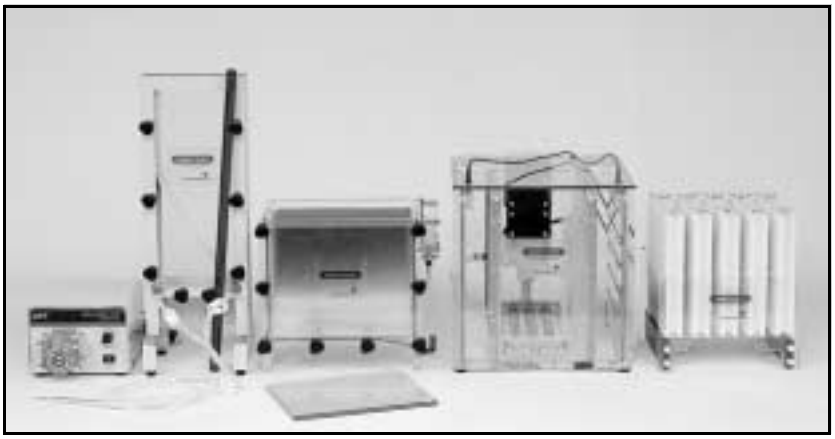
Hoefer DALT System

DALT Electrophoresis Tank

DALT Gradient Maker

DALT Multiple Gel Caster

DALT Blotting Kit



User Manual

80-6431-50 Rev A/12-98

Amersham Biosciences

Important User Information

English

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.

If you have any comments on this manual, please send them to us at:

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Renseignements importants d'utilization

Français

Pour une bonne compréhension et une utilisation en sécurité maximale, il convient de lire entièrement ce manuel.

Dans la documentation qui accompagne l'instrument un point d'exclamation dans un triangle équilatéral a pour but d'attirer l'attention de l'utilisateur sur des instructions importantes de fonctionnment ou de maintenance.

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Deutsch

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Información importante para el usuario

Español

Para comprender el producto y utilizarlo con seguridad es necesario leer este manual en su totalidad.

El signo de admiración en un triángulo equilátero en el manual, advierte al usuario sobre la presencia de instrucciones importantes de operación y mantenimiento del aparato.

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Informazioni importanti per l'operatore

Italiano

Per un utilizzo sicuro del prodotto, leggere attentamente l'intero contenuto del presente manuale.

Il punto esclamativo all'interno di un triangolo equilatero indica all'operatore la presenza di importanti istruzioni di funzionamento e manutenzione nella documentazione allegata al prodotto.

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CONTENTS

Hoefer DALT System Function and Description

In 2-D protein electrophoresis, proteins are separated first according to isoelectric point by isoelectric focusing, most reliably on immobilized pH gradient (IPG) strips. The second dimension electrophoresis separates the proteins on the basis of their molecular mass on a slab gel containing the denaturing detergent, sodium dodecyl sulfate (SDS). The Hoefer DALT System is designed to simplify the handling of multiple second dimension gels and improve the reproducibility of the second dimension separation.

DALT System Components

The Hoefer DALT System comprises:

- a multiple vertical slab electrophoresis tank
- a multiple gel caster
- a gradient maker with peristaltic pump
- gel cassettes
- a blotting kit

