user manual
electrophoresis system

# Ettan DALT*twelve* System

second-dimension gel electrophoresis

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Amersham Biosciences

# Page finder

# Ettan DALT*twelve* System

In 2-D electrophoresis, proteins are separated according to isoelectric point by isoelectric focusing, most reliably on immobilized pH gradient (IPG) strips using the IPGphor™ IEF System. The second-dimension electrophoresis separates the proteins on the basis of their molecular mass using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The Ettan™ DALT*twelve* System is designed to handle multiple large second-dimension gels in a simple, efficient, and reproducible manner.

## Ettan DALT*twelve* System components:

- 12-slot vertical slab separation unit
- 200 W, 600 V, 1 A Power Supply/Control Unit
- gel caster
- gel casting cassettes
- gradient maker with peristaltic pump.

**Fig 1.** Ettan Dalt*twelve* Separation Unit.

### Separation Unit

The Ettan DALT*twelve* Separation Unit accommodates up to twelve 25.5 × 20.5 cm slab gels for separation under identical conditions. A sample, focused in an IPG strip, is placed on the cathodic surface of a slab gel and sealed with agarose. Up to 12 gels are inserted into the separation unit through the buffer seal slots flanked by rubber gaskets. Any unused slots are filled with the blank cassette inserts. The buffer seal is an effective current and liquid barrier.

The platinum wire cathode is attached to the underside of the lid, and the platinum wire anode is in the lower tank. Safety interlocks prevent the application of power to the separation unit unless the lid is closed properly and the pump valve is in the *circulate* position. The lid is easily removed for cleaning by sliding it off its hinges.

A pump located in the lower chassis of the unit circulates the buffer, pumping it up into the main chamber on the left side, between the cassettes, down the right side and over the internal heat exchanger before returning to the pump. The default setting for the pump is *auto;* the pump will come on only when a run is started. Turning the lever at the back of the unit from *circulate* to *drain* drains the tank. The temperature is controlled by Peltier modules attached to the heat exchanger beneath the tank.

**Fig 2.** Power Supply/Control Unit.

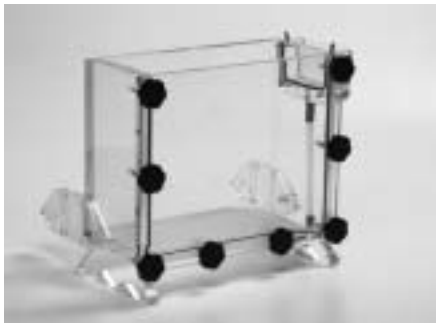

**Fig 3.** Gel Caster.

**Fig 4.** Gel Casting Cassette.

## Power Supply/Control Unit

The Ettan DALT*twelve* System is controlled from the Power Supply/Control Unit (see Fig 2). The unit supplies a maximum power output of 200 W with a maximum of 600 V or 1 A. The temperature control range is 10 °C to 50 °C.

## Gel Caster

The Ettan DALT*twelve* Gel Caster holds up to fourteen 1.0 mm or thirteen 1.5 mm gel cassettes, with separator sheets, for casting homogenous or gradient gels (see Fig 3). Fewer gels can be cast at one time by using blank cassette inserts to occupy unneeded volume. The removable faceplate and separator sheets simplify loading and unloading of cassettes from the unit. The unit also has removable tilt support legs that allow the caster to be tipped back for more convenient loading of gel casting cassettes.

## Gel Casting Cassettes

The gel casting cassettes are pre-assembled. Two glass plates are joined along one edge by a hinge strip of silicone rubber, and the vinyl side spacers (1.0 mm or 1.5 mm thick) are glued in place. To complete assembly, close the two plates like a book and press the plates together over the length of the spacer. Gels are removed by opening the book after the run and carefully lifting out the gel slab. Care must be taken to ensure that the gel does not adhere to the spacers and tear during removal. The cassette is cleaned as a unit and can be stood upright to dry. The cassettes can be cleaned in an automatic dishwasher. Cassettes are 27 × 22 cm and produce a gel about 25.5 × 20.5 cm. A 1.0-mm thick gel has a volume of approximately 52 ml and a 1.5-mm thick gel has a volume of approximately 78 ml.

## Important information

- Connect the instrument to a properly grounded electrical outlet.
- The safety lid must be firmly in place and the pump valve set to *circulate* before power can be applied.
- Stop the run before opening the safety lid.
- Rinse and flush the tank and pumping system with distilled or deionized water before and after use.
- Always disconnect the power cord before servicing.
- Do not run the circulation pump if the separation unit is empty.
- Do not operate with buffer temperature above 50 °C. All plastic parts are rated for 50 °C continuous duty.
- Turn the pump on during electrophoresis to minimize heating. Overheating will cause irreparable damage to the unit.
- For runs near the lower temperature limits (~10–15 °C), it may be necessary to operate the unit in a cold room, especially in laboratories where the ambient temperature is above 25 °C.
- Do not autoclave or boil this unit or any of its parts.
- Use care when lifting and moving the separation unit. It is best to move the unit when empty.
- When filled with glass plates and gel solutions, the casting unit is very heavy. Use caution when moving or lifting the caster.
- If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
- Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining and servicing this product.

***Caution!*** To avoid risk of electrical shock, ensure that all electrode contacts are clean and dry and there is no excess liquid on the upper rim of the separation unit. Inspect the cables for damage before use of the instrument and contact Technical Support if any damage is apparent.

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# Specifications

## Ettan DALT*twelve* Electrophoresis Unit

| | |
|---|---|
| Gel capacity | 12 gels |
| Electrophoresis buffer volume | 10 l |
| Dimensions (h × w × d) | 54 × 46 × 51 cm, lid closed<br>74 × 46 × 51 cm, lid open |
| Weight | 24.5 kg |
| Maximum temperature | 50 °C |
| Environmental operating conditions | Indoor use, 4–40 °C<br>Humidity up to 90%<br>Altitude to 2000 m |
| Buffer circulation pump rate | ~10 l/min |

## Ettan DALT*twelve* Power Supply/Control Unit

| | |
|---|---|
| Maximum wattage | 200 W |
| Maximum voltage | 600 V DC |
| Maximum current | 1.00 A |
| Temperature control range | 10–50 °C |
| Weight | 6.35 kg |
| Dimensions (h × w × d) | 33 × 26 × 19 cm |
| Environmental operating conditions | Indoor use, 4–40 °C<br>Humidity up to 90%<br>Altitude to 2000 m |
| Installation category | II |
| Pollution degree | 2 |
| 115 V~ | 90–130 VAC, 60 Hz, 650 W |
| 230 V~ | 180–230 VAC, 50–60 Hz, 650 W |
| Product and safety certifications* | EN61010-1, UL3101-1, CSA22.2<br>1010.1, EMC EN61326, CE Marked |

## Ettan DALT*twelve* Gel Caster

| | |
|---|---|
| Gel capacity | 14 for 1.0 mm thick gel<br>13 for 1.5 mm thick gel |
| Acrylamide solution volume (total) | 950 ml for 1.0 mm thick gel<br>1200 ml for 1.5 mm thick gel |
| Dimensions (h × w × d) | 26 × 21 × 36 cm |
| Weight | (empty) 5 kg<br>(loaded) 19 kg |

## 1.0 mm Gel Casting Cassette

| | |
|---|---|
| Cassette dimensions (h × w × d) | 21.7 × 27.6 × 0.70 cm |
| Slab gel dimensions (h × w × d) | 20.5 × 25.5 × 0.10 cm |

## 1.5 mm Gel Casting Cassette

| | |
|---|---|
| Cassette dimensions (h × w × d) | 21.7 × 27.6 × 0.75 cm |
| Slab gel dimensions (h × w × d) | 20.5 × 25.5 × 0.15 cm |

## Gradient Maker

| | |
|---|---|
| Dimensions (h × w × d) | 54 × 19 × 18 cm |
| Maximum gradient volume | 2 l |
| Weight | 5.4 kg |

## Peristaltic Pump for Gradient Maker

| | |
|---|---|
| 115 V~ | 37 W, 1.5 A |
| 230 V~ | 37 W, 0.9 A |
| Weight | 4.1 kg |
| Dimensions (h × d × w) | 13.5 × 18 × 22 cm |
| Environmental operating conditions | Indoor use: 0–40 °C<br>Humidity: 10–90% |
| Installation category | II |
| Pollution degree | 2 |
| Product certifications* | EN61010-1, UL508, cUL (115 V), IEC 1010 (230V), CE |

*This declaration of conformity is valid for the instrument only 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.

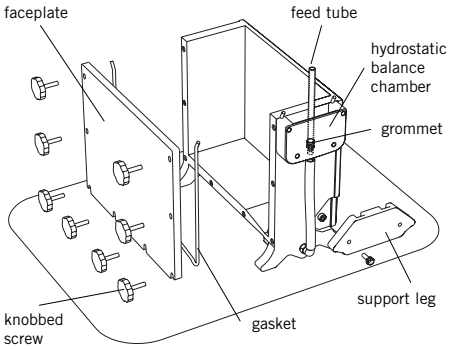



**Fig 5.** Exploded view of caster.

**Fig 6a.** Gel caster with gel cassettes, tipped back.

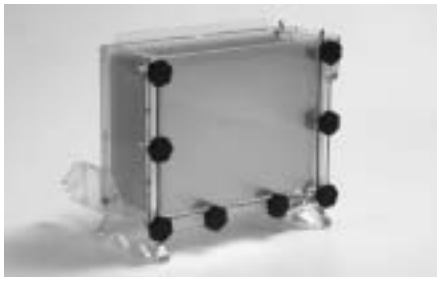


**Fig 6b.** Gel caster filled and level.

# Preparing the gel caster

Set up the gel caster near a sink, in a tray, or on a drainboard so that any liquid that may overflow from the unit or drain out of it during pouring or disassembly can be easily contained.

