Ettan Dalt*six* Electrophoresis System

second-dimension gel electrophoresis

um 80-6492-49/Rev. AD/08-02

Amersham Biosciences

Page finder

Ettan DALT*six* System components

- Six slot vertical slab electrophoresis unit
- Gel caster
- Gel casting cassettes
- Gradient maker

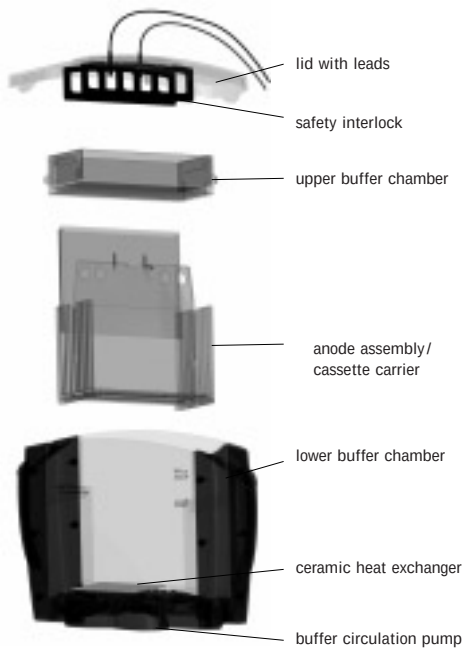


Fig 1. Exploded view of Ettan DALT*six* Electrophoresis Unit.

Fig 2. Ettan DALT*six* Gel Caster for casting 1.0 and 1.5 mm large format gels.

Ettan DALT*six* Electrophoresis System function and description

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

Electrophoresis Unit

The Ettan DALT*six* Electrophoresis Unit accommodates up to six 25.5 × 20.5 cm slab gels, either 1 mm or 1.5 mm thick, in a common tank under identical conditions. A sample, focused in an IPG strip, is placed on the cathodic (upper) surface of a slab gel and sealed in place with agarose. Up to six gel cassettes are inserted into the electrophoresis unit and any unused slots are filled with blank cassette inserts. The Upper Buffer Chamber/Buffer Seal (UBC) is pushed down over the cassettes, holding the gel cassettes in slots flanked by a double rubber gasket.

Power is supplied to the unit by an external source such as an EPS 601 Power Supply. The unit is capable of handling 600 V, 400 mA, or 100 W.

A pump mounted under the lower buffer chamber circulates the buffer, pumping it up into the chamber on the right-hand side, between the cassettes, down the left side, and over the internal heat exchanger before returning to the pump. The pump starts when it is plugged in. For temperature control, the heat exchanger located in the bottom of the unit must be connected to a MultiTemp™ III or similar circulating water bath.

Gel Caster

The Ettan DALT*six* Gel Caster holds up to six 1-mm or 1.5-mm gel cassettes, with separator sheets, for casting homogenous or gradient gels. When desired, fewer gels can be cast at one time by using blank cassette inserts to occupy any unneeded volume. The removable faceplate and separator sheets simplify loading and unloading the casting unit. The groove in the back of the caster provides the channel through which the gel solution is poured into the caster unless gradients are being the cast by displacement through the lower tubing fitting.

Fig 3. Gel Casting Cassettes.

Fig 4. Ettan DALT*six* Gradient Maker.

Gel Casting Cassettes

The Ettan DALT*six* System uses the same cassettes as the Ettan DALT*twelve* Electrophoresis System (see Fig 3). 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 dish-washer. 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.

Gradient Maker

The Ettan DALT*six* Gradient Maker is designed for producing linear gradients of aqueous solutions ranging in volume from 200–1 000 ml. The gradient maker can be used to form convex and concave exponential gradients with the addition of a one-holed rubber stopper, a piece of rigid tubing, and a piece of flexible tubing.

Important user information

- Please read this entire manual to fully understand the safe and effective use of this product.
- ⚠ The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument.
- ⚠ The lightening symbol within an equilateral triangle is intended to alert the user to the risk of exposure to highvoltages.

Should you have any comments on this manual, we will be pleased to receive them at:

Amersham Biosciences (SF) Corp.
Marketing Department
654 Minnesota Street
San Francisco, CA 94107 USA

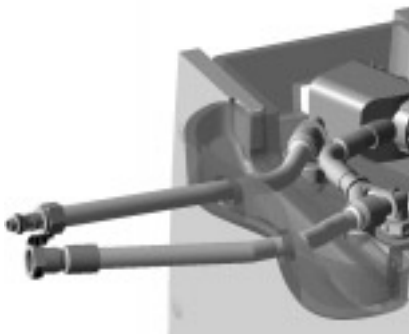


Fig 5. To connect the coolant tubing lines to the tank, remove the lid and the anode rack from the tank and lay the tank fill-label (front) side down. Guide the free ends of the coolant tubes through the holes in the tank base and slip them onto the plastic connector fittings, then pull the connector collar onto the tubing to lock it in place. The end of the tubing can be lubricated with glycerol or a mix of glycerol:water to ease the fit.