Electrophoresis Tank

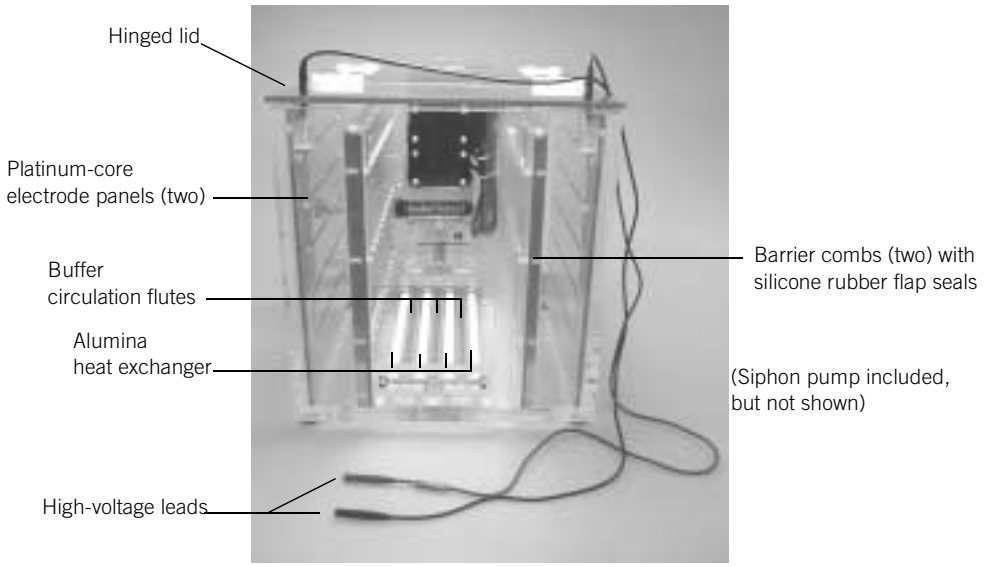


Figure 1. The DALT Electrophoresis Tank

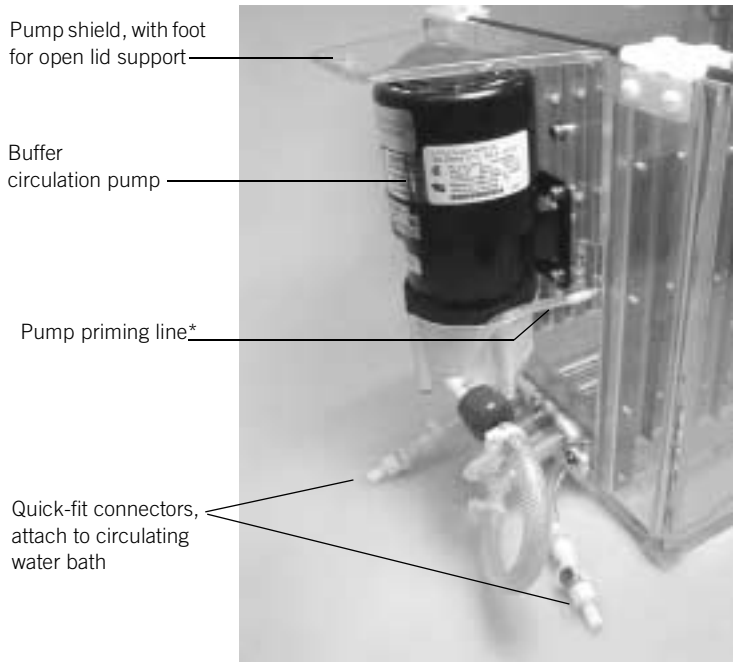
The DALT Electrophoresis Tank accommodates up to ten 23 × 19 cm slab gels for separation under identical conditions. The sample, following focusing in an IPG strip, is attached to the cathodic surface of the gel by embedding in agarose, then the gel is turned 90° and run in a "sideways" orientation, with the IPG strip standing vertically at one edge.

The tank is divided into three chambers by silicone rubber flap seals on the barrier combs. The seals are not designed to be liquid-tight but they do provide a good barrier to electrical current, causing most current to flow through the gels. Although there is

some leakage current bypassing the gels, this has little effect on system operation. The barriers can be raised or removed by loosening the nylon screws which secure them in place.

The left and right chambers contain platinum wire electrodes, cathode (–) and anode (+). The center chamber provides cooling via circulating buffer and a tube-type heat exchanger. Since the gel cassettes are almost entirely exposed to the buffer in the center chamber, with only their ends protruding into the side chambers, cooling of the center chamber buffer provides excellent temperature control of the gel slabs during the run. Electrode panels can be removed by loosening the nylon screws securing them in place. Removal is not typically necessary unless the platinum wire becomes broken and requires replacement. See “Removing the Electrode Panels from the DALT Tank” on page 55.

Figure 2. Rear view of electrophoresis tank



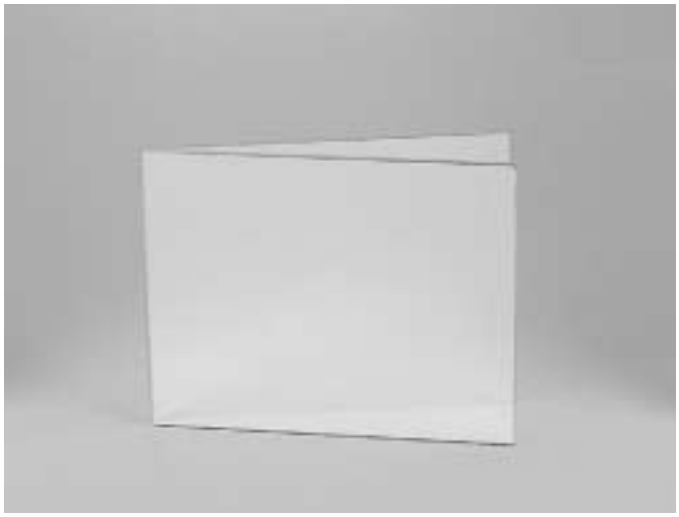
*WARNING The liquid in the tank must cover the opening to the pump priming line when the circulation pump is in operation.

Buffer moves into the pump through the middle of the three flutes running along the length of the tank’s center chamber, and is expelled back into the tank through the left and right flutes. The holes in the output flutes are aligned to generate maximum circulation over both the cassettes and the heat exchanger on the floor of the tank's centre chamber (beneath the flutes). As described on page 24, run the pump only when the tank is filled with liquid, to prevent damage to the pump.

The Quick-fit connectors on the ends of the tank port tubing provide a leak-proof seal when disengaged, eliminating spillage of coolant solution.

Gel Cassettes

Figure 3. DALT gel cassettes



The DALT gel cassettes are pre-assembled. Two glass plates are held together along one edge by a strip of silicone rubber, and the glass spacers (1.5 or 1.0 mm thick) are glued in position. To complete assembly, close the two plates like a book. Gels are removed by opening the book after the run and lifting out the slab. The cassette is easily cleaned as a unit, and can be stood upright in the open to dry. The cassettes are dishwasher-safe. Cassettes are 20.4 × 25.5 cm and produce a gel about 19 × 23 cm: 46 ml for 1-mm thick, 68 ml for 1.5-mm thick. The gels make the best use of the standard 20 × 25 cm X-ray film.

Multiple Gel Caster

Figure 4. The DALT Gel Caster

The DALT Gel Caster holds up to 25 gel cassettes, 1 mm thick, or up to 23 cassettes, 1.5 mm thick, with separating sheets, for casting homogeneous or gradient gels. Fewer cassettes can be filled at one time by inserting filler blocks to occupy unneeded volume.

