- 3 Pull out the upper buffer chamber assembly. Pour out the buffer by inverting the upper buffer chamber over a sink. Install the assembly in the dual gel caster and then release the sandwiches by removing the cams.
- 4 Unscrew the clamps from the sandwiches and remove. Gently loosen and then slide away both spacers. Use the Wonder Wedge plate separator tool to separate the plates.
- 5 Carefully lift one glass plate. *Handle the gel with care to avoid damaging it.* Invert the plate and position the gel low over the stain tray. Lift one corner of the gel and allow it to drop into the tray, or, if the gel is thick enough to handle, lift it and place into the tray. To avoid splashing, add staining or fixative solution to the tray after the gel is transferred.
- 6 Clean the unit as described in the next section.

**Note**

Use only flexible plastic prying tools to avoid chipping the glass plates.

# 4

## Care and Maintenance

### Cleaning

- ▶ **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.**
- ▶ **Do not soak the laminated gasket.**

Immediately after each use, rinse the upper and lower buffer chambers with water and then rinse thoroughly with distilled water. Handle the upper buffer chamber with care to prevent damage to the banana plug. Clean gaskets with mild detergent and rinse with distilled water. Allow to air dry.

Clean glass 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.

### Replacing a heat exchanger glass tube

**Note:** If the old tube is cracked or broken, protect your hand with thick gloves, a piece of cloth, or paper towel before removing the tube.

- 1 Remove the tube by simultaneously twisting and sliding it down as far as possible, until the top end is free of the upper grommet. Carefully guide the tube so that it will clear the assembly, then lift the tube out of the lower grommet.
- 2 Lightly grease the outside of both ends of the new tube with silicone grease. Twist and slide one end of the tube into the lower grommet. Then, slip the other end into the top grommet, gently pushing it with a slight twist into the top grommet until it stops.
- 3 Check that the grommet is not pinched.

## 5 Troubleshooting

### Gel sandwich leaks while casting

- ✓ Plates, spacers, and the gasket must be completely clean. Wash if necessary.
- ✓ Replace chipped plates. (Especially if chipped near the spacers.)
- ✓ Check plate and spacer alignment and realign if necessary.
- ✓ Cam only as far as necessary to create a seal (usually 90–150°, but up to 180°).
- ✓ Apply a *light* film of GelSeal to the bottom **outside** corner only of each spacer. Do not use silicone grease.
- ✓ Check the caster gasket for cuts or cracks and replace if necessary.

### Sample wells damaged or irregular

- ✓ Remove air bubbles before inserting combs. Slide comb into solution at an angle. If comb must be removed, add more monomer solution before reinserting the comb.
- ✓ Allow acrylamide gels to set for a minimum of 1 hour.
- ✓ Rinse out unpolymerized gel with sample buffer.
- ✓ Remove the comb at a slight angle and very slowly to prevent damage to the gel.
- ✓ Agarose gels: Lower the comb no more than 1 cm into the gel.

### Incomplete gel polymerization

- ✓ Use only recent stock of the highest quality reagents.
- ✓ If the dry ammonium persulfate does not crackle when added to buffer, replace with fresh stock.
- ✓ Solutions with extreme pH values (especially acidic) may not polymerize.
- ✓ Remove oxygen from the gel environment: Degas the monomer solution 5 to 10 minutes before pouring and then overlay the gel surface with water-saturated n-butanol.
- ✓ Adjust the gel solution temperature to a minimum of 20 °C, especially for low % T gels.
- ✓ Increase TEMED or APS concentration, or both.

### Upper buffer chamber leaks

- ✓ Check that the glass plates, spacers, and clamps are aligned and fit snugly into the upper chamber gasket.
- ✓ Check that both gaskets are centered and that the positioning ridges fit inside the grooves.
- ✓ Check that the gasket is not damaged or pinched.

### Power supply detects current leak

- ✓ Add more silicone grease to seal heat exchanger grommets.
- ✓ Check for leaks or cracks in the heat exchanger. Replace worn grommets.



## Dye front curves up (smiles) at the edges

- ✓ Fill the lower buffer chamber to the level appropriate for the run. (See section 3.2.)
- ✓ Circulate coolant.
- ✓ Prechill the buffer.
- ✓ Decrease the current or voltage setting.
- ✓ Run the gel in the cold room.