The Ettan DALT*twelve* Gel Caster (see Fig 5) can accommodate up to fourteen 1.0 mm or thirteen 1.5 mm gel cassettes with separator sheets between them. If you are not planning to cast a full set of gels, use the blank cassette inserts, with separator sheets in between (provided with the separation unit) to occupy the extra space.

Gel labels, for easy indexing of gels and samples, can be placed in the cassettes at any time during the assembly of the caster.

❶

Check that the caster is level. Remove the faceplate and tip the caster back so that it rests on the removable tilt support legs. If casting gradient gels, place the triangular sponge in the base of the V-shaped feed channel; otherwise, it may be omitted (see Fig 6a).

❷

Start filling the gel caster by placing a separator sheet against the back wall to make it easier to remove the last cassette from the unit after polymerization. Fill the caster by alternating cassettes with separator sheets. The rubber hinge should be on the left side of the caster, with the offset end of the cassette up. End with a separator sheet then use the thicker spacer sheets to bring the level of the stack even with the edge of the caster.

❸

Lubricate the foam gasket with a light coating of GelSeal compound to help ensure a liquid-tight seal. Place the gasket in the groove on the faceplate. Avoid stretching the gasket by seating it from the ends toward the middle.

❹

Turn four black-knobbed screws into the four threaded holes across the bottom until they are well engaged (two to three full turns). Carefully place the faceplate onto the caster with the bottom slots resting on their respective screws. Screw the four remaining black-knobbed screws into the holes at the sides of the faceplate and tighten all eight evenly. Be sure the sealing gasket is compressed evenly by the faceplate and forms a tight seal with the caster. Do not overtighten the screws (see Fig 6b).

❺

Insert the end of the rigid plastic feed tube, supplied with the gel caster, into the rubber grommet in the floor of the hydrostatic balance chamber on the side of the caster. The feed tube must be snug so that there is no leakage from the balance chamber into the caster. The feed tube should be connected with flexible tubing directly to either a peristaltic pump or a funnel (see Fig 7).

# Casting homogeneous gels

***Warning!*** Acrylamide is a neurotoxin. Never pipette by mouth and always wear protective gloves when working with acrylamide solutions, IPG strips, or surfaces that come into contact with acrylamide solutions.

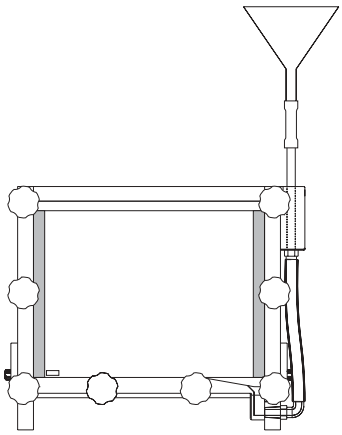


**Fig 7.** Gel caster, in use.

❶

Be sure the entire gel casting system is clean, dry, and free of any polymerized acrylamide.

❷

Prepare a sufficient volume of gel overlay solution (water-saturated *n*-butanol). You need 1 ml of overlay for each 1.0 mm cassette and 1.5 ml for each 1.5 mm cassette.

❸

Make up 100 ml of displacing solution (0.375 M TrisCl pH 8.8, 50% (v/v) glycerol, bromophenol blue).

❹

For a full 14-gel 1.0 mm cassette set, make up 0.9 l of acrylamide gel stock solution without adding the 10% (w/v) ammonium persulphate (APS) or 10% (v/v) N,N,N',N'-tetramethylethylenediamine (TEMED). For a full 13-gel 1.5 mm cassette set, make up 1.2 l of acrylamide gel stock solution as above. This amount of gel solution will provide you with sufficient volume to cast gels using either a funnel or a peristaltic pump.

❺

Assemble the gel caster as described in the preceding section; the caster should be placed on a level bench or on a leveling table so that gel tops are level. The white triangular sponge may be omitted.

❻

Connect the feed tube to either a funnel held in a ring-stand above the top of the gel caster (about 30 cm) or a peristaltic pump. Insert the other end of the feed tube into the grommet in the bottom of the balance chamber (see Fig 7).

❼

Fill the balance chamber with 100 ml of the displacing solution.

❽

Add the appropriate volumes of APS and TEMED only when ready to pour the gels, not before. Once these two components are added, polymerization begins and the gel solution should be completely poured within 10 min.

❾

Pour the gel solution into the funnel, taking care to avoid introducing any air bubbles into the feed tube. If a peristaltic pump is being used, the flow rate should be increased slowly to the desired speed to avoid introducing any air bubbles into the feed tube.

❶

Pump gel solution into the caster until it is about 1 to 2 cm below the final desired gel height. Stop the flow of acrylamide and remove the feed tube from the balance chamber grommet. Once the feed tube is removed, the dense displacing solution flows down the connecting tube, filling the V-well and sloped trough at the bottom of the caster. The remaining acrylamide solution is forced into the cassettes to the final gel height. The amount of gel solution required will be 800 to 850 ml for gels 1.0 mm thick and ~1150 ml for 1.5 mm thick gels.

⓫

Immediately pipette 1 ml of water-saturated butanol onto each gel. If you are using a peristaltic pump to pour the gels, rinse the gel solution from the pump before it begins to polymerize.

⓬

Allow the homogeneous gels to polymerize for at least 1 h before disassembling the caster.

# Casting gradient gels

## Preparing for gradient gel casting

Successful gradient gel casting requires planning, timing, and practice. A full cast with the Ettan DALT*twelve* Gel Caster requires approximately 0.9–1.2 l of acrylamide stock. Polymerization begins as soon as TEMED and APS are added to the acrylamide stock. At this point there is no time to adjust the gradient maker divider, or the cassettes and separators in the gel caster. To familiarize yourself with the gel caster and gradient maker before casting gels, we recommend setting up the unit and configuring the gradient divider as described below. Follow the gradient pouring procedure on pages 11–12, substituting water for the appropriate volume of light solution, and a mixture of glycerol and water for the appropriate volume of heavy solution. When the angle of the gradient divider is adjusted correctly and you are comfortable with the gel casting procedure, clean all parts of the caster and gradient maker, including sponges, separator sheets and filler blocks, with a solution of mild detergent, followed by a deionized water rinse.

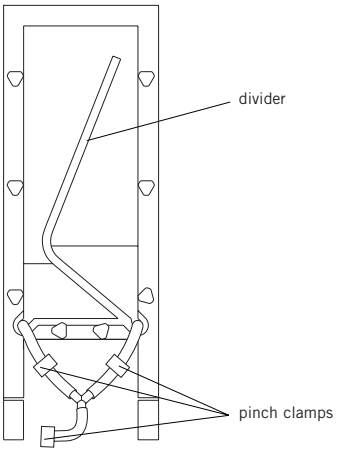



**Fig 8.** Configuration of the gradient maker.

## Configuring the gradient divider

You can adjust the shape of the gradient divider to suit the number of gels to be cast and the shape of the gradient you want. When casting a full tank of gels, the angle of the adjustable divider to the floor of the gradient maker should be about 40° (see Fig 8). For fewer gels, decrease the divider angle. Whatever angle you use, a straight divider gives a linear gradient.

❶

Determine the amount of gel solution needed to cast the desired number of gels.

❷

Loosen the faceplate screws on the empty gradient maker to adjust the gasket angle. Refer to the divider configuration in Figure 8 as a guideline for the angle you should use. Pull the divider slightly to help move it into position. Use the red adjuster rod provided with the gradient maker to push the gradient divider down.

❸

For a linear gradient, straighten the divider, with its upper end almost touching the left wall at the height determined by the volume of water.

❹

To make a funnel for introducing heavy solution into the left side, bend the remaining top of the divider to the right.

*continues*

❺

Tighten the faceplate screws and close the pinch clamps on the outflow tubing at the bottom of the gradient maker before adding water or gel solutions.

❻

Using water in place of gel solution, put half of the required volume in the right chamber and the other half in the left chamber.

❼

Adjust the angle of the gradient divider so that the level of liquid in the "heavy", or left, chamber is about 2 cm below the level of liquid in the "light" chamber. You can use tape or wax pencil on the outside of the gradient maker to mark the angle of the gradient divider.

❽

Open the pinch clamps to remove the water, or pour the water out of the top of the gradient maker. Repeat this procedure whenever the required volume of acrylamide solution changes as a result of changing the number of gels you are casting.

## Calibrating the peristaltic pump

Install the pump head and tubing on the pump controller as directed in the User Manual supplied with the pump. Calibrate the pump flow rate before the first use and after every 10 to 20 uses, to ensure proper flow rates. This calibration procedure assumes a desired flow rate of 335 ml/min for 14 gels.

**Example.** You want to cast a full tank of gels and determine that the flow rate is 500 ml/min when you set the flow rate at 3. For a flow rate of 335 ml/min:

$(335 \div 500) \times 3 = 2$ = the appropriate flow rate setting to deliver 335 ml/min

❶

Place the inlet side of the tubing in a beaker that contains 1 l of water.

❷

Place the outlet side of the tubing in a 1 l graduated cylinder.

❸

Set the flow speed at 3 on the dial and start the pump.

❹

Stop the flow of liquid after exactly 2 min.

❺

Measure the water in the outlet cylinder and divide by 2 to determine the flow in ml/min.

❻

To determine the appropriate flow setting, divide the desired flow rate by the flow rate in step 5; multiply this result by the flow speed used in step 3.