A removable front plate and cassette separating sheets simplify loading and unloading cassettes from the unit. A hydrostatic balance chamber allows accurate gradient positioning and provides a means to flush the fill tubing before polymerization occurs. The chamber also provides a "make up solution" to accommodate the volume shrinkage during polymerization.

Pump-Assisted Gradient Maker

Figure 5. The DALT Gradient Maker

The DALT Gradient Maker casts gradients of arbitrary shape, up to 2.0 liters total volume. The solution is delivered at a controlled rate by a peristaltic pump. A flexible liquid-tight divider partitions the gradient maker into two chambers of complementary shapes for casting both linear and non-linear gradients. With the divider adjusting rod, you can configure the gradient divider to adapt to your gradient gel requirements.

Blotting Kit

Figure 6. The DALT Blotting Kit

The DALT Blotting Kit consists of a five-place acrylic cassette-holding rack and five transfer cassettes, complete with cassette packing material— sponges and blotting paper. The cassettes hold the gel and the transfer membrane secure, with just enough pressure to assure even transfer.

Unpacking

Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part is missing, contact your local Amersham Biosciences 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.

Rinse the DALT Tank and Gel Caster with distilled water and let air dry. Before initial use, carefully clean glass plates and spacers with a dilute solution of laboratory cleanser to remove fingerprints. Rinse thoroughly with tap and distilled water. See “Care and Maintenance” on page 55 for more detailed instructions.

Important Information

- Plug the instruments into a properly grounded outlet.
- The safety lid must be in place before connecting the power leads to a power supply.
- Turn all power supply controls off and disconnect the power leads before opening the safety lid.
- Rinse only the electrodes (not the banana plugs) with distilled water before and after use.
- Always disconnect the power cord before servicing.
- Do not operate the circulation pump if the DALT Tank is empty. The pump is not self-priming and can be damaged if run dry.
- Circulate only water or 50/50 water/ethylene glycol through the heat exchanger. Never introduce anti-freeze or any organic solvent into any part of the instrument. Organic solvents will cause irreparable damage to the unit!
- Do not connect the heat exchanger to a water tap or any coolant source where the pressure is unregulated.
- Do not operate with buffer temperature above 45 °C. All plastic parts are rated for 45 °C continuous duty.

  Circulate coolant through the heat exchanger during electrophoresis to minimize heating. Overheating will cause irreparable damage to the unit!

  For longer runs you can control heating somewhat by chilling the buffer before use, running the unit in a cold room, or both.
- Do not lift the DALT Tank by the buffer pump.
- Do not autoclave or boil this unit or any of its parts.
- The casting unit, when filled with glass plates and gel solutions, is very heavy. Use caution when trying to move or lift the casting unit.
- 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.

Informations Importantes

- Raccorder l'instrument à une prise de terre appropriée.
- Le couvercle de sécurité doit être en place avant de brancher les prises au générateur.
- Eteindre le générateur et débrancher les prises avant d'ouvrir le couvercle de sécurité.
- Rinser seulement les électrodes (pas les "banana-plugs") avec de l'eau distillée avant et après l'utilisation.
- Toujours déconnecter le cordon d'alimentation avant de réparer l'instrument.
- Ne pas faire fonctionner la pompe de circulation tampon, lorsque la cuve d'électrophorèse DALT est vide. La pompe ne s'amorce pas automatiquement et peut-être endommagée si elle tourne à sec.
- Faire circuler seulement de l'eau ou 50/50 d'eau et d'éthylène glycol dans l'échangeur vertical à cirulation d'eau. Ne jamais utiliser d'anti-gel ou tout autre solvant organique avec cet instrument. Les solvants organiques causeraient des dommages irréparables à l'appareil.
- Ne pas connecter l'échangeur vertical à circulation d'eau à un robinet ou quelque source de refroidissement dont la pression n'est pas régulière.
- Ne pas utiliser avec un tampon à une température au dessus de 45 °C. Toutes les piéces en plastique sont prévues pour résister à une température constante de 45 °C.

  Faire circuler l'eau dans l'échangeur vertical durant l'électrophorèse pour minimiser l'échauffement afin d'éviter des dommages irréparables à l'instrument.

  Pour des coulages plus long, on peut aussi contrôler la température en refroidissant le tampon avant l'utilisation et/ou en utilisant l'instrument dans une chambre froide.
- Ne pas soulever la Cuve DALT en s'appuyant sur la pompe.
- Ne pas autoclaver ou stériliser cette unité, ni aucune de ses pièces détachées.
- L'unité de coulage des gels, quand elle comprend les plaques de verre et la solution d'acrylamide, est très lourde. Prenez toute vos précautions,quand vous déplacez ou soulevez l'unité.
- Si l'instrument n'est pas utilisé en conformité avec les recommandations du fabriquant, les protections de sécurité qui équipent cet appareil peuvent être rendues inéfficaces.
- Seulement les accessoires et piéces detachées approuvés ou fournis par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