Important information

- Connect the instrument to a properly grounded electrical outlet.
- The safety lid must be firmly in place before power can be applied.
- Turn off the power to the gels before opening the safety lid.
- Do not connect the heat exchanger to a water tap or any other water source with unregulated pressure that may exceed 82 kPa (12 PSI).
- 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 electrophoresis tank is empty.
- Do not operate with buffer temperature above 40 ºC. All plastic parts are rated for 40 ºC continuous duty.
- Turn the buffer circulation pump on during electrophoresis to minimize uneven heating even if not connected to a thermostatted circulator.
- Connect the heat exchanger to an external thermostatted circulating bath. Overheating will cause irreparable damage to the unit.
- Do not autoclave or boil this unit or any of its parts.
- Use care when lifting and moving the electrophoresis unit. It is best to move the unit when empty.
- The casting unit, when filled with glass plates and gel solutions, is very heavy. Use caution when trying to move or lift the caster.
- The protection provided by the equipment may be impaired if this equipment is used in a manner not specified by the manufacturer.
- Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining and servicing this product.

Unpacking, inventory, and set-up

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.

Heat exchanger

The heat exchanger is built into the base of the separation tank. The white alumina ceramic heat exchange surface is *fragile* and the user should avoid dropping anything directly on surface.

When connected to a circulator bath, coolant passes through a serpentine chamber beneath the ceramic plate. The thin ceramic permits rapid heat exchange between the coolant and the buffer in the electrophoresis tank. The ceramic plate is attached with silicone rubber adhesive. The heat exchanger tubing connectors are 13-mm o.d.

The heat exchanger is rated to a maximum of 0.8 atmospheres above ambient (12 psig). Do not connect it to a water tap. Connect only to coolant sources with regulated pressure.

Deutsch

Wichtige benutzerinformationen

- Für ein vollständiges Verständnis und eine sichere Handhabung dieses Produktes ist es notwendig, daß der Benutzer dieses Handbuch vollständig durchliest.
- ⚠ Ein Ausrufezeichen in einem gleichseitigen Dreieck soll den Benutzer auf die Anwesenheit wichtiger Betriebs und Wartungsanweisungen in der dem Gerät beiliegenden Dokumentation hinweisen.
- ⚠ Ein Blitzsymbol in einem gleichseitigen Dreieck soll den Benutzer auf die Gefahr anliegender Hockspannungen hinweisen:

Wenn Sie Anmerkungen zu diesem Handbuch haben, dann senden Sie diese bitte an:

Amersham Biosciences (SF) Corp.
Marketing Department
654 Minnesota Street
San Francisco, CA 94107 USA

Amersham Biosciences behält sich das Recht vor, die Spezifikationen ohne vorhergehende Ankündigung zu ändern.

Français

Renseignements importants d'utilisation

- Pour une compréhension totale d'utilisation de ce produit en conditions de sécurité maximale, il convient de lire entièrement ce manuel.
- ⚠ Dans la documentation qui accompagne le produit, un point d'exclamation dans un triangle équilatéral a pour but d'attirer l'attention de l'utilisateur sur des instructions importantes de fonctionnement ou d'entretien.
- ⚠ Le symbole de l'éclair dans un triangle équilatéral a pour objet d'attirer l'attention de l'utilisateur sur un danger d'exposition à de hautes tensions.

Tous vos commentaires sur ce manuel sont les bienvenus et nous vous serions gré de les adresser à:

Amersham Biosciences (SF) Corp.
Marketing Department
654 Minnesota Street
San Francisco, CA 94107 USA

Amersham Biosciences se réserve le droit d'effectuer des modifications de ces spécifications sans aucun préavis.

Wichtige informationen

- Schliessen Sie das Gerät ausschliesslich an eine geerdete Steckdose an.
- Der Sicherheitsdeckel muss korrekt sitzen bevor die elektrische Spannung angelegt werden kann.
- Schalten Sie die elektrische Verbindung zu den Gelen ab bevor Sie den Sicherheitsdeckel öffnen.
- Schliessen Sie den Wärmetauscher niemals an eine Wasserleitung oder eine unkontrollierbare Druckquelle an, welche 82 kPa (12 PSI) übersteigen kann.
- Vor und nach jedemGebrauch: Waschen und spülen Sie den Puffertank und das Pumpensystem mit destilliertem oder entionisiertem Wasser.
- Vor Reinigung oder Reparatur der Kammer: Entfernen Sie stets das Stromkabel vom Netz.
- Betreiben Sie die Pumpe nie bei leerem Puffertank.
- Verwenden Sie das Gerät nie mit einer Puffertemperatur über 40 ºC. Alle Plastikteile sind für Temperaturen unter 40 ºC spezifiziert.
- Lassen Sie während der Elektrophorese die Pufferumwälzpumpe auch dann laufen, wenn die Kammer nicht an einen Kühlthermostaten angeschlossen ist: Dies minimiert eine ungleichmässige Temperaturverteilung.
- Verbinden Sie den Wärmetauscher mit einem externen Kühlthermostaten. Überhitzung verursacht eine irreparable Schädigung des Gerätes.
- Autoklavieren Sie niemals das Gerät oder Teile davon.
- Üben Sie Vorsicht wenn Sie das Gerät anheben und transportieren. Am besten transportieren Sie das Gerät nur im leeren Zustand.
- Die Gelgiesseinheit ist sehr schwer, wenn sie mit Glasplatten und Gellösung gefüllt ist. Üben Sie Vorsicht, wenn Sie versuchen die Giesseinheit anzuheben oder zu transportieren.
- Der Sicherheitsschutz des Gerätes kann unter Umständen nicht funktionieren, wenn das Gerät in einer Weise benützt wird, für die es vom Hersteller nicht spezifiziert wurde.
- Nur Originalzusatzteile und Ersatzteile, die vom Amersham Biosciences genehmigt oder geliefert wurden, dürfen zum Betrieb, zur Erhaltung und zur Reparatur dieses Produktes verwendet werden.