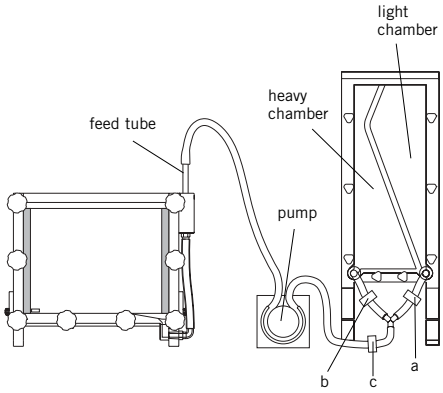



**Fig 9.** Pump connected to gradient maker.

## Casting gradient gels

The gradient maker is a simple unit with two chambers that are defined by an adjustable silicone rubber gasket clamped between two acrylic plates. You can modify the shape of the gel gradient by adjusting the movable divider. Solutions flow out of the two chambers in proportion to the relative widths at the surface of the liquid, join at the Y-connector, and then are thoroughly mixed in an in-line "bow-tie" mixer that has no moving parts. Three pinch clamps control the flow at the exits from the light (a) and heavy (b) chambers, and after the mixer (c) to control flow into the peristaltic pump (see Fig 9).

A gradient gel results from using two gel solutions of different acrylamide concentrations and densities—a light solution and a heavy solution. The heavy gel solution contains glycerol. During the gradient pouring procedure, the mixing ratio of heavy solution to light solution gradually increases, with the heavier solution underlaying the light solution. This generates a downward gradient of increasing gel percentage. To ensure balanced flow, when the gradient maker is filled with equal volumes on each side of the divider, the height of the heavy gel solution in the gradient maker should be 1 to 2 cm less than the height of the light solution. Under these conditions, the two solutions are in hydrostatic equilibrium. See "Configuring the gradient divider" on page 8. Hydrostatic equilibrium can also be achieved by using equal masses, instead of volumes, of the heavy and light solutions.

***Warning!*** Acrylamide is a neurotoxin. Never pipette by mouth and always wear protective gloves when working with acrylamide solutions, IPG strips, or surfaces that come into contact with acrylamide solutions.

## Gradient casting setup

❶

Be sure the entire gel casting system is clean, dry, and free of any polymerized acrylamide. Place the white sponge in the base of the V-shaped feed channel of the caster. The caster should be placed on a level bench to ensure that the gels and gradients are even and level.

❷

Configure the gradient divider for the number of gels you are casting. If necessary, calibrate the gradient pump flow rate. See "Calibrating the peristaltic pump" on page 9.

❸

Be sure that the faceplate screws on the gradient maker are fingertight and all the gradient maker lines are clamped off. There are three clamps: one coming from each chamber and one after the bow-tie mixer. Close all three.

❹

Prepare a sufficient volume of gel overlay solution (water-saturated *n*-butanol). You need 1 ml of overlay for each 1.0 mm cassette, and 1.5 ml for each 1.5 mm cassette.

❺

Make up 100 ml of displacing solution.

❻

Make up the gel acrylamide solutions from the stock mixes, but do not add the 10% ammonium persulphate (APS) and 10% N,N,N',N',-tetramethylethylenediamine (TEMED). See "Gradient gel solutions" on page 28.

# Pouring gel solutions for gradient gels

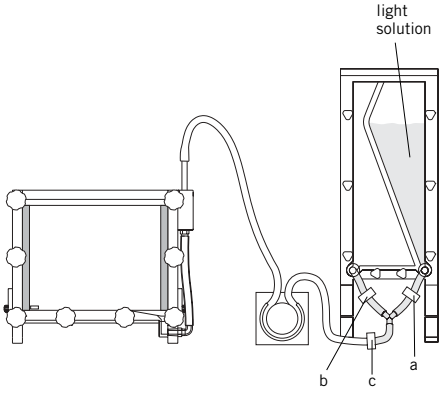



**Fig 10.** Priming the gradient maker with light solution.

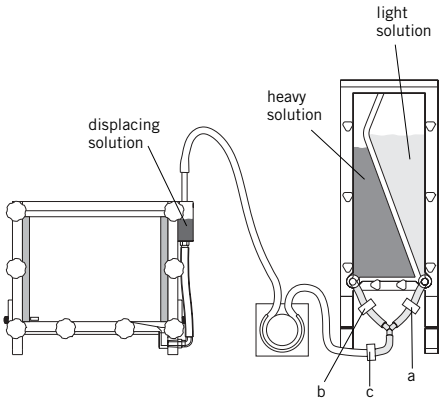



**Fig 11.** Properly filled caster and gradient maker.

❶

Prepare the gel caster, as described on page 6, placing gel labels in each cassette.

❷

When you are ready to cast the gels, add the APS and TEMED and mix each gel solution thoroughly. Vary the amount of TEMED added to control the rate of polymerization. Once these reagents are added, polymerization begins. You have about 10 min to cast the gradient before the gels begin to solidify at the top. Work rapidly and carefully.

❸

Pour the light solution into the right side of the gradient maker (the chamber that is narrower at the bottom—"Light in right").

❹

Fill the tubing between the light and heavy chambers with light solution. Carefully open the clamp on the light chamber exit tube (a) and then very slowly open the heavy chamber exit tube clamp (b). Allow light solution to fill the tube coming from the light chamber all the way to the Y-connector and back up to the point at which the heavy tube enters the heavy chamber. Fill the entire tube with light solution (no bubbles), but do not allow light solution into the heavy chamber itself (see Fig 10).

❺

Close both clamps again. All three clamps should now be closed.

❻

Add the heavy solution to the heavy (left) chamber (the chamber that is wider at the bottom) until the liquid level reaches a point about 2 cm below the level of light solution in the adjacent chamber.

❼

Load the balance chamber on the side of the caster with 75 ml of displacing solution (see Fig 11). The feed tube should be seated securely in the grommet seal to prevent leakage of displacing solution into the caster.

❽

Open the clamp below the mixer (c) to start flow to the gel caster via the peristaltic pump.

❾

Carefully open the clamp on the light chamber exit tube (a) and turn on the pump to bring a small amount of solution into the caster. Light solution should begin to flow through the feed tube and mixer toward the caster. At this point a small amount of light solution can enter the caster.

*continues*

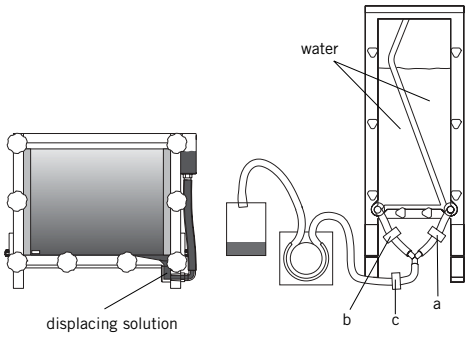



**Fig 12.** Finished cast.

❶

When the light solution level in the gradient maker falls to about 1 cm above the level of the heavy solution, open the heavy chamber exit tube clamp (b).

⓫

Watch the gradient enter the caster.

⓬

When the caster is filled to about 1–2 cm below the final desired gel height or the gradient maker is empty, turn off the pump and close the feed tube clamp (c). Stop the pump before air enters the feed tube.

⓭

Pull the feed tube out of the balance chamber grommet. Place the end in a waste container to collect the excess polymerizing acrylamide. As soon as the feed tube is removed, the dense blue displacing solution flows down the connecting tube to the unit. It should completely fill the V-well and the sloped trough at the bottom of the caster. If the V-well is not completely filled and the level of gel in the cassettes is more than 1 cm below the top of the cassettes, you can add up to 25 ml more displacing solution to the balance chamber. The gradient is now in hydrostatic equilibrium in the unit, ready to polymerize (see Fig 12).

⓮

Immediately pipette gel overlay solution (water-saturated *n*-butanol) onto each gel. Apply 1 ml of overlay for each 1.0 mm cassette and 1.5 ml for each 1.5 mm cassette. Allow gels to polymerize at least 2 h.

⓯

Quickly reopen clamp (c) and restart the pump to empty the gradient maker of any excess polymerizing acrylamide. Collect the excess in a waste container. Dispose of unpolymerized acrylamide according to applicable safety guidelines.

⓰

Rinse the gradient maker well to prevent polymerization within the tubing lines. Place the feed tube in a larger waste vessel or a sink drain. Pour a litre of water into each chamber of the gradient maker and open all clamps.

⓱

Start the pump to flush the system. Flush another 2 l of water through the gradient maker and tubing.

## Polymerization

Allow nongradient gels to polymerize for at least 1 h; allow gradient gels 2 h to polymerize. Gradient gel polymerization should proceed from the top down. You can observe this through the front and sides of the caster. The level of the dense displacing solution falls farther as the gels contract upon polymerization.

# Unloading the gel caster

❶

Make sure the caster is either near a sink or on a tray so that any liquid leaking out can be contained.

❷

Remove the front of the gel caster by loosening and removing the black-knobbed screws.

❸

Carefully unload the cassettes from the unit by pulling forward on the separator sheets.

❹

Rinse the top surface of each gel with distilled water to remove the butanol and any unpolymerized acrylamide. Remove the separator sheet if still attached and rinse the glass cassettes with water to remove any acrylamide adhering to the glass plates.

❺

Examine the gels for polymerization defects and discard any unsatisfactory gels.

❻

Store the good gels in an airtight container at 4 ºC with a small amount of gel storage solution to keep the gels from drying out.

❼

Rinse the gel caster and all tubing with mild detergent, then rinse thoroughly with deionized water. Clean the separator and spacer sheets with a mild detergent and rinse with deionized water.