Specifications

DALT Electrophoresis Tank with Buffer Circulation Pump

Gel capacity, 1.0 or 1.5 mm thick	10 gels
Electrophoresis buffer volume	20 liters
Blotting buffer volume	22 liters
Dimensions (h × w × d)	lid closed: 38 × 48 × 33 cm (15 × 19 × 13 in) lid open: 50 × 48 × 33 cm (20 × 19 × 13 in)
Weight	16.8 kg
Maximum wattage	200 W
Maximum voltage	500 V DC
Maximum amperage	1000 mA
Maximum temperature	30 °C
Environmental operating conditions	Indoor use: 4 – 40 °C Humidity up to 90% Altitude up to 2000 m
Installation category	II
Pollution degree	2
Buffer circulation pump rate	20 liters/min
115 V~	50/60 Hz, 2.4 A, 180 W
230 V~	50/60 Hz, 1/0.83 A
Product certifications*	EN61010-1, UL3101-1, CSA C22.2 1010.1, CE

DALT Multiple Gel Caster

Gel Capacity	
1.0 mm thick	25 gels
1.5 mm thick	23 gels
Acrylamide solution volume (total)	1,800 ml
Dimensions (h × w × d)	29 × 34 ϖ 25 cm
Weight	33.5 kg

DALT Gel Cassettes

Glass cassette dimensions (w × h)	25.5 × 20.4 cm
Slab gel dimensions	1.0 mm × 23.4 × 19.5 cm 1.5 mm × 23.4 × 19.5 cm

DALT Gradient Maker

Dimensions (h × w × d)	54 × 19 × 18 cm
Maximum gradient volume	2,000 ml
Weight	5.4 kg

Peristaltic Pump for Gradient Maker

115 V~	37 W, 1.5A
230 V~	37 W, 0.9A
Weight	4.1 kg (9 lbs.)
Dimensions (h × w × d)	13.5 × 18 × 22 cm
Environmental operating conditions	Indoor use: 0 – 40 °C Humidity: 10 – 90% Altitude: up to 2000 m
Installation category	II
Pollution degree	2
Product certifications*	EN61010-1, UL508, cUL (115 V), IEC 1010 (230 V), CE

DALT Blotting Kit

Capacity	5 gels
Rack dimensions (h × w × d)	28 × 23 × 26 cm
Weight	3.2 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 of products recommended or approved by Amersham Biosciences.

Required or Convenient, But not Supplied

Refrigerated Cooling Bath

DALT Tank cooling is provided by circulating chilled liquid through the heat exchanger on the floor of the central chamber. With the aid of Quick-fit connectors, you can connect a refrigerated circulating bath, such as the MultiTemp III, to the fittings at the back of the DALT Tank. We strongly recommend active cooling for protein transfers.

Focused IPG Strips

Immobiline DrySrips provide a stable gradient pH gradient (IPG) immobilized onto an acrylamide matrix and supported by a plastic film backing. They are readily available, easy to handle and not prone to breakage and stretching. IPG strip focusing is accomplished on the IPGphor or Multiphor II with the Immobiline DryStrip tray accessory.

Power Supply

For overnight or full-day runs, a conventional electrophoresis power supply delivering 600 V at 400 mA, such as the EPS 601, is suitable. For multiple tanks or faster runs (8 – 9 h) a higher current power supply, such as the EPS 2A200, is desirable.

Transfer Membranes

The DALT Blotting kit includes 50 pieces of blotting paper. For electrophoresis tank blotting, Amersham Biosciences offers a complete line of transfer membranes, including pure nitrocellulose, supported nitrocellulose, PVDF and nylon. Choose the appropriate transfer membrane for your application.

Small Gadgets

- **A small plastic spatula or thin plastic ruler** to insert and seat IPG gels on the tops of the slab gels between the glass plates
- **Plastic-covered dish racks (Rubbermaid or equivalent)** for holding the DALT gel cassettes during the loading process
- **A microwave oven or 100 °C heating block** for preparing the agarose sealing solution
- **A Wonder Wedge (80-6127-88)** for prying open the gel cassettes after the DALT run
- Screw-cap culture tubes, 25 × 200 mm, for storing IPG strips at –40 °C
- GelSeal for lubricating the gradient gasket, to assure a leakproof seal
- **A peristaltic pump** is recommended for more rapid removal of buffer than the small siphon pump supplied with the DALT Tank. The pump can be set up to deliver used buffer that is not radioactive directly to a lab drain.
- **A vacuum pump** for degassing solutions
- Thick, latex dishwashing gloves, with textured fingers

Preparing the Gel Caster

Set up the gel caster near a sink, in a tray or container that can act as a catch basin for any liquid that may overflow the unit or empty out of it when you open it to remove the gels. Alternatively, place the unit on a plastic or rubber kitchen drain board that empties into a sink.

The DALT Gel Caster can accommodate up to twenty-five 1-mm, or twenty-three 1.5-mm gel cassettes, with separator sheets between each cassette, before the first cassette and after the last cassette. If you are not preparing the maximum number of gels, use filler blocks and separator sheets to take up the excess caster volume. Place separator sheets between the filler blocks.

Cast at least 22 cassettes for two 10-sample runs to allow for the possibility of one or two imperfect gels.

1. Check that the caster is level. Remove the front plate and rest the caster on its back, with its feet facing you. Place the triangular sponge in the base of the V-shaped feed channel.

   Plates are more easily loaded in this orientation. Check that the caster is clean, and free of dust.

2. Lubricate the foam sealing gasket with a light coating of GelSeal to help assure leak-proof sealing. Place the gasket in the groove along the front side of the caster. Avoid stretching the gasket.

3. Start filling the gel caster by placing a separator sheet against the inside wall to make it easier to remove the cassettes and filler blocks after polymerization.