Renseignements importants d'utilisation

- Brancher l'appareil à une prise de courant électrique correctement reliée à la terre.
- Le couvercle de sécurité doit être parfaitement en place avant de mettre l'appareil sous tension.
- Eteindre le générateur et débrancher les prises avant d'enlever le couvercle de sécurité
- Ne pas connecter l'échangeur de chaleur (vertical) à circulation d'eau à un robinet ou quelque source d'eau dont la pression est non regulée et pourrait dépasser 82 kPa (12PSI).
- Rincer et vider la cuve d'électrophorèse et le système de pompage avec de l'eau distillée ou déionisée avant et après utilisation.
- Débrancher toujours la prise avant tout entretien.
- Ne pas allumer la pompe de circulation lorsque la cuve d'électrophorèse est vide
- Ne pas utiliser avec un tampon à une température au dessus de 40 °C. Toutes les pièces en plastique sont prévues pour résister à une température constante de 40 °C.
- Allumer la pompe de circulation du tampon pendant l'électrophorèse afin de réduire au minimum un échauffement non uniforme, même si non reliée à un bain à circulation d'eau et à thermostat.
- Relier l'échangeur de chaleur (vertical) à circulation d'eau à un bain à circulation d'eau et à thermostat. La surchauffe endommagera l'unité de façon irréparable.
- Ne pas autoclaver ou faire bouillir cet appareil, ni aucunes de ses pièces détachées.
- Soulever et déplaçer l'unité d'électrophorèse avec beaucoup de soin. Il vaut mieux déplacer l'unité une fois vide. L'unité de coulage des gels, une fois remplie de plaques de verre et de solutions de gel, est très lourde. Soulever et déplacer l'unité de coulage des gels avec beaucoup d'attention.
- La protection fournie par cet équipement peut etre altérée si l'équipement est utilisé d'une façon non indiquée par le constructeur.
- Seuls les accessoires et piéces detachées approuvés ou fournis par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

Specifications

Ettan DALT*six* Electrophoresis Unit

Maximum voltage	600 V DC
Maximum current	400 mA
Maximum power	100 W
Gel capacity	6 gels
Electrophoresis buffer volume	5.5 l
Dimensions (h × w × d)	40.3 × 54.2 × 16.0 cm
Weight (empty)	11.1 kg
Maximum temperature	40 °C
Environmental operating conditions	Indoor use, 4–40 °C Humidity up to 90% Altitude to 2 000 m
Installation category	II
Pollution degree	2
115 V~ model	104–127 VAC, 60 Hz, 8 W
230 V~ model	207–264 VAC, 50 Hz, 7 W
Product and safety certifications	EN61010-1, UL3101-1, CSA22.2 1010.1, EMC EN61326, CE marked

Ettan DALT*six* Gel Caster

Gel capacity	6 gels, 1.0 mm or 1.5 mm thick
Acrylamide solution volume (total)	425 ml for 1.0 mm thick gels, 550 ml for 1.5 mm thick gels
Dimensions (h × w × d)	27 × 37 × 8 cm
Weight (empty)	3.6 kg

1.0 mm Gel Casting Cassette

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

1.5 mm Gel Casting Cassette

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

Ettan DALT*six* Gradient Maker

Volume	1 000 ml
Weight	0.73 kg

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.

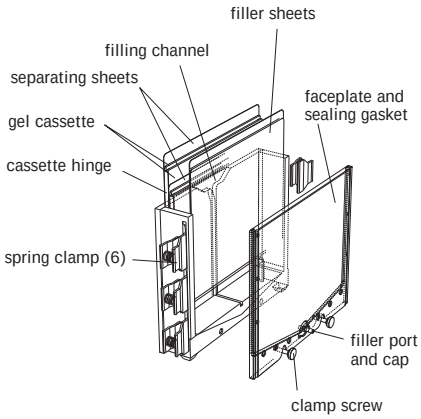


Fig 6. Gel caster with parts labeled.

Preparing the Ettan DALT*six* Gel Caster

Set up the gel caster near a sink, in a tray, or on a drainboard so that any liquid that overflows, spills, or drains out of the unit during pouring or disassembly can be easily contained.

The Ettan DALT*six* Gel Caster accommodates up to six 1-mm or 1.5-mm gel cassettes with separator sheets (0.5 mm) between them. If you are not planning to cast a full set of gels, use the blank cassette inserts (purchased separately) with separator sheets between them 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 lay the caster on its back. If casting gels by the displacement method, remove the triangular wedge in the V-shaped base.

❷

Fill the gel caster starting with a separator sheet against the back wall. The separator sheets make it easier to remove the cassettes 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 matching ends of the cassette down. End with a separator sheet then use the thicker filler sheets (1.0 mm) to bring the level of the stack of cassettes and spacers even with the edge of the caster.

❸

Remove the gray foam seal from the groove in the faceplate and lubricate it with a light coating of GelSeal compound to help ensure a liquid-tight seal. Place the gasket back in the groove on the faceplate. Avoid stretching the gasket by seating it from the ends first, working toward the middle.

❹

Turn two black knobbed screws into the two 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. Clamp both sides of the faceplate with six spring clips and tighten the screws. Be sure the sealing gasket is compressed evenly by the faceplate and forms a tight seal with the caster. Do not overtighten the screws.

❺

If casting using the filling channel in the back plate of the caster, be sure to plug the barbed fitting in the faceplate. If casting using the displacement method, attach the tubing from the peristaltic pump to the barbed fitting.

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.