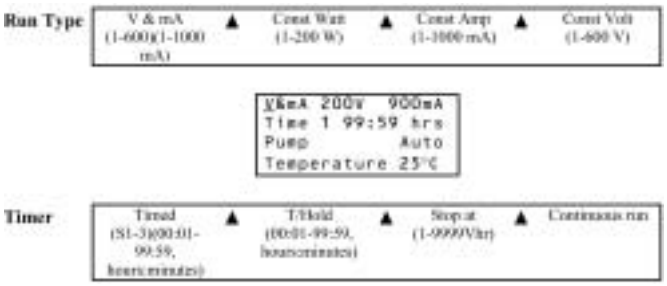
# Electrophoresis

The unit should be placed close to a sink for easy rinsing and draining. A length of rubber or vinyl tubing sufficiently long to reach a sink should be attached to the drain port on the back of the unit before use. The unit should not be placed on bench paper or any other material that might be pulled in by the air intake fans, as any hindrance to air circulation will reduce the cooling capacity.

## Programming the power supply/control unit

The control unit has four programmable parameters: Run Type, Timer, Pump, and Temperature. When the unit is turned on, the default settings of constant power, continuous run, auto pump, and 25 °C are shown on the display. The *set/read* button toggles the controller between the set and read modes. The *start/stop* button starts and stops the execution of the programmed electrophoresis run. The ◀ and ▶ buttons move the cursor between run parameters, and the ▲ and ▼ buttons change the settings of the parameters (see Fig 13).

**Fig 13.** Controller interface in programming mode.




**Run Type** determines the method of power regulation. The options for run type are constant power, constant current, constant voltage, and crossover mode. In crossover mode the voltage and current limits for the run are set manually, instead of using the instrument defaults of 600 V and 1 000 mA. As the run progresses, the power supply operates in the mode that is limiting.

**Timer** controls the duration of the electrophoresis run. The options are continuous run, timed step, timed and hold, and volt-hours. Up to three timed steps of up to 100 h each can be programmed using the timed step mode. With timed step mode, all the steps must have the same run type.

**Pump** controls buffer circulation through the separation unit. The options for the pump are on, off, and auto. Auto mode activates the pump only when power is applied.

**Temperature** controls the cooling or heating of the buffer in the separation unit. The temperature range is from 10–50 °C. On the display, cooling is indicated by ↓ and heating is indicated by ↑. The pump must be on to properly cool or heat buffer in the tank. The lower temperature limit is a function of the ambient temperature and the power reading. To reach the lower temperature limit (10 °C) or in laboratory environments where the ambient temperature is elevated, it may be necessary to place the unit in a cold box or cold room.

### Sample program

Twelve gels electrophoresed at 180 W, constant power, and 25 °C with a 5 W/gel entry phase. The second phase is extended longer than required to ensure that the dye front runs off the gel.

**Step #1**

| | |
|---|---|
| Const Watt | 60 W |
| Time 1 | 00:45 hrs |
| Pump | Auto |
| Temperature | 25°C |

**Step #2**

| | |
|---|---|
| Const Watt | 180 W |
| Time 2 | 04:00 hrs |
| Pump | Auto |
| Temperature | 25°C |

## Preparing the electrophoresis unit for lab cast gels

The Ettan DALT*twelve* Electrophoresis Unit requires a total volume of about 10 l of electrophoresis buffers to fill both the upper and lower chambers. For lab cast Laemmli gels, the lower buffer chamber is filled with 7.5 l of 1× SDS electrophoresis buffer while the upper buffer chamber is filled with 2.5 l of 2× SDS electrophoresis buffer. With the full volume of the lower buffer in the unit, the liquid buffer serves as a lubricant for inserting the glass cassettes through the buffer seal. Lubrication of the cassettes and the rubber surfaces of the buffer seal is vital when loading the unit. Forcing dry cassettes through the slots can severely damage the seal. The electrophoresis buffer can be made within the tank using the internal circulating pump to mix the solution.

**Fig 14.** Circulation valve.

❶

Before filling the tank, turn the pump valve to *circulate* (see Fig 14).

❷

Fill the electrophoresis tank to the 7.5 l fill line with 1× SDS electrophoresis buffer. Turn on the pump/control unit, and turn the pump on. If the pump fails to begin circulating buffer immediately, the pump must be primed; turn the pump valve to drain then back to circulate while the pump is on.

❸

On the control unit, adjust the temperature to the desired setting.

## Preparing second-dimension gels: equilibration and loading

For a detailed description of the components of the SDS equilibration solution and the equilibration process, please consult *2-D Electrophoresis: Using Immobilized pH Gradients* (80-6429-60).

❶

Prepare SDS equilibration buffer. Just prior to use, add DTT to the buffer to a concentration of 1% (w/v).

❷

Place the IPG strips in individual tubes with the support film toward the wall.

❸

Add 5–10 ml of the DTT-containing solution to each tube. Typically, two 18-cm strips can be equilibrated with 10 ml of buffer.

❹

Incubate the strips for 10–15 min with gentle agitation. Do not overequilibrate, as proteins can diffuse out of the strip during this step.

❺

Second equilibration (optional): Prepare SDS equilibration buffer with iodoacetamide added to 2.5% (w/v) and equilibrate the strips with this solution, as in steps 3–4.

❻

Before equilibration is completed, prepare the gel cassettes for loading by rinsing the top of the gel with deionized water and draining. Before loading the IPG strips, make sure that the gel surface and plates are dry.

❼

Lay the prepared gel flat on a clean surface.

❽

Using forceps, remove the equilibrated IPG strip from the equilibration solution and rinse with fresh SDS electrophoresis buffer.

❾

Holding one end of the IPG strip with forceps, carefully draw it across the long gel plate until the strip is completely on the glass plate and centered (see Fig 15a). Using a thin plastic spatula, ruler, or spacer, push against the plastic backing of the IPG strip — *not* the gel itself — and slide the strip between the two glass plates and down into contact with the surface of the slab gel (see Fig 15b). The strip should just rest on the surface of the gel. Avoid trapping air bubbles between strip and the slab gel and avoid piercing the second-dimension gel with the strip. By convention, the acidic, or pointed, end of the IPG strip is on the left. The gel face of the strip should not touch the opposite glass plate.

*continues*

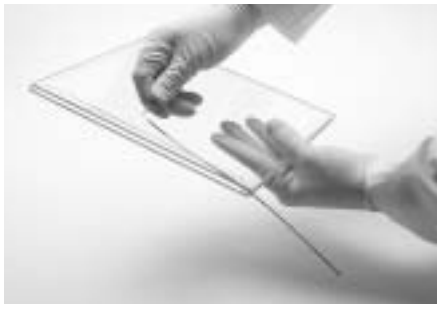


**Fig 15a.** IPG strip being positioned on cassette.

**Fig 15b.** IPG strip being seated against slab gel.

❶

Apply molecular weight marker proteins (optional): Apply the markers to a sample application piece in a volume of 15–20 µl, then cover the piece with 50 µl of agarose sealing solution. Pick up the application piece with forceps and place next to one end of the IPG strip. The markers should contain 0.2–1.0 mg of each component for Coomassie blue staining or about 10–50 ng of each component for silver staining.

⓫

Seal the IPG strip in place. For each IPG strip, melt an aliquot of agarose sealing solution in a heating block or boiling water bath. (***Tip:*** An ideal time to carry out this step is during IPG strip equilibration.) Allow the agarose to cool slightly and slowly pipette the solution across the length of the IPG strip, taking care not to introduce bubbles. It will flow down between the glass plate and the support film and seal the IPG strip in place (see Fig 16). Agarose should also be used to seal any gap between the side of the gel and a side spacer. Allow a minimum of 1 min for the agarose to cool and solidify.

**Fig 16.** Adding agarose sealing solution.

## Loading the separation unit

❶

Once the electrophoresis tank has reached the desired temperature and the gels are ready, carefully slide the gels, one-by-one, into the tank. Until the buffer reaches the bottom of the rubber sealing tubes, the cassettes should be lubricated with buffer or water to prevent the rubber tubing from sticking to the cassettes. Once the buffer level reaches the sealing tubes, the gels should slide in easily.

**Note:** Forcing cassettes through the rubber tubes of the buffer seal without sufficient lubrication can damage the buffer seal.

❷

Fill any unused slots with the blank cassette inserts. When the last cassette is put into place, buffer will be pushed out of the lower tank into the upper tank via the two air vents at the corners of the sealing assembly. The final level of electrophoresis buffer in the upper tank should not be above the openings for the air vents.

**Recommendation:** For electrophoresis runs of six or fewer gels, it is helpful to alternate gel cassettes with blank cassette inserts. Alternating cassettes will make it considerably easier to remove the cassettes from the unit following the run. The blank cassette inserts are easily removed first, leaving a larger gap that makes it easier to grasp and remove the gel cassettes.

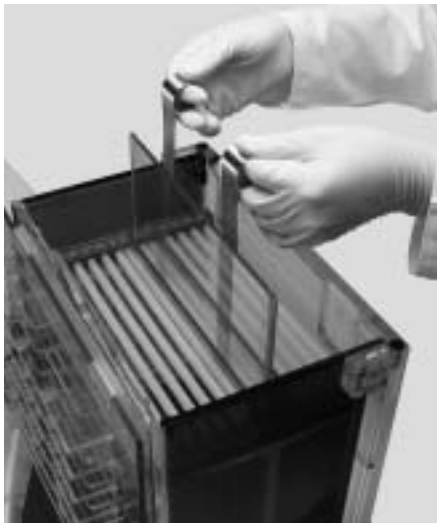


**Fig 17.** Using the cassette removal tools.

**Fig 18.** Removing cassettes by hand.

## Unloading and cleaning the separation unit

❶

After the run has been completed, remove one or more of the blank cassette inserts or gels and drain enough of the electrophoresis buffer from the tank to expose 2–3 cm of the gel cassettes. This will ease removing the remaining cassettes. When the first cassette, either blank or gel, is removed, a sucking sound will be heard as air is drawn into the lower chamber. For runs of six or fewer gels, alternating gel cassettes with blank cassettes also eases the removal of the gel cassettes at the end of a run. Leave enough of the electrophoresis buffer in the tank to act as a lubricant between the glass cassettes and the buffer seal.