## Protein streaks vertically

- ✓ Centrifuge or filter sample before loading to remove particulates.
- ✓ Dialyze or desalt the sample.
- ✓ Load less sample.
- ✓ Add protease inhibitor, such as PMSF.

## Unusually slow (or fast) run

- ✓ Check for leaks; all plates and spacers must be aligned and free of grease.
- ✓ If used, the buffer dam must be secure.

*Adjust the solutions*

- ✓ If the required pH of a solution is exceeded, do not back-titrate. Prepare fresh buffer.
- ✓ Check recipes, gel concentrations, and buffer dilution. (For instance, do not use Tris-HCl instead of Tris for Laemmli tank buffer.)
- ✓ Dispose of older acrylamide solutions and use only stock of the highest quality.
- ✓ Only use freshly deionized urea.
- ✓ Decrease the salt concentration of samples.

*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.

## Protein bands are diffuse or broader than usual

- ✓ Use only high quality acrylamide and bis.
- ✓ Ensure that polymerization is complete.
- ✓ Fully equilibrate sample.
- ✓ Make fresh sample buffer. Add a protease inhibitor such as PMSF.
- ✓ Reduce TEMED or APS concentration.

Stained sample collects:

*Near the buffer front*

- ✓ Molecules are not sufficiently restricted by the resolving gel pore size; increase the %T.
- ✓ Proteins may be degraded by endogeneous proteases; use protease inhibitors during the isolation step.

*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 (or stacking) gel.
- ✓ The protein has precipitated. Heat the sample at a lower temperature (70 °C or less) for 1–2 minutes.

*At each end of the gel*

- ✓ The molecular weight range of the sample requires an acrylamide concentration gradient to resolve the full range of proteins.

Poor band resolution

- ✓ Begin electrophoresis as soon as the sample is loaded to prevent low molecular weight species from diffusing.
- ✓ Conduct the separation at a lower current or voltage setting.
- ✓ Allow gel to polymerize fully.

*Reagent quality and gel preparation*

- ✓ Use only the highest quality reagents.
- ✓ Only use freshly deionized urea. (Deionize a 10 ml solution to  $\leq 10 \mu\text{S}$ .)
- ✓ Only use gels that were recently prepared.
- ✓ Add a stacking gel. Prepare the resolving gel surface by first rinsing it with stacking gel monomer before pouring the stacking gel to ensure continuity between both gels.
- ✓ Check pH values of the resolving and stacking gel solutions. Do not back-titrate buffers.

*Sample preparation*

- ✓ Store sample on ice before it is denatured.
- ✓ Dialyze or desalt the sample.
- ✓ Heat samples in SDS sample buffer for no more than 1–2 minutes at 100 °C to improve dissociation of subunits. Store on ice after heating.
- ✓ Adjust the sample volume or concentration.
- ✓ Add more mercaptoethanol or dithiothreitol; check sample treatment.
- ✓ Add protease inhibitors such as PMSF if necessary to prevent proteolytic degradation of sample.
- ✓ Increase glycerol or sucrose to increase sample density.
- ✓ Store samples to be frozen in aliquots to prevent repeated thawing. Store at -40 to -80 °C.

Tracking dye 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 salt concentrations.

## A

## Laemmli System Gels

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:

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

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

The total percent of acrylamide (% T) in the resolving 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.

	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 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 <sup>†</sup>	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. 28.

<sup>†</sup>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 <sub>2</sub> O		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, 1 liter)

Tris (FW 121.1)	1.5 M	181.6 g
4 N HCl		to pH 8.8
Deionized H <sub>2</sub> O		to 1000 ml

**3 0.5 M TrisCl, pH 6.8**

(4X Stacking gel buffer, 500 ml)

Tris (FW 121.1)	0.5 M	30.3 g
4 N HCl		to pH 6.8
Deionized H <sub>2</sub> O		to 500 ml

**4 10% SDS solution**

(100 ml)

Sodium dodecylsulfate (SDS) (FW 288.4)	0.35 M	10.0 g
Deionized H <sub>2</sub> O		to 100 ml

**5 10% APS**

(Initiator, 1 ml)

Ammonium persulfate (APS) (FW 228.2)	0.44 mM	0.1 g
Deionized H <sub>2</sub> O		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 <sub>2</sub> O		to 100.0 ml

—OR—

**Water-saturated n-butanol**

Shake n-butanol and deionized H<sub>2</sub>O in a separatory funnel. Remove the aqueous (lower) phase. Repeat this procedure several times. Use the upper phase.