Casting homogeneous gels

❶

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

❷

Prepare a sufficient volume of gel overlay solution (water-saturated *n*-butanol). You need 1.0 ml of overlay for each 1-mm cassette and 1.5 ml for overlay for each 1.5-mm cassette. Mixing 1-mm and 1.5-mm cassettes, in one casting is not advised.

❸

If casting using the displacement method, make up 200 ml of displacing solution (0.375 M Tris-Cl pH 8.8, 30% (v/v) glycerol, bromophenol blue).

❹

For a full 6-gel, 1.5-mm cassette set, make up 600 ml of acrylamide gel stock solution without ammonium persulfate (APS) or N,N,N',N'-tetramethylethylenediamine (TEMED). For a full 6-gel, 1-mm cassette set, make up 450 ml of acrylamide gel stock solution as above. This amount of gel solution will provide you with sufficient volume to cast gels using either the filling channel or a peristaltic pump.

❺

Assemble the gel caster as described in the proceeding section; the caster should be placed on a level bench or on a leveling table so that gel tops are level.

❻

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.

❼

Pour the gel solution into the filling channel. If displacement casting is being used, the flow rate on the peristaltic pump should be increased slowly to the desired speed to avoid introducing any air bubbles.

❽

For displacement casting, pump the gel solution into the caster until it is about 7–11 cm below the final desired gel height. Stop the flow of acrylamide and transfer the feed tube to the container holding the displacement solution. Restart the pump to displace the gel solution in the tubing and the V-trough at the bottom of the caster. The remaining acrylamide solution is forced into the cassettes to the final gel height. When the final desired gel height is reached, stop the pump and clamp off the tubing near the barbed fitting at the bottom of the caster.

For filling channel casting, pour the gel solution into the channel until the solution reaches the final desired height, about 1 cm below the top edge of the short plate.

The amount of gel solution required in either case will be ~550 ml for six 1.5-mm gels and ~425 ml for six 1-mm gels.

❾

Immediately pipette the water-saturated *n*-butanol onto each gel. Avoid wetting adjacent plastic surfaces of the caster with *n*-butanol.

❶

Allow the homogeneous gels to polymerize for at least one hour before disassembling the caster. If the peristaltic pump outlet tubing was connected directly to the caster, it should not be removed until polymerization is complete. Do not leave the water-saturated *n*-butanol on the gels overnight as long term exposure of acrylic to butanol will damage the caster.

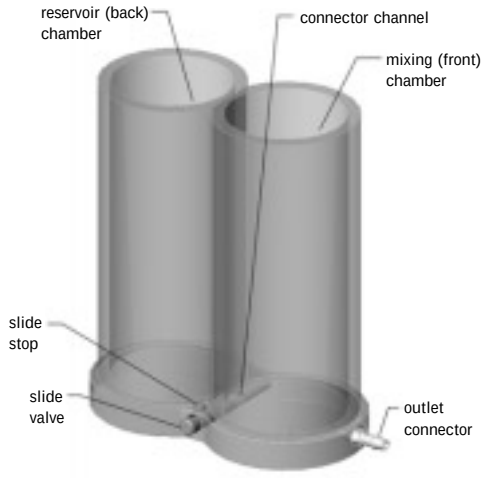


Fig 7. DALT*six* gradient maker.

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.

Casting gradient gels

Preparation for gradient gel casting

Successful gradient gel casting requires planning, timing, and practice. A full cast with the Ettan DALT*six* Gel Caster requires 420–550 ml 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, or the cassettes and separators in the gel caster. To familiarize yourself with the gel caster and gradient maker before casting gels, you should set up the unit and carry out a practice gradient cast, substituting water for the appropriate volume of light solution, and a mixture of glycerol and water for the appropriate volume of heavy solution.

A pore gradient gel results from mixing varying proportions of two solutions of different acrylamide concentrations and densities, a light solution and a heavy solution. The heavy gel solution contains glycerol and a higher concentration of acrylamide. During the gradient pouring procedure, the mixing ratio of high concentration acrylamide solution to low concentration solution gradually changes, with the heavier (high concentration) solution sinking below the light solution. This generates a downward gradient of increasing acrylamide (gel) percentage.

Gradient casting setup

❶

Be sure the entire gel casting system is clean, dry, and free from any polymerized acrylamide. Remove the rubber insert from 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

Add a magnetic stir bar of the appropriate dimensions (20–30 mm long) to the mixing chamber and place the unit on a magnetic stirrer. If volumes will be less than half the capacity of the unit, an identical stir bar should be placed in the reservoir chamber as well to balance the displacement and prevent backflow into the reservoir when the chambers are first connected.

❸

Connect tubing to the outlet connector and pump, and adjust pump speed, if used. Connect the tubing to the gel casting unit.

❹

Close the connecting valve between the reservoir (heavy) and mixing (light) chambers and clamp off the exit line.

❺

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

❻

Make up 200 ml of displacing solution.

❼

Make up the gel acrylamide solutions from the stock mixes, but do not add the 10% ammonium persulfate (APS) and 10% N,N,N',N',-tetramethylethylenediamine (TEMED). See “Gradient gel solutions” on page 16.

Pouring gel solutions for gradient gels

❶

Prepare the gel caster, as described on page 6, and place identifying gel labels inside 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

Close the slide valve (out on side of white slide stop button, Fig 8). If the outlet tubing is not controlled by a pump, clamp it off near the gradient former. Add the required volume of the final solution to the reservoir (back) chamber.

❹

Carefully open the slide valve (valve in on button side) and allow just enough solution to flow through the connector channel to fill it to the edge of the mixing chamber, then close the valve. Be sure no large bubbles remain to obstruct flow through the channel.