There are two methods for removing the first cassettes from the unit: using (a) the cassette removal tool or (b) the hands.

Ⓐ Carefully insert the cassette removal tool between the cassette and the buffer seal, with the folded tip facing the cassette, until the tip is beneath the bottom edge of the cassette. Verify that the tool is caught on the bottom edge of the cassette, then lift it out slowly with the tool (see Fig 17).

Ⓑ By hand, apply upward pressure alternately to each side of the cassette, gradually shifting it up until you can grasp it and remove it (see Fig 18).

❷

Open the cassettes using a Wonder Wedge (80-6127-88) and carefully transfer the gels to a staining tray (80-6468-17), for example. Take care to ensure that the gel does not adhere to the spacers.

**Note:** Vinyl gloves are less sticky than latex gloves and make it easier to handle large gels.

❸

When all of the gels and blank cassette inserts have been removed, drain the buffer by turning the pump valve to *drain* with the pump on. Emptying will take about 1 min.

❹

After the buffer has been removed, pour 3–4 l of distilled or deionized water into the unit and allow it to drain. Rinse the unit with 5–7 l of distilled or deionized water in circulate mode, empty again, and repeat if necessary.

❺

Remove the lid from the unit by sliding it to the left and rinse it with distilled or deionized water. Slide the lid back on its hinges before using the unit again.

❻

In most cases thorough rinsing is all the cleaning that is necessary. If a more thorough cleaning is required, see “Care and maintenance” for a detailed description of the removal of all the internal components.

# Electrophoresis on pre-cast gels

Ettan DALT Gel, 12.5 is a pre-cast polyacrylamide gel for the second-dimension of two-dimensional electrophoresis. The gel is cast onto a plastic support film. The gel size is 255 × 196 × 1 mm.

The gel is a homogeneous 12.5% polyacrylamide gel cross-linked with bisacrylamide. It is intended to be used in the Ettan DALT*twelve* System together with the Ettan DALT Buffer Kit. The gel is formulated for long shelflife and, when used with the buffer kit, generates a discontinuous buffer system yielding rapid runs with sharp, reproducible results. Performance and capacity of this gel and buffer system are similar to the widely used Laemmli (Tris-glycine) buffer system.

These instructions describe how to use Ettan DALT Gel, 12.5 together with the Ettan DALT Buffer Kit for the second-dimension of 2-D electrophoresis.

## Ettan DALT Gel, 12.5 and Ettan DALT Buffer Kit

### Package contents

Each gel package contains six gels and instructions. The buffer kit contains four bottles of buffer and 12 tubes of sealing solution. The solutions are sufficient for a single run of up to 12 gels.

| product | quantity | product number |
|---|---|---|
| **Ettan DALT Gel 12.5%** | 6 | 17-6002-36 |
| Instructions | 1 | 71-5019-56 |
| **Ettan DALT Buffer Kit** | enough to run 12 gels | 17-6002-50 |
| Anode buffer | 1 bottle (75 ml) | |
| Cathode buffer | 2 bottles (2 × 125 ml) | |
| Gel buffer | 1 bottle (60 ml) | |
| Sealing solution | 12 tubes (12 × 1 ml) | |

### Technical data

| | |
|---|---|
| Gel composition | T = 12.5%, C = 3%<br>(12.125% acrylamide, 0.375% bisacrylamide) |
| Separation range | $M_r$ 12 000–120 000 |
| Gel dimensions | 255 × 196 × 1 mm |
| Buffer in gel | Piperidinopropionamide (PPA)* |
| Gel backing | Polyester film, 265 × 211 mm |
| Shelf life | 6 months |
| Storage | +4 °C to +8 °C |
| 100× anode buffer | 5 M diethanolamine (DEA), 5 M acetic acid |
| 10× cathode buffer | 0.25 M Tris, 1.92 M glycine, 1% (w/v) SDS |
| Gel buffer | Piperidinopropionamide (PPA)* |
| Sealing solution | Gel buffer with 0.5% agarose and 0.002% bromophenol blue |

*The buffer system in this gel and buffer kit is covered by United States Patent 6,090,252 and others.

**Fig 19.** Ettan DALT*twelve* Gel, 12.5 and buffer kit components.

**Fig 20.** Pre-cast gel cassette.

## Description of the system

The Ettan DALT Gel, 12.5 gel is a pre-cast polyacrylamide gel for the second-dimension of large-format 2-D electrophoresis. It is bound to a plastic support film, which provides ease of handling and dimensional stability. The gel is intended for use in the Ettan DALT*twelve* System. The gel is inserted into a cassette that allows it to be run in a vertical mode with liquid buffers. The gel is used with the Ettan DALT Buffer Kit that includes concentrated buffers for running the gels, gel buffer for seating the gel in the pre-cast gel cassette, and sealing solution for attaching the IPG strip to the top of the slab gel.

The pre-cast gel cassette holds the Ettan DALT gel vertically in the Ettan DALT Electrophoresis Unit. It consists of a glass plate with spacers glued to the vertical edges and connected to a rigid plastic frame along one edge by a flexible hinge. The gel is placed against the glass plate between the spacers. When the cassette is closed and snapped together, the frame presses the gel evenly against the glass plate. The glass plate extends 5 mm higher than the plastic frame at the cathodic (–) edge providing a surface for sliding the IPG strip into position.

The buffer in the gel is part of a unique buffer system that gives longer shelf life and shorter run times than the conventional Laemmli (Tris-glycine) system, while retaining the protein capacity and robustness of that system. Separations performed using Ettan DALT Gel, 12.5 are similar to those seen with a 12.5% Laemmli gel.

The associated buffer kit contains all the reagents necessary for a single run of up to 12 Ettan DALT Gel, 12.5 gels in the Ettan DALT*twelve* Electrophoresis Unit.

**Tip:** The subsequent steps of electrophoresis unit preparation, insertion of the gel into the pre-cast gel cassette, and melting of the Sealing Solution can be performed as the IPG strips are equilibrating.

**Fig 21.** Addition of 100× anode to the separation unit solution.

**Fig 22.** Pre-cast gel cassette open.

**Fig 23.** Gel buffer.

## Preparing the Ettan DALT*twelve* Electrophoresis Unit

Ensure that the valve on the Ettan DALT Electrophoresis Unit is set to *circulate*. Fill the tank to the 7.5 l fill line with distilled or deionized water. Add the entire contents (75 ml) of the bottle of 100× anode solution from the buffer kit. Avoid pouring the 100× anode solution on the buffer seal tubing by spreading it slightly with one hand while pouring the solution (see Fig 21). Turn on the pump to mix. Set the unit to the desired temperature (25 °C is recommended).

### Inserting the Ettan DALT Gel, 12.5 into the pre-cast gel cassette

❶

Open the gel package. Cut around the gel on two sides at about 1 cm from the edge to avoid cutting the gel or the support film. Remove the gel from the package.

The gel is cast onto a plastic support film and does not cover the film entirely. The gel is covered with a protective plastic sheet. Markings on the protective sheet indicate the orientation of the gel and the direction of electrophoresis. The bottom (+ or anodic) edge of the gel is flush with the edge of the support film. The support film protrudes approximately 15 mm beyond the top (– or cathodic) edge of the gel and approximately 5 mm at either side.

❷

Open an Ettan DALT Pre-cast Gel Cassette and place it on the bench top with the hinge down (see Fig 22).

❸

Pipette 2–4 ml of gel buffer onto the centre of the glass plate (see Fig 23).

*continues*

**Fig 24.** Initial placement of the gel.

**Fig 25.** Final placement of the gel.

**Fig 26.** Removing air bubbles.

**Fig 27.** Closing the cassette.

**Fig 28.** Snapping the cassette.

❹

Remove the protective plastic sheet from the gel. Handling the gel only by the side support film margins, hold it, gel-side down, over the glass plate. Ensure that it is oriented with the cathodic (–) edge of the gel toward the cathodic (–) edge of the cassette. Flex the centre of the gel downward slightly and lower it toward the glass plate so that the middle of the gel contacts the puddle of gel buffer. The gel buffer will lubricate the gel and allow it to be moved and placed in the proper position (see Fig 24).

❺

Move the gel and allow it to fall against the glass so that the edges of the gel are against (not overlapping) the side spacers and so that the bottom (anodic) edge of the gel is flush (within 1 mm) of the bottom (anodic) edge of the glass plate. The protruding side support film margins (but not the gel) should rest on top of the side spacers (see Fig 25).

❻

Use the roller to press out any bubbles and excess buffer from between the gel and the glass. Press firmly against the plastic support film with the roller and roll over the entire gel (see Fig 26). After rolling, the gel should adhere firmly to the glass and resist further movement.

❼

Close the cassette and snap the plastic frame to the glass plate (see Fig 27 and 28).

❽

Repeat the procedure for each second-dimension gel to be run.

**Fig 29.** Placing of the IPG strip.

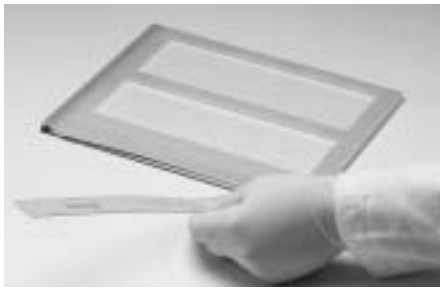


**Fig 30.** Seating the IPG strip against the gel.

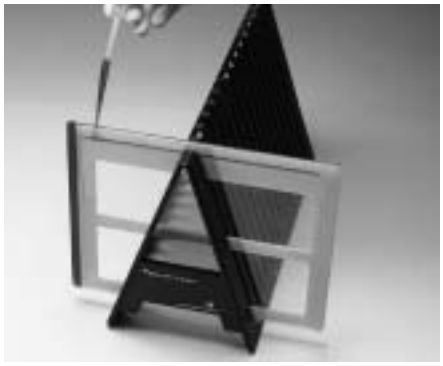


**Fig 31.** Sealing the IPG strip with agarose.

## Applying the IPG strip

❶

Leave the loaded gel cassette lying flat on the bench top with the glass plate down and the plastic frame up.