   If you are casting fewer than the maximum number of gels, first add a selection of filler blocks, with a separator sheet between each block. For example, to cast twelve 1.0-mm gels, you may need four 25-mm blocks and one 3-mm block, with separator sheets between each block. To cast twelve 1.5-mm gels, you may need three 25-mm blocks, one 12-mm block, one 6-mm block, and one 3-mm block, with separator sheets between each block.

   Next add the cassettes, with all hinge strips vertical and aligned on the side of the unit opposite the feed tube. Insert a gel separator sheet between each cassette pair.

   End with a separator sheet between the final cassette and the gel caster face plate. If necessary, use additional separator sheets to bring the level of the stack even with the edge of the caster.

4. Put the removable front plate of the gel caster in place and screw on the black knurled knobs. Tighten these hand tight (not over-tight), then carefully place the gel caster in an upright position.

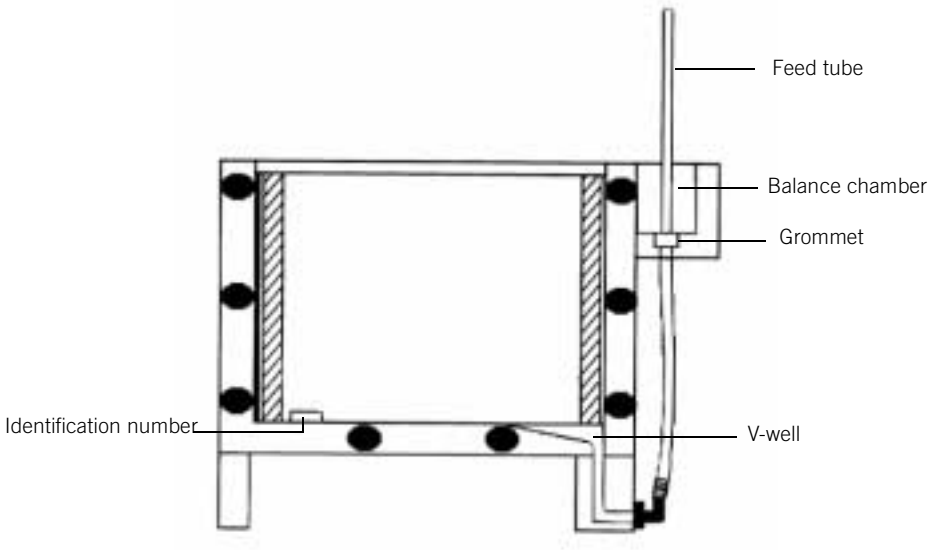
   Be sure the sealing gasket on the front plate forms a tight seal against the face plate.

5. Prepare a set of gel labels on filter paper, as described on page 53.

   Cut the labels apart, leaving little excess around the characters, and place them in order in front of the gel caster. Then, taking care to keep track of which cassette will be numbered next, drop the numbers, in order, into the cassettes, on the side opposite the gradient inlet port. As the gradient is introduced, the numbers fall to the floor of the caster but remain in the respective cassettes and ultimately are polymerized into the gels.

6. Insert the end of the plastic feed tube, supplied with the gel caster, into the grommet in the floor of the side hydrostatic balance chamber of the caster (Figure 7).

Figure 7. Attaching the feed tube to the gel caster



The feed tube must be snugly in place so that there is no leakage from the balance chamber into the caster at this point. The feed tube should be connected directly to the gradient maker tubing or to a funnel with flexible vinyl tubing.

Casting Homogeneous (Non-Gradient) Gels

WARNING Acrylamide is a neurotoxin. Always use mechanical pipettes and wear protective gloves when working with acrylamide solutions, IPG strips or surfaces that come in contact with acrylamide solutions.

1. Be sure the entire gel casting system is clean, dry, and free from any polymerized acrylamide.
2. Prepare a sufficient volume of gel overlay solution (water-saturated *n*-butanol). You need 0.75 ml of overlay for each cassette, or about 20 ml for a set of 25 cassettes.
3. Make up 200 ml of displacing solution (page 42).
4. Make up the gel acrylamide stock solution, without adding the 10% ammonium persulfate (APS) or 10% N,N,N',N',-tetramethylethylenediamine (TEMED). See "Homogeneous Gel Solutions" on page 46.
5. Load the gel caster with cassettes, separator sheets and filler blocks, if necessary. Place a gel label in each cassette. See page 12 for directions.
6. Connect the feed tube to a funnel held in a ring-stand at a level about 12 inches above the top of the gel caster (Figure 8). Insert the other end of the feed tube in the grommet in the bottom of the balance chamber.

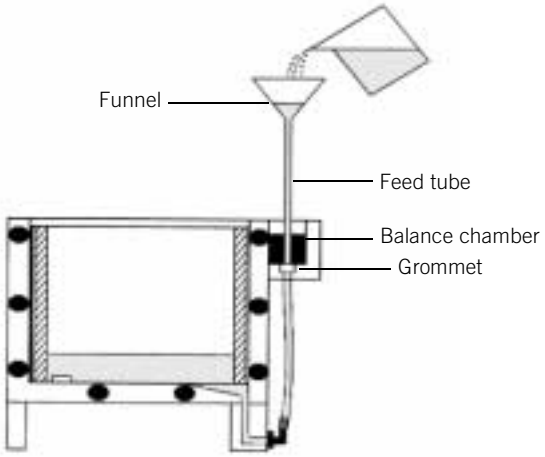


Figure 8. Setting up the caster for a non-gradient gel

7. Load the balance chamber with 150 ml heavy displacing solution.
8. Add the appropriate volumes of APS and TEMED only when you are ready to pour the gel, not before.