❺

Add the required volume of the light solution to the mixing chamber and start the magnetic stirrer.

❻

Simultaneously, open the outlet tubing if clamped off, and start the pump. Adjust the pump rate so that the solution is not forced up in a “fountain” that mixes with the overlying solution.

❼

Immediately open the connecting valve between the mixing and the reservoir chamber.

❽

Watch the gradient solution enter the caster. It is important that no bubbles disturb the gradient—watch the delivery carefully.

❾

Just as the last of the gradient mix is pumped out of the mixing chamber, add 200 ml of displacement solution to the mixing chamber and pump it through until almost all of the gradient mix has entered the cassettes. It is convenient to include a dye in the displacement solution to visually track the boundary between the gradient mix and the displacement solution.

❶

When the gels have reached the desired height and before air is introduced, stop the peristaltic pump. It is important that no air bubbles disturb the gradient.

⓫

Immediately pipette the water saturated *n*-butanol onto each gel. Avoid wetting adjacent plastic surfaces of the caster with *n*-butanol.

⓬

Allow the gradient gels to polymerize for 2 hours before disassembling the caster. Gradient gel polymerization should proceed from the top down. If the peristaltic pump outlet tubing was connected directly to the caster, it should not be removed until polymerization is complete. Do not leave the water-saturated *n*-butanol on the gels overnight as long term exposure of acrylic to butanol will damage the caster.

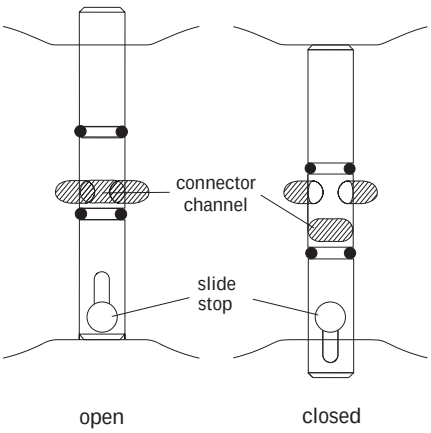


Fig 8. Open and closed positions of slide valve.

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 and spring clamps.

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 *n*-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 acceptable 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.

Using Ettan DALT*six* for electrophoresis

The unit should be placed close to a sink for easy rinsing and draining. The tubing leading to and from the heat exchanger should be connected to a circulating water bath such as the MultiTemp III; the heat exchanger should not be connected to a water tap or any other coolant supply that lacks pressure regulation. An EPS 601 Power Supply should be placed conveniently near the electrophoresis unit.

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

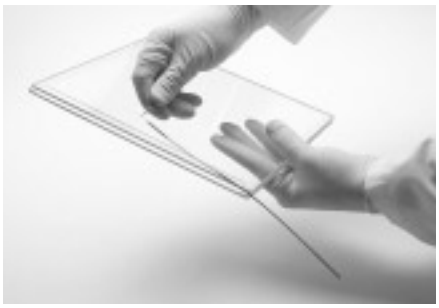
Prepare SDS equilibration buffer. Just prior to use, add DTT to the buffer to a concentration of 1% (w/v).

❷

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

❸

Add 10–15 ml of the DTT-containing solution to each tube. Typically, two 18 cm strips can be equilibrated with 10 ml of buffer or two 24 cm strips can be equilibrated with 15 ml of buffer.



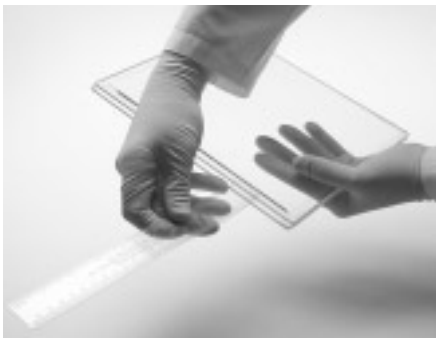


Fig 9A and B. IPG strip are loaded onto cassette and slid into place.

Fig 10. Adding agarose overlay.

❹

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

❺

Second equilibration. Prepare SDS equilibration buffer with iodoacetamide added to 2.5 % (w/v) and repeat steps 3 and 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, short glass plate side up.

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 exposed top part of the long gel plate until the strip is completely on the glass plate and centered. 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. The strip should just rest on the surface of the gel. Avoid trapping air bubbles between strip and the slab gel or 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. (See Fig 9).

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 µg of each component for Coomassie blue staining and 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 or trap bubbles. It will flow down between the glass plate and the support film and seal the IPG strip in place (see Fig 10). 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.

Unloading the gels and cleaning the unit

There are three alternatives for removing the completed gels from the electrophoresis unit:

Alternative A:

1. Without removing the UBC, carefully lift the anode assembly out of the unit.
2. Carefully pour off the upper buffer then remove the UBC.
3. Remove the gel cassettes from the anode assembly.
4. Open the gel cassettes using a Wonder Wedge (80-6127-88) to separate the plates on the side opposite the hinge.
5. For lab cast gels, run an edge of the Wonder Wedge down each side of the cassette along the spacer and carefully lift the gel out of the cassette. For pre-cast gels, lift the gel out by grasping the GelBond backing.
6. Rinse all the components with distilled or deionized water. Flush the pump with distilled or deionized water by filling the unit and turning the pump on.