❷

Rinse the IPG strip. Pour some of the diluted (1×) cathodic buffer into a 100 ml graduated cylinder or similar vessel. Using forceps, remove the equilibrated IPG strip from the equilibration solution and dip it into the cathodic buffer in the cylinder. (This step lubricates the IPG strip and washes off any particulate material that may be precipitated on the surface of the IPG strip.)

❸

Place the IPG strip on the top (cathodic) surface of the gel. Holding one end of the IPG strip, carefully draw it across the shelf formed by the extension of the glass plate beyond the plastic frame, until the strip is completely on the glass plate and centered, gel face upward (see Fig 29).

❹

Push the IPG strip into place. Using a thin spatula or ruler, push against the plastic backing of the IPG strip to slide it a short distance into the gap between the glass plate and the support film and plastic frame. Be sure to push against the backing of the IPG strip, not the gel itself (see Fig 30). Place the cassette upright in the cassette rack with the glass plate forward. Continue to slide the IPG strip down until it contacts the surface of the second-dimension gel. The strip should just rest on the surface of the gel. Avoid trapping bubbles between the strip and the slab gel and avoid piercing the second-dimension gel with the strip. Note the orientation of the IPG strip relative to the gel (conventionally, the acidic [pointed] end of the IPG strip points to the left). The gel face of the strip should not touch the plastic support film.

❺

Seal the IPG strip in place. For each IPG strip, melt an aliquot of sealing solution from the buffer kit in a 100 °C heating block or boiling water bath. (***Tip:*** An ideal time to carry out this step is during IPG strip equilibration.) Allow the solution to cool slightly, then slowly pipette the solution across the length of the IPG strip, taking care not to introduce bubbles. It will flow down between the glass plate and the support film and seal the IPG strip in place (see Fig 31). There may be a gap of up to 2 mm between the edge of the gel and the side spacer. Any gap should be plugged by allowing some of the sealing solution to flow down the gap. Allow a minimum of 1 min for the agarose to cool and solidify).

❻

Repeat the procedure for each second-dimension gel to be run.

**Fig 32.** Loading gels into the separation unit.

## Inserting gels into the separation unit

❶

When the lower tank buffer has reached the desired temperature, insert the loaded gel cassettes with the IPG strips in place (see Fig 32). Push blank cassette inserts into any unoccupied slots. Load the unit from back to front. Gel cassettes and blank cassette inserts slide much more easily into the unit if they are wet. Distilled or deionized water from a squirt bottle can be used to wet the cassettes and inserts as they are being loaded into the unit. When all 12 slots are filled, the buffer level should be slightly below the level of the buffer seal gaskets.

❷

Dilute the cathode buffer to working strength by adding both bottles of 10× cathode buffer (total volume 250 ml) to 2.25 l of distilled or deionized water.

❸

Pour the diluted (1×) cathode buffer into the top of the tank to the fill line. (Some of this buffer may drip through the gasket and mix with the lower anode buffer during the run, but this will not affect performance or results.)

❹

Program the desired run parameters into the control unit, close the lid of the electrophoresis tank, and press *start/stop* to begin electrophoresis.

## Detection

The Ettan DALT Gel, 12.5 gel can be stained or visualized with a variety of commonly used techniques, including Coomassie Blue and silver staining. When using the PlusOne™ Silver Staining Kit, Protein, a modified staining protocol should be used. Prepare the staining reagents (250 ml per gel) as indicated in the kit instructions with the following exceptions:

- Prepare twice the fixing solution called for (500 ml per gel rather than 250 ml).
- Prepare the developing solution with twice the formaldehyde called for (100 µl per 250 ml of developing solution rather than 50 µl). Stain the gels according to the following protocol:

| | |
|---|---|
| Fixing | 2 × 60 min* |
| Sensitization | 60 min |
| Water wash | 5 × 8 min |
| Silver | 60 min |
| Wash | 4 × 1 min |
| Developing | 10 min† |
| Stop | 60 min |
| Wash | 2 × 30 min |
| Preserve | 40 min |

*The first fixing step may be prolonged up to 3 days if desired for the sake of convenience.

† Approximate time: this step may be visually monitored. The gels should be transferred to stop solution when the spots have reached the desired intensity and before the background staining becomes too dark.

# Recommended running conditions

**Note:** The run times provided should only be used as guidelines or estimates. Decreasing the number of gels per run allows increased watts per gel, which reduces run times. The maximum power specified for each temperature is the limit of the cooling system capacity at that temperature.

| | step | power (w/gel) | approximate run duration* (h:min) |
|---|---|---|---|
| **Day runs (set temperature to 25 ºC)** | | | |
| 1 mm-thick gels | 1 | 5 | 0:30 |
| (lab-cast and precast) | 2 | 17 (max 180) | 4:00 |
| 1.5 mm-thick gels | 1 | 5 | 0:30 |
| | 2 | 17 (max 180) | 6:00 |
| **Overnight runs† (set temperature to 30 ºC and power supply to continuous run)** | | | |
| 1.0 mm-thick gels | | 1 | 17:00 |
| 1.5 mm-thick gels | | 1.5 | 17:00 |

*The time shown is approximate. Stop electrophoresis when the dye front is 1 mm from the bottom of the gel.

† For the best possible resolution, faster separation times should be used. Use the faster (<6 h) protocols instead.

# Recipes

***Warning!*** Acrylamide is a neurotoxin. Always use mechanical pipettes and wear gloves when working with acrylamide solutions.

### Acrylamide stock (30.8 %T)

| | final conc. | amount |
|---|---|---|
| Acrylamide (MW 71.08) | 30% | 900 g |
| Bis (N,N' methylenebisacrylamide, MW 154.17) | 0.8% | 24 g |
| Distilled or deionized water | | to 3 000 ml |

May need filtration. Weigh acrylamide and bis in a hood; avoid contact with dust. Filter and store at 4 °C.

### 1.5 M TrisCl, pH 8.8

| | final conc. | amount |
|---|---|---|
| Tris (MW 121.14) | 1.5 M | 545 g |
| 6 M HCl to pH 8.8 | | about 150 ml |
| Distilled or deionized water | | to 3 000 ml |

Adjust to pH 8.8 and store at 4 °C.

### 10% (w/v) SDS

| | final conc. | amount |
|---|---|---|
| Sodium dodecylsulphate (MW 288.38) | 10% | 10 g |
| Distilled or deionized water | | to 100 ml |

Store at room temperature.

### 10% (w/v) Ammonium persulphate

| | final conc. | amount |
|---|---|---|
| Ammonium persulphate (MW 71.08) | 10% | 2 g |
| Distilled or deionized water | | to 20 ml |

Prepare fresh.

### 10% (v/v) TEMED

| | final conc. | amount |
|---|---|---|
| TEMED (MW 116.2) | 10% | 0.5 ml |
| Distilled or deionized water | | 4.5 ml |

Prepare fresh.

### Displacing solution

*(0.375 M TrisCl, pH 8.8, 50% (v/v) glycerol, bromophenol blue, 100 ml)*

| | amount |
|---|---|
| TrisCl (1.5 M, pH 8.8) | 25 ml |
| Glycerol | 50 ml |
| Bromophenol blue | 2 mg |
| Distilled or deionized water | 25 ml |

Should be made fresh; stored solution may support microbial growth.

# **Recipes** (continued)

### Water-saturated butanol

| | amount |
|---|---|
| *n* or *t*-butanol | 50 ml |
| Distilled or deionized water | 5 ml |

Combine in a bottle and shake. Use the top phase to overlay gels.
Store at room temperature indefinitely.

### Gel storage solution

*(0.375 M TrisCl, pH 8.8, 0.1% (w/v) SDS, 2 l)*

| | final conc. | amount |
|---|---|---|
| TrisCl (1.5 M, pH 8.8) | 0.375 M | 500 ml |
| 10% (w/v) SDS | 0.1% (w/v) | 20 ml |
| Distilled or deionized water | | to 2000 ml |

Store at 4 °C.

### 10 × SDS electrophoresis buffer

*(250 mM Tris, 1.92 M glycine, 1% (w/v) SDS, approximate pH 8.3, 10 l)*

| | final conc. | amount |
|---|---|---|
| Tris (MW 121.14) | 250 mM | 303 g |
| Glycine (MW 75.07) | 1.92 M | 1440 g |
| SDS (MW 288.38) | 1% (w/v) | 100 g |
| Distilled or deionized water | | to 10 l |

Do not adjust the pH of this solution.

### SDS equilibration buffer

*(50 mM TrisCl, pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, bromophenol blue, 200 ml)*

| | final conc. | amount |
|---|---|---|
| TrisCl (1.5 M, pH 8.8) | 50 mM | 6.7 ml |
| Urea (MW 60.06) | 6 M | 72.07 g |
| Glycerol (87% [v/v], MW 92.09) | 30% (v/v) | 69 ml |
| SDS (MW 288.38) | 2% (w/v) | 4.0 g |
| Bromophenol blue | trace | a few grains |
| Distilled or deionized water | | to 200 ml |

Store at -20 °C. This is a stock solution. Add DTT or iodoacetamide before using.