   Once these reagents are added, polymerization begins. You have about 10 minutes before the gels begin to solidify. Vary the amount of TEMED added to control the rate of polymerization.
9. Slowly pour the gel solution into the funnel, taking care to avoid introducing any bubbles into the feed tube line.

10. Once the pouring is complete, remove the feed tube from the balance chamber grommet.

    As soon as the feed tube is removed, the dense blue displacing solution flows down the connecting tube to the unit, fills 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 casettes is more than 1 cm below the top of the cassettes, you may add up to 50 ml more displacing solution to the balance chamber.

    The displacing solution pushes the remaining acrylamide solution out of the V-well into the gel cassettes. As gels contract during polymerization, the displacement solution is drawn in from the bottom.

    IMPORTANT Apply overlay immediately! See "Applying Overlay to DALT Slab Gels" on page 22.

11. Allow non-gradient gels to polymerize for at least 1 hour.

Preparation for Gradient Gel Casting

Successful gel casting requires planning, timing and practice.

A full DALT Gel Caster requires approximately two liters of acrylamide stock. Polymerization begins as soon as you add TEMED and APS to the acrylamide stock. Once you have added these reagents, 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 you set up the unit, as described on page 12, and configure the gradient divider, as described on this page. Follow the gradient pouring procedure on page 18, 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 correctly adjusted and you are comfortable with the gel casting procedure, clean all parts of the caster and gradient maker, including sponges, separating sheets and filler blocks, with a solution of mild detergent, followed by a fresh water rinse.

Configuring the Gradient Divider

You can adjust the shape of the gradient divider to suit the number of gels to be cast and 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 70 °. For fewer gels, decrease the divider angle (Figure 9). Whatever angle you use, a straight divider gives a linear gradient.

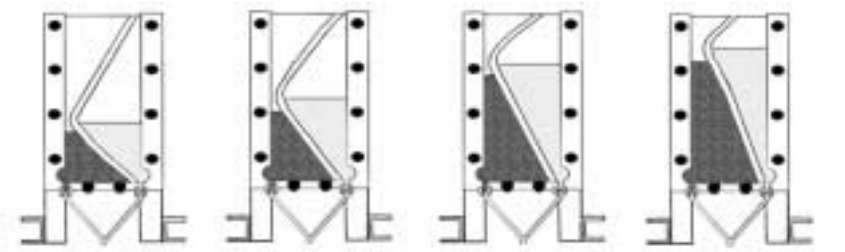


Figure 9. Gradient divider configurations for half-full and full Gel Caster tanks.

Divider angle (approx.)	40 °	50 °	60 °	70 °
Number/thickness	12 gels/1.0 cm	12 gels/1.5 cm	25 gels/1.0 cm	23 gels/1.5 cm

1. Determine the amount of gel solution needed to cast the desired number of gels.
2. Loosen the faceplate screws on the empty gradient maker to adjust the gasket angle. Refer to the divider configurations in Figure 9 as a guideline to 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.

3. 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.
4. To make a funnel for introducing heavy solution into the left side, bend the remaining top of the divider to the right.
5. Tighten the faceplate screws and close the pinch clamps on the tubing that runs out of the gradient maker before adding water or gel solutions.
6. Using water in place of gel solution, put half the required volume in the right chamber and the other half in the left chamber.
7. Adjust the angle of the gradient divider so that the level of liquid in the "heavy" chamber is about 2 cm below the level of liquid in the "light" chamber.

   You may use tape or wax pencil on the outside of the gradient maker to record the angle of the gradient divider. Avoid using marker ink that can only be removed with methanol. Most solvents, including methanol, can craze the plastic parts of the gradient maker.
8. 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 or thickness of gels you are casting.

Calibrating the Peristaltic Pump

Install the pump head and tubing to the pump controller, as directed in the manual supplied with the pump. Calibrate the pump flow before the first use, and after every ten to twenty uses, to assure proper flow rates.

This calibration procedure assumes a desired flow rate of 440 ml/min for a full tank of gels (2000 ml acrylamide) and 325 ml/min for 12 gels.

1. Place the inlet side of the tubing in a beaker that contains one liter of water.
2. Place the outlet side of the tubing in a 1-liter graduated cylinder.
3. Set the flow speed at approximately 3 – 3.5 on the dial and start the pump.
4. Stop the flow of liquid after exactly 2 minutes.
5. Measure the water in the outlet cylinder and divide by 2 to determine the flow in ml/min.
6. To determine the appropriate flow setting, divide the desired flow rate by the flow rate in step 5; multiply this result times the flow speed used in step 3.