—OR—

If an overlay interferes with the preferred protocol, isolate the gel from atmospheric oxygen by placing a preparative comb or resolving gel former on the gel.

**7 2X Sample treatment buffer***(0.125 M TrisCl, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol, pH 6.8, 10 ml)*

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 <sub>2</sub> O		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)*

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, 5.0 liters)*

Tris (FW 121.1)	0.025 M	15.1 g
Glycine (FW 75.07)	0.192 M	72.0 g
SDS (FW 288.4)	3.5 mM	5.0 g
Deionized H <sub>2</sub> O		to 5.0 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****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 <sub>2</sub> O		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 <sub>2</sub> O		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 <sub>2</sub> O		to 1.0 liter

**Note**

The Processor Plus with gel staining tray, used together with PlusOne staining kits simplifies and automates staining protocols, especially the sensitive but time- and labor-consuming silver staining process.

## Gel recipes

The Laemmli gel recipes are for 30 ml of a single concentration solution (enough for one 1.5 mm, 18 x 16 cm gel). Tabulated are ingredients and volumes for relatively large pore gels (7.5–10% T range) as well as smaller pore gels (12.5–15% 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 resolving 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 <sub>2</sub> O	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 <sub>2</sub> O	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

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## mation

### 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 representative.

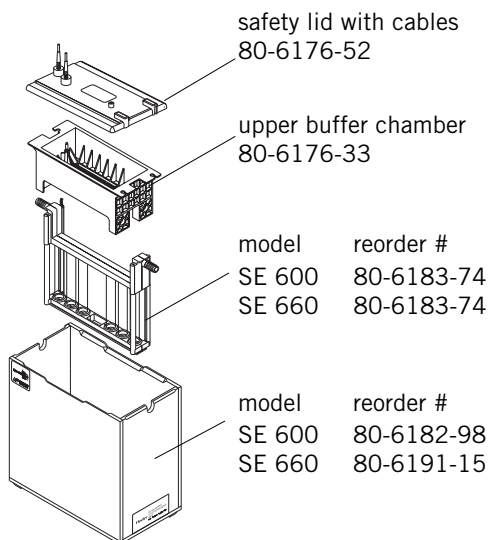
**Important:** Request a copy of the Amersham Biosciences “Health and Safety Declaration” Form before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

### Ordering information

Product	Qty.	Code No.
<b>SE 600 Series Electrophoresis Units</b>		
for 18x16 cm gels		
<b>SE 600 Dual Cooled Vertical Unit, basic.</b>	1	80-6171-58
Includes: 3 sets of glass plates, 6 cams, dual gel casting stand with leveling base and level, buffer dam, Spacer-Mate alignment template and WonderWedge plate separation tool. (Order 2 combs and 2 sets of 16 cm spacers separately.)		
<b>SE 600 Dual Cooled Vertical Unit, complete.</b>	1	80-6171-96
Includes: basic unit plus two 15-well comb and 2 spacers 1.5-mm thick.		
for 18x24 cm gels		
<b>SE 660 Dual Cooled Vertical Unit, basic.</b>	1	80-6189-82
Includes: 3 sets of glass plates, four 16-cm and four 8-cm clamp assemblies, 6 cams, dual gel casting stand with leveling base and level, buffer dam, Spacer-Mate alignment template and WonderWedge plate separation tool. (Order 2 combs and 2 sets of 24 cm spacers separately.)		