### Sealing solution

*(0.25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, bromophenol blue, 0.5% (w/v) agarose, 25 ml)*

| | final conc. | amount |
|---|---|---|
| SDS electrophoresis buffer (see above) | | 25 ml |
| Agarose (IEF, NA, or M) | | 125 mg |
| Bromophenol blue | trace | a few grains |

Combine all ingredients in a 250 ml Erlenmeyer flask. Swirl to disperse. On a low setting, heat in a microwave oven until the agarose is completely melted, about 1 min. Do not allow the solution to boil over.

Allow the agarose to cool slightly before using. Do not adjust pH.

# Homogeneous gel solutions

### 900 ml

| | volume required for (ml) | | |
|---|---|---|---|
| **final %T** | **10%** | **12.5%** | **15%** |
| Acrylamide stock | 300 | 375 | 450 |
| 1.5 M TrisCl, pH 8.8 | 225 | 225 | 225 |
| Water | 356 | 281 | 206 |
| 10% SDS | 9 | 9 | 9 |
| 10% APS | 9 | 9 | 9 |
| 10% TEMED | 1.54 | 1.24 | 1.03 |

*Note:* The amounts of TEMED (0.025–0.09% (v/v)) and APS (0.1% (w/v)) suggested here are based on our experience. You may want to change volumes for your laboratory because of differences in temperature and reagent quality. Perform a small-scale test before using a new composition to check that your solution polymerizes in about 10 min.

The gel recipes are based on Laemmli, U.K. *Nature* **227**, 680–685 (1970).

# Gradient gel solutions

### Light solution, 450 ml

| | volume required for (ml) | | | | |
|---|---|---|---|---|---|
| **final %T** | **8%** | **10%** | **12%** | **14%** | **16%** |
| Acrylamide stock | 120 | 150 | 180 | 210 | 240 |
| 1.5 M TrisCl, pH 8.8 | 113 | 113 | 113 | 113 | 113 |
| Water | 207 | 178 | 148 | 118 | 88 |
| 10% SDS | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| 10% APS | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| 10% TEMED | 0.96 | 0.77 | 0.64 | 0.55 | 0.48 |

### Heavy solution, 450 ml

| | volume required for (ml) | | | | |
|---|---|---|---|---|---|
| **final %T** | **12%** | **14%** | **16%** | **18%** | **20%** |
| Acrylamide stock | 180 | 210 | 240 | 270 | 300 |
| 1.5 M TrisCl, pH 8.8 | 113 | 113 | 113 | 113 | 113 |
| Water | 120 | 90 | 60 | 30 | 0 |
| 10% SDS | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| Glycerol | 31 | 31 | 31 | 31 | 31 |
| 10% APS | 2.3 | 2.3 | 2.3 | 2.3 | 2.3 |
| 10% TEMED | 0.21 | 0.18 | 0.16 | 0.14 | 0.13 |

# Troubleshooting

| symptom | possible causes | possible solutions |
|---|---|---|
| **Electrical and mechanical** | | |
| No current at start of run | Insufficient volume of buffer in upper reservoir. | Ensure that the unit contains enough buffer to contact the upper electrode. |
| Buffer not circulating | Pump is not primed. | Turn circulation valve to *drain* to fill with buffer then back to *circulate*. |
| | Pump is off or set to *auto*. | On control unit, set pump to *on*. |
| | Pump is broken. | Service call. |
| Display on the control unit blank | Unit is not turned on. | Turn unit on at power switch in back. |
| | Unit is not plugged in. | Plug in unit. |
| | Display is broken. | Service call. |
| Control unit display malfunctions | | Turn the AC power switch off for a few seconds, then on again. If the problem persists, Service call. |
| Open-circuit warning | Safety interlocks not engaged. | Make sure that the lid is completely closed. |
| | | Make sure that the pump valve is turned completely to *circulate*. |
| | | Service call. |
| **Gel casting** | | |
| Gel caster leaks | | Apply a light film of GelSeal compound to the foam gasket before casting. |
| | | Check the foam gasket for cracks or nicks and replace if necessary. |
| | | If the stack is too thick, the front plate may not seat firmly against the gasket. Remove one or more of the filler sheets until the gasket seals. |
| Incomplete gel polymerization | | Use only recent stocks of the highest-quality reagents. |
| | | If the dry ammonium persulphate does not crackle when water is added to it, replace with fresh reagent. |
| | | Use fresh ammonium persulphate. |
| | | Solutions of extreme pH may not polymerize. |
| | | Degas the monomer solution. Oxygen inhibits polymerization. |
| | | Increase both ammonium persulphate and TEMED by 30 to 50%. |
| | | Adjust the gel solution temperature to a minimum of 20 ºC. |
| Gel is too soft, too brittle, or white | | Check and adjust crosslinker concentration. Standard SDS gels should have a crosslinker concentration of 2.6% (%C = (g bis × 100)/(g monomer + g bis)). |
| | | Make up fresh acrylamide stock solution. |
| Gel exhibits swirls | | If gel polymerized too fast (<10 min), reduce the concentration of catalyst (APS and TEMED) by 25%. |
| | | If gel polymerized too slowly (>50 min), increase the concentration of catalyst (APS and TEMED) by 50%. |
| | | Make up fresh acrylamide stock solution. |
| Dye front curves up “smiles” | | Check circulation of the buffer. |
| | | Pre-chill the buffer. |
| | | Decrease power, voltage, or current. |

# **Troubleshooting** (continued)

| symptom | possible causes | possible solutions |
|---|---|---|
| **Gel casting (continued)** | | |
| Gels cast simultaneously are different sizes | | Allow the solution to settle, or reach equilibrium, before adding the overlay. |
| | | Add equal amounts of overlay solution to each gel. |
| | | Add overlay as quickly as possible. |
| Gradient gels — uneven layering | | Add sucrose (15% (w/v)) or glycerol (25% (v/v)) to the high-percentage monomer solution. |
| | | Add a very small amount of bromophenol blue to the high-percentage monomer solution to track gradient formation. *Note:* Excessive bromophenol blue will inhibit polymerization. |
| Unusually slow or fast run | Check for leaks. | All plates, spacers, and gaskets must be clean, dry, and free of grease. |
| | | Make sure buffer is at the fill level and not covering the vent holes. |
| | | Check the pH of the buffer. If the pH is exceeded, make fresh buffer; do not back-titrate. |
| | | Check recipes, gel concentrations, and buffer dilutions. (For example, do not use TrisCl in place of Tris base for the electrophoresis buffer.) |
| | | Discard older acrylamide solutions and use only reagents of the highest quality. |
| | | Only use freshly deionized urea of the highest quality. |
| | | Adjust power, current, or voltage. |
| **Pre-cast gels** | | |
| Second-dimension separation proceeds slowly with high current | All of the slots in the buffer seal are not occupied by either gel cassettes or blank cassettes. | Ensure that all 12 slots in the buffer seal are occupied. Do not pour more than the suggested volume (7.5 l) into the lower reservoir. |
| | Anodic buffer has mixed with cathodic buffer from overfilling of either the cathodic or the anodic reservoir. | Ensure that the level of the anode (lower) buffer does not come above buffer seal when the separation unit is fully loaded. Remove any excess anode buffer from the upper reservoir. Ensure that the level of cathode buffer is not above the air vents in the upper reservoir. |
| Dye front is irregular | The top surface of the gel has been damaged during application of the IPG strip. | Take care during application of the IPG strip that neither gel is damaged. |
| | Bubbles or liquid between the gel and the glass plate. | Use the roller to remove any bubbles or excess liquid between the gel and the glass plate. Ensure that no visible bubbles remain and that the gel adheres firmly to the glass and resists movement. |
| | Interfering substances in the first dimension. | Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. Modify sample preparation to limit these contaminants. See *2-D Electrophoresis Using Immobilized pH Gradients — Principles and Methods* (80-6429-60). |
| Pronounced downward curving of the dye front on one side of the gel | There is an unfilled gap between the gel and one of the spacers. | When sealing the IPG strip into place, ensure that some of the agarose sealing solution flows down any gap that may exist between the gel and the spacer. |

# **Troubleshooting** (continued)

| symptom | possible causes | possible solutions |
|---|---|---|
| **Stained gels** | | |
| Protein spots are diffuse or broader than usual | | Use only the highest-quality reagents. |
| | | Make sure that polymerization is complete. |
| | | Check equilibration time of IPG strips. Too long can lead to diffusion, and too short can lead to incomplete equilibration. |
| | | Make sure the IPG strip rests on the slab gel surface without damaging. |
| | | Problems with first dimension—see troubleshooting guides for IPGphor or Multiphor™ units, or *2-D Electrophoresis: Principles and Methods.* |
| Protein spots are poorly resolved | | Allow gel to polymerize completely. |
| | | Begin electrophoresis as soon as the IPG strips are loaded, to prevent diffusion of low-molecular-weight proteins. |
| | | Running too fast. Reduce the power, current, or voltage. |
| | | Reduce the temperature setting. |
| | | Problems with the first dimension. |
| Protein spots are near the buffer front | | Pore size of the gel is too large. Increase the %T. |
| | | Proteins degraded during sample preparation. Add protease inhibitors during sample preparation. |
| | | Check the pH of the 4× gel buffer. It should be pH 8.8. Proteins will migrate faster below pH 8.8. |
| Protein spots have not entered the gel when buffer front has reached the bottom of the gel | | The gel pore size is too small. Decrease the %T. |
| | | Check the pH of the 4× gel buffer. It should be pH 8.8. Proteins will migrate more slowly above pH 8.8. |
| Protein spots are at both extremes but not in center | | The molecular weight range of the sample requires an acrylamide concentration gradient to resolve the full range of proteins. |
| Vertical protein streaks | IPG strip not properly placed on gel surface. | Make sure IPG strip uniformly contacts the gel surface along its entire length. Avoid gouging the surface of the separating gel. |
| Spots skewed or distorted | Gels run too fast—uneven migration. | Run at a lower power setting. Use a two-step program: Start at a low power setting until the proteins enter the gel, then increase the power for the remainder of the run. |
| | Uneven gel surface. | Overlay the running gel with water-saturated butanol before polymerization begins to avoid forming an uneven gel surface. |
| | | Uneven gel polymerization or gradient formation. |
| Heavy background after silver staining | | Use reagents of the highest purity, preferably electrophoresis grade. |
| | | Use deionized, double-distilled water. |