Example. You want to cast a full tank of gels and determine the flow rate is 500 ml/min when you set the flow rate at 3. For a flow rate of 440 ml/min:

$(440 \div 500) \times 3 =$ **2.6** = the appropriate flow rate setting to deliver 440 ml/min

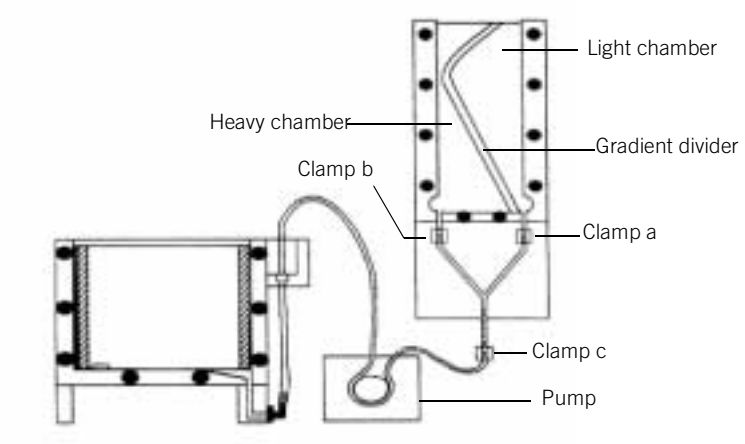
Casting Gradient Gels

The gradient maker is a simple unit with two chambers, defined by a silicone rubber gasket clamped between two acrylic plates. The chambers are separated by a movable divider, which you can modify to define the shape of the gel gradient.

Solutions flow out of the two chambers, join at the Y-connector and then thoroughly mix in a "bow-tie" in-line pipe 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 (Figure 10).

Figure 10. The pump-assisted gradient maker



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 assure 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 – 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 16.

Gradient Casting Setup

WARNING Acrylamide is a neurotoxin. Always use mechanical pipettes and wear protective gloves when working with acrylamide solutions, IPG strips or surfaces that come in contact with acrylamide solutions.

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

2. 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 17.
3. Be sure that the faceplate screws on the gradient maker are tightened hand tight and the gradient-maker lines are all clamped off. There are three clamps: one coming from each chamber and one after the bow-tie mixer. Close all three.
4. Prepare a sufficient volume of gel overlay solution (water-saturated *n*-butanol). You need 0.75 ml of overlay for each cassette, or about 20 ml for a set of 25 cassettes.
5. Make up 200 ml of displacing solution (page 42).
6. 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',-tetramethylethylene-diamine (TEMED). See “Gradient Gel Solutions” on page 49.

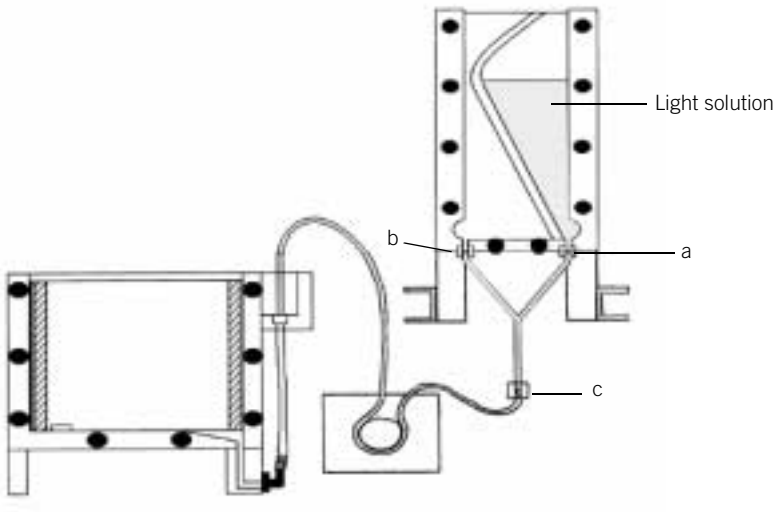
Pouring Gel Solutions for Gradient Gels

1. Prepare the gel caster, as described on page 12, placing gel labels in each cassette.
2. 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 minutes to cast the gradient before the gels begin to solidify at the top. Work rapidly and carefully.
3. Pour the light solution into the right side of the gradient maker (the chamber that is wider at the top—“Light in Right”).
4. Fill the tubing between the light and heavy chambers with light solution.

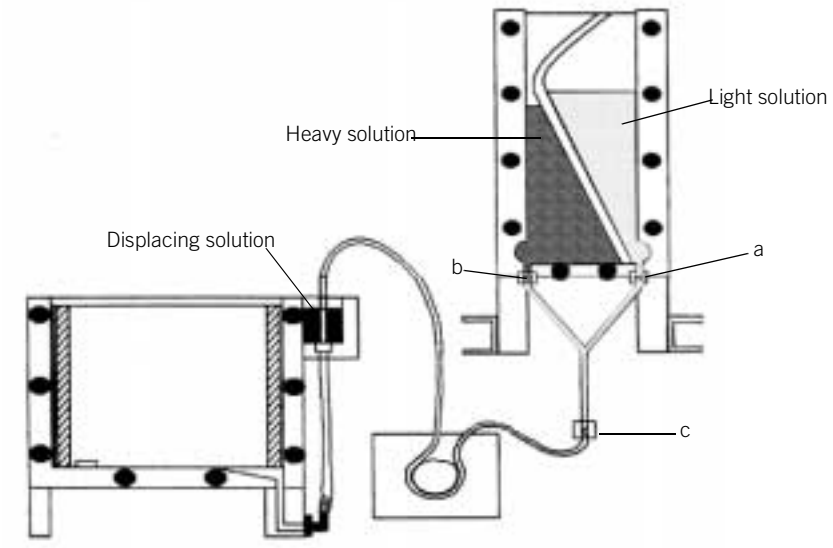
   Carefully open the clamp on the light chamber exit tube (a) and then very slowly open the heavy chamber exit tube clamp (b). Allow light solution to fill the tube coming from the light chamber all the way to the “Y” connector and back up to the point at which the heavy tube enters the heavy chamber. Fill the entire tube with LIGHT solution (no bubbles), but do not allow light solution into the heavy chamber itself (Figure 11).