Alternative B:

1. Carefully pull the UBC upward using the two grooves at each end.
2. As the UBC is pulled upward make sure that the cassettes remain in the anode assembly.
3. Completely remove the UBC, then remove the gel cassettes from the anode assembly.
4. Open the gel cassettes using a Wonder Wedge (80-6127-88) to separate the plates on the side opposite the hinge.
5. For lab cast gels, run an edge of the Wonder Wedge down each side of the cassette along the spacer and carefully lift the gel out of the cassette. For pre-cast gels, lift the gel out by grasping the GelBond backing.
6. Rinse all the components with distilled or deionized water. Flush the pump with distilled or deionized water by filling the unit and turning the pump on.

Alternative C:

1. Insert the cassette removal tool carefully 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 slowly with the tool.
2. Remove all the cassettes from the unit then lift the UBC off the anode assembly.
3. Open the gel cassettes using a Wonder Wedge (80-6127-88) to separate the plates on the side opposite the hinge.
4. For lab cast gels, run an edge of the Wonder Wedge down each side of the cassette along the spacer and carefully lift the gel out of the cassette. For pre-cast gels, lift the gel out by grasping the GelBond backing.
5. Rinse all the components with distilled or deionized water. Flush the pump with distilled or deionized water by filling the unit and turning the pump on.

Preparing the electrophoresis unit

The Ettan DALT*six* electrophoresis unit requires a total volume of about 5.5 l of electrophoresis buffer to fill both the upper and lower chambers. For lab cast Laemmli gels, the upper chamber is filled with 0.8 l of 2× buffer while the lower chamber is filled with approximately 4.5 l of 1× buffer. For the Ettan DALT II Pre-cast Gels and Buffer Kit, only half of the 100× Anode (lower) Buffer is used for each run; the slightly reduced buffer concentration should not affect the run conditions. A single bottle of 10× Cathode (upper) Buffer should be diluted to a final volume of 0.8 liter with deionized water with the full amount added to the upper chamber. Please consult the user instructions for the Ettan DALT II Pre-cast Gels for detailed technical information regarding the assembly of gels in their cassettes.

❶

Insert the anode assembly into the tank so that the circulation ports are properly aligned. The anode assembly is keyed so that it may only be inserted in one orientation and the bottom edge of the assembly should fit into the slot in the bottom of the tank.

❷

Fill the electrophoresis unit with 4.0 liters of 1× SDS electrophoresis buffer and turn the pump on.

❸

Turn on the MultiTemp III, adjust the temperature to the desired setting.

❹

Insert the prepared gels into the unit. Fill any unused slots with blank cassette inserts

❺

Add 1× SDS electrophoresis buffer until the buffer level is at or just below the "LBC start fill" line marked on the unit.

❻

Carefully slide the UBC over the gels and blank cassette inserts.

❼

Fill the upper (cathode) chamber with 2× SDS electrophoresis buffer to between the fill lines (about 0.8 liters).

❽

Fill the LBC with 1× buffer to the same level as the upper chamber.

❾

Place the safety lid on the unit and begin electrophoresis.

Recommended running conditions

The maximum electrical input for the electrophoresis unit is 600 V, 400 mA, and 100 W and has a maximum operating temperature of 40 ºC. To remain below this temperature for most runs the unit should to be connected to a refrigerated water circulator. The recommended electrophoresis conditions are 2.5 W/gel for 30 min then 100 W total until the run is completed. Under these conditions, a three gel run will finish in about 3.5 hours and a six gel run will finish in 4.5 to 5 hours. With the MultiTemp III set at 10 ºC, the electrophoresis tank temperature stays at about 25 ºC. For overnight runs, the total power should be set at 1–2 W/gel.

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

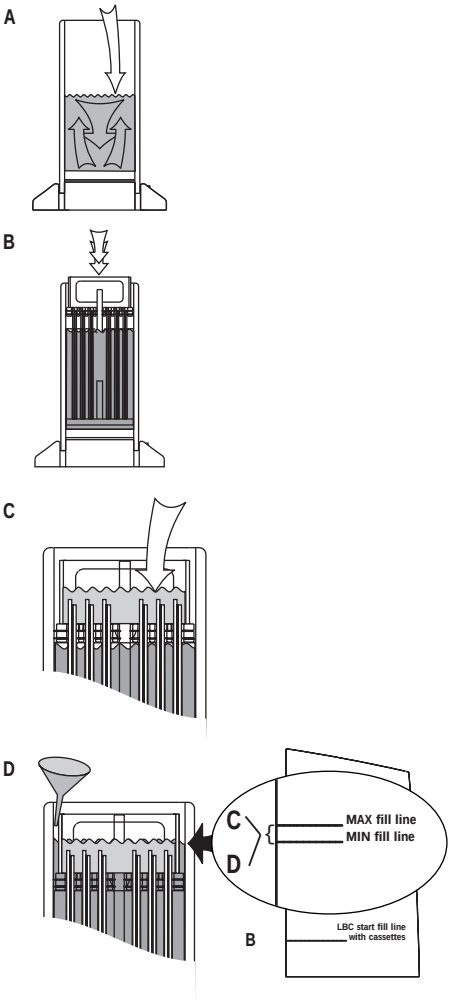


Fig 11. Filling the buffer chambers.

The unit can be assembled and filled in several ways. The standard method is described in the text on the preceding page. Another variant is illustrated above. A. Add the anode (lower) buffer (or buffer concentrate and sufficient water) to the LBC to make 4.5 l for 1 mm gels, 4 l for 1.5 mm gels. Start the pump to mix the buffer if needed. B. Insert the cassettes into the cassette carrier, seat the UBC over the gels and place the assembly in the LBC. The liquid should not be above the "LBC start fill" mark. C. In a separate container, dilute the cathode (upper) buffer concentrate to 0.8 l, mix and pour into the UBC. D. Immediately add enough anode buffer or water to the LBC to bring the level of the liquid to match the level in the UBC.