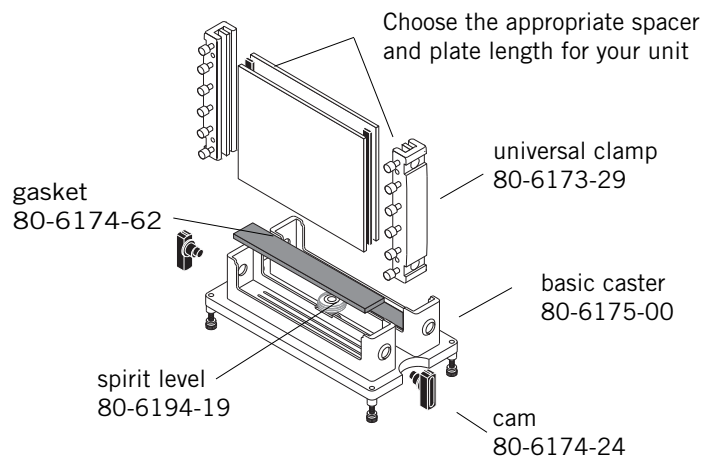
*continued*

Product	Qty.	Code No.
<b>Replacement Parts</b>		
Wonder Wedge gel plate separation tool	1	80-6127-88
Slotted silicone rubber gaskets for upper buffer chamber	2	80-6174-43
Laminated silicone rubber gaskets for casting stand	2	80-6174-62
Buffer dam	1	80-6175-19
Upper buffer chamber for SE 600, SE 660	1	80-6176-33
Lid with high voltage leads for SE 600, SE 660	1	80-6176-52
High-voltage safety lead set	1	80-6177-09
Lower buffer chamber for SE 600	1	80-6182-98
Heat exchanger for SE 600 and SE 660	1	80-6183-74
Lower buffer chamber for SE 660	1	80-6191-15
Grommets for heat exchanger/lower electrode assembly	4	80-6177-28
Banana plug, gold, with 2 washers	1	80-6177-47
SE 600, 660 Heat exchanger/lower electrode assembly	1	80-6183-74
Glass tube with 2 grommets for heat exchanger/lower electrode assembly	1	80-6183-93
Heat exchanger/lower electrode assembly for SE 660	1	80-6183-74
Glass tube with 2 grommets for heat exchanger/lower electrode assembly for SE 660	1	80-6183-93
Lower buffer chamber for SE 660	1	80-6191-15
Spirit level	1	80-6194-19
GelSeal, 1/4 oz. tube	1	80-6421-43



continued

Product	Qty.	Code No.
<b>Gel Casters</b>		
For 1 or 2 gels:		
<b>Dual Gel Caster, basic, 2 gels, 18 cm wide.</b>	1	80-6175-00
Includes: 2 blank gaskets for 1 or 2 gels. (One included with each SE 600 Series unit.)		
<b>Spirit level</b>	1	80-6194-519
For up to 4 gels:		
<b>Gel Caster Kit, 4 gels, 18x16 cm.</b>	1	80-6191-34
Includes: 8 glass plates, 3 space-saver plates, 5 filler sheets, 100 sheets of wax paper, Space Mate alignment template, and filler plugs. (Order combs and spacers separately.)		
<b>Gel Caster only, 4 gels, 18x16 cm.</b>	1	80-6191-53
(Order combs, spacers, and glass plates separately.)		
For up to 10 gels:		
<b>Multiple Gel Caster Kit, 10 gels, 18x16 cm.</b>	1	80-6182-79
Includes: 20 glass plates, space-saver plate, 5 filler sheets, 100 sheets of wax paper, and Space Mate alignment template. (Order combs and spacers separately.)		
<b>Multiple Gel Caster only, 10 gels, 18x16 cm.</b>	1	80-6183-36
(Order combs, spacers, and glass plates separately.)		



**Clamps and Cams**

Clamp and Cam Kit, four 16-cm clamps and 8 black cams	1	80-6173-67
Replacement thumbscrews for clamps	12	80-6172-48
Cams, black, for clamps with cam holes	4	80-6174-24
Clamp assemblies, 8 cm.	2	80-6187-35
Clamp assemblies, 16 cm.	2	80-6173-29

*continued*

Product	Qty.	Code No.
<b>Glass Plates</b>		
<b>18x8 cm</b>		
Glass plates	2	80-6186-59
Glass plates, frosted	2	80-6186-97
Glass plate, club sandwich divider, notched	1	80-6186-78
<b>18x16 cm</b>		
Glass plates	2	80-6178-99
Glass plates, frosted	2	80-6179-37
Glass plate, club sandwich divider, notched	1	80-6179-18
<b>18x24 cm</b>		
Glass plates	2	80-6190-01
Glass plate, club sandwich divider, notched	1	80-6190-20

**Teflon Combs**

**Adjustable comb back** 1 **80-6163-22**

Required to convert any 25-mm deep comb to 10 or 15 mm depth.