# **Troubleshooting** (continued)

| symptom | possible causes | possible solutions |
|---|---|---|
| Distortion in the 2-D pattern | Bubbles between the gel and the glass plate. | Use the roller to remove any bubbles or excess liquid between the gel and the glass plate. |
| | Liquid between the gel and the glass plate. | Ensure that no visible bubbles remain and that the gel adheres firmly to the glass and resists movement. |
| | Interfering substances in the first dimension. | Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. These distortions can result in disturbances in the second-dimension. |
| Vertical gap in the 2-D pattern | Bubble between IPG strip and top surface of second dimension gel. | Ensure that no bubbles are trapped between the IPG strip and the top surface of the second-dimension gel. |
| Vertical streaking | Incorrectly prepared equilibration solution. | Prepare equilibration solution according to instructions. |
| | Poor transfer of protein from IPG strip to second dimension gel. | Use low power for sample entry phase. Extend entry phase if necessary. |
| | Insufficient equilibration | Prolong equilibration time. |
| Spots are vertically doubled, or “twinned” | IPG strip is not placed properly. | Ensure that the plastic backing of the IPG strip is against the glass plate of the second-dimension cassette. |
| Poor representation of higher-molecular-weight proteins | Incorrectly prepared equilibration solution. | Prepare equilibration solution according to instructions. |
| | Poor transfer of protein from IPG strip to second dimension gel. | Use low power for sample entry phase. Extend entry phase if necessary. |

# Care and maintenance

## Cleaning

For day-to-day operation of the unit, the cleaning procedure outlined in unit operation—thoroughly rinsing the separation tank with distilled or deionized water—is sufficient. If desired, the internal components of the separation unit can be removed for a more thorough cleaning (see below). The unit can also be periodically cleaned with a dilute solution of a mild detergent.

Clean the gel casting cassettes and pre-cast gel cassettes with a dilute solution of a laboratory cleanser such as RBS-35, from Pierce Chemical Company. Rinse the cassettes thoroughly with distilled or deionized water.

- DO NOT autoclave or heat any part above 50 ºC.
- DO NOT expose the unit or its parts to organic solvents, including >20% ethanol
- If using radioactive reagents, decontaminate the unit with a cleaning agent such as CONTRAD 70 or Decon 90 from Decon Laboratories, Inc.

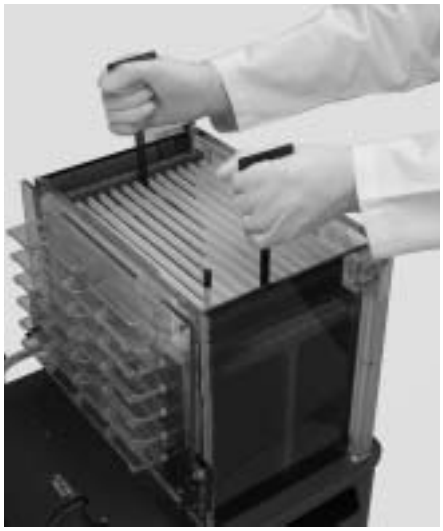


**Fig 33.** Using the buffer seal removal tools.

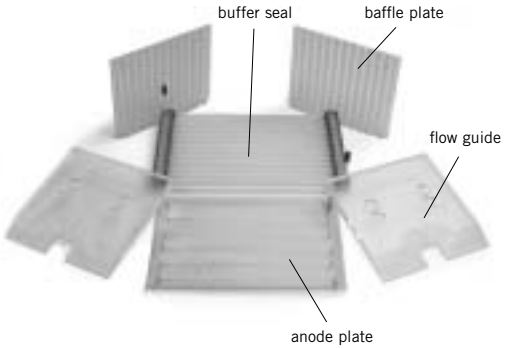



**Fig 34.** Internal components.

## Replacing internal components

To remove the anode plate or any of the other internal components, follow the directions below.

❶

Insert the buffer seal removal tools through the fifth or sixth slot, with the wider end underneath the tubes. Turn the tools 90º and place as close to the end blocks as possible. Carefully pull upward on the buffer seal until it is removed from the unit (see Fig 33).

❷

Slide the two baffle plates upward and out of the unit.

❸

The two flow guides are removed by pulling out the retaining pins and lifting the blocks out.

❹

The anode plate then can be removed by unscrewing the flathead nylon screw and lifting the plate out.

❺

To reassemble the unit, replace the anode plate and screw, making sure that the sealing sleeve is in place. Spread a small amount of GelSeal compound on the plug with a swab applicator to prevent corrosion.

❻

Replace the flow guides and baffle plates.

❼

Put a light film of GelSeal compound on the gasket of the buffer seal and reinsert it into the unit using even pressure. Make sure that it is fully seated before using the unit.

# 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 Amersham Biosciences Technical Support.

***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 | code number |
|---|---|
| **Ettan DALT*twelve* Electrophoresis Unit and Power Supply/Control Unit** | |
| 115 V~ | 80-6466-46 |
| 230 V~ | 80-6466-27 |
| Replacement Lid | 80-6473-11 |
| Replacement Buffer Seal | 80-6473-30 |
| Buffer Seal Removal Tool (2/pkg) | 80-6474-63 |
| Replacement Baffle Plate | 80-6473-49 |
| Replacement Flow Guide | 80-6473-68 |
| Replacement Anode Plate | 80-6473-87 |
| **Ettan DALT Cassettes** | |
| Pre-cast Gel Cassette | 80-6466-65 |
| Gel Casting Cassette, 1.0 mm | 80-6466-84 |
| Gel Casting Cassette, 1.5 mm | 80-6488-69 |
| Blank Cassette Insert | 80-6466-03 |
| Cassette Removal Tool (2/pkg) | 80-6474-82 |
| **Ettan DALT Pre-cast Gels** | |
| Pre-cast Gel, 12.5% (6/pkg) | 17-6002-36 |
| Buffer Kit (one run of 12 gels) | 17-6002-50 |
| **Ettan DALT*twelve* Gel Caster** | |
| Complete with Separator Sheets (16 pcs) and Filler Sheets (6 pcs) | 80-6467-22 |
| Separator Sheets (16/pkg) | 80-6467-41 |
| Filler Sheets (6/pkg) | 80-6467-60 |
| Black-Knobbed Screws (4/pkg) | 80-6437-58 |
| Triangular Sponge | 80-6474-06 |
| Acrylic Feed Tube | 80-6437-20 |
| Foam Sealing Gasket | 80-6023-76 |
| Silicon Tubing Set, two pieces/pkg: 9 mm o.d., 178 mm long; and 12.5 mm o.d., 16 mm long | 80-6437-39 |
| Replacement Tilt Leg with Nylon Screw | 80-6474-25 |
| Replacement Faceplate | 80-6474-44 |

| product | code number |
|---|---|
| **DALT Gradient Maker with Peristaltic Pump** | |
| 115 V~ | 80-6067-65 |
| 230 V~ | 80-6067-84 |
| Gradient Maker Gasket/Divider | 80-6068-41 |
| Gasket Adjuster Rod | 80-6068-60 |
| Knobs (4/pkg) | 80-6437-58 |
| Plastic Feed Tubing (1.8 m) | 80-6068-03 |
| Bow-Tie Mixer Kit | 80-6068-22 |
| Acrylic Feed Tube | 80-6437-20 |
| **Accessories** | |
| *2-D Electrophoresis: Using Immobilized pH Gradients* | 80-6429-60 |
| Cassette Rack (2/pkg) | 80-6467-98 |
| Equilibration Tubes (12/pkg) | 80-6467-79 |
| Stainless Steel Staining Tray Set | 80-6468-17 |
| GelSeal, 1/4 oz. tube | 80-6421-43 |
| Roller | 80-1106-79 |
| Fluorescent Rulers (2/pkg) | 80-6223-83 |
| Wonder Wedge Plate Separation Tool | 80-6127-88 |
| **PlusOne Electrophoresis Chemicals and Reagents** | |
| Urea, 500 g | 17-1319-01 |
| Dithiothreitol (DTT), 1 g | 17-1318-01 |
| Bromophenol Blue, 10 g | 17-1329-01 |
| Glycerol (87%), 1 l | 17-1325-01 |
| Acrylamide IEF (acrylic acid <0.002%), 1 kg | 17-1300-02 |
| Acrylamide IEF 40% solution, 1 l | 17-1301-01 |
| N,N',-Methylene bisacrylamide, 25 g | 17-1304-01 |
| N,N',-Methylene bisacrylamide 2% solution, 1 l | 17-1306-01 |
| Agarose IEF, 10 g | 17-0468-01 |
| N,N,N',N',-tetramethylethylenediamine (TEMED), 25 ml | 17-1312-01 |
| Ammonium Persulphate (APS), 25 g | 17-1311-01 |
| Tris, 500 g | 17-1321-01 |
| Glycine, 500 g | 17-1323-01 |
| Sodium Dodecylsulphate (SDS), 100 g | 17-1313-01 |
| Silver Staining Kit, Protein | 17-1150-01 |
| **Molecular Weight Markers** | |
| MW Range 2 512–16 949, 2 mg/vial, 1 vial | 80-1129-83 |
| MW Range 14 400–94 000, 575 mg/vial, 10 vials | 17-0446-01 |
| MW Range 53 000–212 000, 175 mg/vial, 10 vials | 17-0615-01 |

**Notes:**

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