Recipes

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 3000 ml

May need filtration. Weigh acrylamide and bis under a hood; avoid contact with dust. Filter and store at 4 ºC.

1.5 M Tris-Cl, 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 3000 ml

Adjust to pH 8.8 and store at 4 ºC.

10% (w/v) SDS

	final conc.	amount
Sodium dodecylsulfate (MW 288.38)	10%	10 g
Distilled or deionized water		up to 100 ml

Store at room temperature.

10% (w/v) Ammonium persulfate

	final conc.	amount
Ammonium persulfate (MW 71.08)	10%	2 g
Distilled or deionized water		up 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 Tris-Cl, pH 8.8, 30% (v/v) glycerol, bromophenol blue, 200 ml)

	amount
Tris-Cl (1.5 M, pH 8.8)	50 ml
Glycerol	60 ml
Bromophenol blue	2 mg
Distilled or deionized water	90 ml

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

Water-saturated butanol

	amount
n, i, or *t*-butanol	50 ml
Distilled or deionized water	10 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 Tris-Cl, pH 8.8, 0.1% (w/v) SDS, 2.0 l)

	final conc.	amount
Tris-Cl (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 2 000 ml

Store at 4 ºC.

10× SDS electrophoresis buffer

(250 mM Tris, 1.92 M glycine, 1.0% (w/v) SDS, approximate pH 8.3, 20 l)

	final conc.	amount
Tris (MW 121.14)	250 mM	605.0 g
Glycine (MW 75.07)	1.92 M	2 882.0 g
SDS (MW 288.38)	1.0 % (w/v)	200.0 g
Distilled or deionized water		to 20 l

Do not adjust the pH of this solution.

SDS equilibration buffer

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

	final conc.	amount
Tris-Cl (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 ºC. This is a stock solution. Add DTT or iodoacetamide before using.

Agarose sealing solution

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

	final conc.	amount
1× SDS electrophoresis buffer (see above)		25 ml
Agarose (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

600 ml

	volume required for (ml)		
final %T	10%	12.5%	15%
Acrylamide stock	200	250	300
1.5 M Tris-Cl, pH 8.8	150	150	150
Water	237	187	137
10% SDS	6.00	6.00	6.00
10% APS	6.00	6.00	6.00
10% TEMED	1.03	0.83	0.69

450 ml

	volume required for (ml)		
final %T	10%	12.5%	15%
Acrylamide stock	150	188	225
1.5 M Tris-Cl, pH 8.8	113	113	113
Water	178	140	103
10% SDS	4.5	4.5	4.5
10% APS	4.5	4.5	4.5
10% TEMED	0.77	0.62	0.52

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 changes these 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

For six 1 mm gels:

Light solution, 210 ml

	volume (ml) required for				
final %T	**8%**	**10%**	**12%**	**14%**	**16%**
Acrylamide stock	55	68	82	95	109
1.5 M Tris-Cl, pH 8.8	52.5	52.5	52.5	52.5	52.5
Water	98	85	71	58	44
10% SDS	2.1	2.1	2.1	2.1	2.1
Glycerol	0	0	0	0	0
10% APS	2.1	2.1	2.1	2.1	2.1
10% TEMED	0.45	0.36	0.30	0.25	0.22

Heavy solution, 210 ml

	volume required for (ml)				
final %T	**12%**	**14%**	**16%**	**18%**	**20%**
Acrylamide stock	82	95	109	123	136
1.5 M Tris-Cl, pH 8.8	52.5	52.5	52.5	52.5	53
Water	55	41	27	14	0.0
10% SDS	2.1	2.1	2.1	2.1	2.1
Glycerol	18	18	18	18	18
10% APS	1.05	1.05	1.05	1.05	1.05
10% TEMED	0.09	0.08	0.07	0.06	0.06

For six 1.5 mm gels:

Light solution, 275 ml

	volume required for (ml)				
final %T	**8%**	**10%**	**12%**	**14%**	**16%**
Acrylamide stock	73	92	110	128	147
1.5 M Tris-Cl, pH 8.8	69	69	69	69	69
Water	127	109	91	73	54
10% SDS	2.75	2.75	2.75	2.75	2.75
Glycerol	0	0	0	0	0
10% APS	2.75	2.75	2.75	2.75	2.75
10% TEMED	0.59	0.47	0.39	0.34	0.29

Heavy solution, 275 ml

	volume required for (ml)				
final %T	**12%**	**14%**	**16%**	**18%**	**20%**
Acrylamide stock	110	128	147	165	183
1.5 M Tris-Cl, pH 8.8	69	69	69	69	69
Water	73	55	37	18	0
10% SDS	2.75	2.75	2.75	2.75	2.75
Glycerol	19	19	19	19	19
10% APS	1.4	1.4	1.4	1.4	1.4
10% TEMED	0.13	0.11	0.10	0.09	0.08

Troubleshooting

Electrical and mechanical

symptom	possible causes	possible solutions
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 pump off and on to purge air bubbles.
	Pump is off.	Turn on pump.
	Pump is broken.	Service call.