No. of wells	Thickness (mm)	Width (mm)	Qty.	Code No.
10	0.75	8.3	1	80-6159-99
10	1.00	8.3	1	80-6160-18
10	1.50	8.3	1	80-6160-37
10 <sup>a</sup>	.75	8.3	1	80-6092-54
10 <sup>a</sup>	1.50	8.3	1	80-6092-73
12	0.75	7.6	1	80-6160-75
12	1.00	7.6	1	80-6459-24
12	1.50	7.6	1	80-6160-94
15	0.75	5.7	1	80-6161-13
15	1.00	5.7	1	80-6161-32
15	1.50	5.7	1	80-6161-51
20	0.75	4.1	1	80-6161-70
20	1.00	4.1	1	80-6161-89
20	1.50	4.1	1	80-6162-08
28 <sup>b</sup>	0.75	2.7	1	80-6162-27
28 <sup>b</sup>	1.00	2.7	1	80-6162-46
28 <sup>b</sup>	1.50	2.7	1	80-6162-65

<sup>a</sup>Also for use with the PR 200 Deca-Probe Incubation Manifold.

<sup>b</sup>Comb depth 15 mm, all others 25 mm

**Preparative Combs**

These combs are 25 mm deep, adjustable to 10 or 15 mm.

No. of wells prep/ref	Thickness (mm)	Width (mm) prep/ref	Qty.	Code No.
1/1	0.75	121/6	1	80-6164-17
1/1	1.00	121/6	1	80-6164-36
1/1	1.50	121/6	1	80-6164-55
1/2	0.75	113/6	1	80-6163-41
1/2	1.00	113/6	1	80-6163-60
1/2	1.50	113/6	1	80-6163-79

Product	Qty.	Code No.
<b>Spacers</b>		
<b>Thickness (mm)</b>	<b>Length</b>	<b>Width (cm)</b>
0.75	8	2
1.00	8	2
1.50	8	2
0.75	16	2
1.00	16	2
1.50	16	2
0.75	24	2
1.00	24	2
1.50	24	2
0.75	16	1
1.0	16	1
1.5	16	1
<b>Companion products</b>		
Buffer Saver displacement insert for SE 600	1	80-6183-17
SE 100 Plate Mate washing and storage unit.	1	80-6116-29
<b>MultiTemp III Thermostatic circulator</b>		
115 VAC	1	18-1102-77
230 VAC	1	18-1102-78
<b>Processor Plus, base unit</b>	<b>1</b>	<b>80-6444-04</b>
19x29 cm staining tray	1	80-6444-99
29x35 cm staining tray	1	80-6445-37
<b>2-D Electrophoresis</b>		
<b>Tube Gel Adaptor Kit (for use with carrier ampholytes)</b>	<b>1</b>	<b>80-6175-38</b>
Includes: 24 tubes, 1.5 mm i.d. x 15 cm, and 24 stoppers		
<b>IPGphor Isoelectric Focusing System</b>	<b>1</b>	<b>80-1124-82</b>
QuickFit connectors, female 3/8"	2	80-6115-15
QuickFit connectors, male 3/8"	2	80-6115-53
Protocol Guide: 2-Electrophoresis, 1st Dimension Immobiline DryStrip, IPGphor Isoelectric Focusing System	1	80-6415-73
<b>Multiphor II Electrophoresis System</b>	<b>1</b>	<b>18-1018-06</b>
<b>Power Supplies</b>		
EPS 2A200 (200 V, 2000 mA, 200 W)	1	80-6406-99
EPS 301 (300 V, 400 mA, 80 W)	1	18-1130-01
EPS 601 (600 V, 400 mA, 100 W)	1	18-1130-02
<b>PlusOne Reagents</b>		
Acrylamide PAGE	250 g	17-1302-01
Acrylamide PAGE 40% Solution	1 L	17-1303-01
Acrylamide IEF	250 g	17-1300-01
Acrylamide IEF	1 kg	17-1300-02
Acrylamide IEF 40% Solution	1 L	17-1301-01
N,N'-Methylene-bisacrylamide	25 g	17-1304-01
Ammonium persulphate	25 g	17-1311-01
TEMED	25 ml	17-1312-01
Tris	500 g	17-1321-01
Glycine	500 g	17-1323-01
Sodium dodecylsulphate	100 g	17-1313-01
Coomassie Blue R tablets	40	17-0518-01
Silver Staining Kit, Protein	1	17-1150-01
Bromophenol Blue	10 g	17-1329-01

# Notes

Amersham Biosciences UK Limited Amersham Place Little Chalfont Buckinghamshire England HP7 9NA  
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 D-79021 Freiburg

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