Gel casting

symptom	possible solutions
Gel caster leaks	Apply a light film of GelSeal compound to the foam gasket on the front plate before clamping and 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 persulfate does not crackle when water is added to it, replace with fresh reagent.
	Use fresh ammonium persulfate
	Solutions of extreme pH may not polymerize.
	Degas the monomer solution. Oxygen inhibits polymerization.
	Increase both ammonium persulfate and TEMED by 30–50%.
	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.
Vertical protein streaks	IPG strip not properly placed on gel surface. Make sure IPG strip contacts the gel surface uniformly along its entire length. Avoid gouging the surface of the separating gel.
	Include iodoacetamide equilibration step for IPG strip.
Gels cast simultaneously are different sizes	Allow the solution to settle, or reach equilibrium, before applying the overlay.
	Apply equal amounts of overlay solution to each gel.
	Apply overlay as quickly as possible.
Gradient gels—uneven layering	Add sucrose [15% (w/v)] or glycerol (25% (v/v)) to the high-percent monomer solution.
	Add a very small amount of bromophenol blue to the high-percent monomer solution to track gradient formation. Excessive bromophenol blue will inhibit polymerization.
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.
Unusually slow or fast run	Check for leaks. All plates, spacers, and gaskets must be clean, dry, and free of grease.
	Check the UBC. It should be free of nicks or tears.
	Check the pH of the buffer. If the pH is wrong, make fresh buffer, do not back-titrate.
	Check recipes, gel concentrations, and buffer dilutions. (For example, do not use Tris·HCl in place of Tris base for the electrophoresis buffer.)
	Discard older acrylamide solutions and use only reagents of highest quality.
	Only use freshly deionized urea of highest quality.
	Adjust power, current, or voltage.

Troubleshooting (continued)

Pre-cast gels

symptom	possible causes	possible solutions
Second dimension electrophoresis proceeds slowly with high current	One of the slots in the upper buffer chamber is open.	All 6 slots in the UBC should be occupied by either a gel cassette or a blank cassette.
	The UBC is damaged.	Carefully fill both buffer chambers to the same level.
	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 level of the buffer in the UBC when the electrophoresis unit is fully loaded.
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 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.
	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 spacer.
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 turn 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 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	Extend 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.

Troubleshooting (continued)

Stained gels

symptom	possible solution
Protein spots are diffuse or broader than usual	Use only 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 it.
	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.
Smeared or comet shaped spots	Check pH of cathode buffer. Should be between 8.3 and 8.8.
	Make sure that 2× Laemmli buffer is used in the upper (cathode) chamber.
	Buffer or SDS depleted.
Protein spots are near the buffer front	Buffer depleted. Check pH of upper (cathode) buffer. Should be below pH 8.3–8.8. Be sure that 2× Laemmli buffer is being used in the upper (cathode) chamber.
	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 slower 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.

Care and maintenance

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.

Cleaning

For day-to-day operation of the unit, the cleaning procedure outlined in unit operation is sufficient, thoroughly rinsing the electrophoresis tank with distilled or deionized water. If desired the unit can 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 40 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.

Replacement of components

If any of the components becomes cracked or broken it should be replaced. The upper buffer chamber (UBC) should be replaced if any of the rubber flaps become cut or torn. To prevent damage to the flaps, ensure that all sharp edges on glass plates are smoothed and exercise care when employing the cassette removal tool. With normal use the UBC ribs may become bowed upward; this bowing should not affect the performance of the seal.

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 contact your local Amersham Biosciences representative for more information.

Ordering information

product	code no.
Ettan DALT*six* Electrophoresis Unit	
115 V~	80-6485-08
230 V~	80-6485-27
Replacement Lid	80-6490-40
Replacement Upper Buffer Chamber	80-6490-78
Replacement Anode Assembly/Cassette Carrier	80-6491-35
Ettan DALT*six* and DALT II Cassettes	
Pre-Cast Gel Cassette	80-6466-65
Gel Casting Cassette, 1-mm	80-6466-84
Gel Casting Cassette, 1.5-mm	80-6488-69
Blank Cassette Insert	80-6467-03
Cassette Removal Tool (2/pkg)	80-6474-82
Ettan DALT II 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*six* Gel Caster	
Complete with Separator Sheets (7 pcs) and Filler Sheets (6 pcs)	80-6485-46
Rubber Insert	80-6493-44
Small Black Knobbed Screws	80-6493-63
Ettan DALT*six* Gradient Maker	80-6487-36
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
Funnel 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 Face Plate	80-6474-44

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	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 NA, 10 g	17-0422-01
N,N,N',N', -tetramethylethylenediamine (TEMED), 25 ml	17-1312-01
Ammonium Persulfate (APS), 25 g	17-1311-01
Tris, 500 g	17-1321-01
Glycine, 500 g	17-1323-01
Sodium Dodecylsulfate (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

Companion Products	
EPS 601 Power Supply 6 – 600 V, 1– 400 mA, 1–100 W	18-1130-02
MultiTemp III Thermostatic Circulator, 230 V	18-1102-78
MultiTemp III Thermostatic Circulator, 115 V	18-1102-77

Printed in the USA.

Amersham Biosciences
Amersham Place, Little Chalfont,
Buckinghamshire, England HP7 9NA

Amersham Biosciences AB
SE-751, 84 Uppsala, Sweden

Amersham Biosciences Corp.
800 Centennial Avenue, PO Box 1327,
Piscataway, NJ 08855 USA

Amersham Biosciences GmbH
Munzinger Strasse 9, D-79111, Freiburg, Germany

Amersham Biosciences (SF) Corp.
654 Minnesota Street, San Francisco, CA
94107 USA

www.amershambiosciences.com

Amersham
Biosciences