Figure 11. Priming with light solution



5. Close both clamps again. All three clamps should now be closed.
6. 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.
7. Load the side balance chamber with 150 ml dense displacing solution (Figure 12). The grommet seal and gradient feed tube should prevent leakage of displacing solution into the caster.

Figure 12. Both chambers of the gradient maker filled and the balance chamber of the casting box loaded with overlay solution.



8. Open the clamp after the mixer (c), to open the feed tube to the gel caster via the peristaltic pump.
9. 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 towards the caster. At this point, a small amount of light solution can enter the caster.

10. When the light solution level in the gradient maker falls to a level about 1 cm above the level of the heavy solution, open the heavy chamber exit tube clamp (b).
11. Watch the gradient enter the caster.
12. When the caster is filled to within 4 cm from the top of the cassettes, or the gradient maker is empty, whichever comes first, turn off the pump and close the feed tube clamp (c). Stop the pump before air enters the feed tube.
13. Pull the gradient feed tube out of the balance chamber grommet. Place its end in a waste container to collect 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 casettes is more than 1 cm below the top of the cassettes, you may add up to 50 ml more displacing solution to the balance chamber. The gradient is now in hydrostatic equilibrium in the unit, ready to polymerize (Figure 13). Apply overlay as soon as possible (page 22).

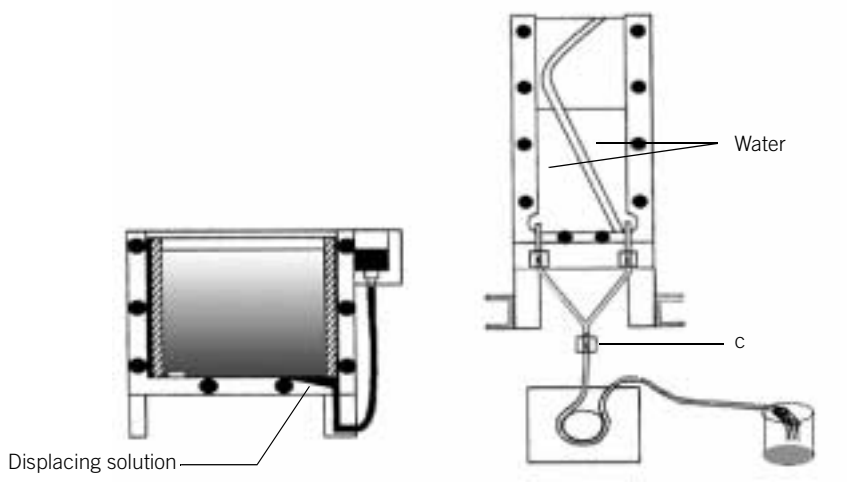


Figure 13. The gradient in hydrostatic equilibrium with the feed tube removed. Displacing solution fills the sloped trough at the bottom of the caster.

14. Quickly reopen clamp c and restart the pump to empty the gradient maker completely of any excess polymerizing acrylamide from the gradient maker.

    Collect the excess in a waste container. Dispose of unpolymerized acrylamide according to applicable safety guidelines.
15. 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 about a liter of water into each chamber of the gradient maker and open all clamps.
16. Start the pump to flush the system. Flush 2 liters more water through the gradient maker and tubing.

Applying Overlay to DALT Slab Gels

The flatness of the top gel surface is a major determinant of the quality and resolution of SDS slab gels. Imperfect gel tops can lead to irreproducible protein spot 2-D gel patterns. A convex or tilted slab top surface can give rise to double spots, as protein at the front and back gel surfaces starts moving at different points. To avoid this, quickly and carefully pipette identical volumes of water-saturated *n*-butanol overlay solution onto each gel. This results in very flat slab gel tops.

1. Immediately after removing the feed tube from the caster, slowly deliver 0.75 ml of water-saturated *n*-butanol to the surface of each gel.

   The overlay should spread evenly across the cassette with a minimum of mixing, resulting in a smooth, flat gel top surface. Apply equal volumes of overlay to each gel to produce gels of consistent heights.

2. Cover the top of the gel caster with plastic wrap and let the gels polymerize.

Polymerization

Allow non-gradient gels to polymerize for at least 1 hour; allow gradient gels 2 hours 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

1. Remove the front of the gel caster.

   The dense displacing solution will leak out into the tray or drain board beneath the casting unit.

2. Carefully unload the cassettes from the unit.

   Pull forward on the separator sheets to easily separate gels.

3. Quickly rinse the top surface of each gel with water to remove *n*-butanol and unpolymerized acrylamide.

4. Wash the cassette glass plates carefully with water and use a dish brush to remove any acrylamide adhering to the outer surfaces.

   As each cassette is washed, place it, hinged-side-up, in a dish rack standing in a plastic container with about 0.5 cm of tap water in the bottom. The container retains the excess liquid as it drains from the surface of the gel. The water in the container helps maintain humidity near the gels. A dry gel breaks and is useless for electrophoresis.

5. Remove and clean the thin separator sheets.

6. Examine the gels for air spaces, uneven top surfaces or other defects and discard any unsatisfactory gels.

7. Put the extra good gels in gel storage solution (see page 42) at 4 °C for later use. Extra gels may also be stored by wrapping the cassettes individually in plastic wrap and putting them in a sealed plastic box with several milliliters of diluted Tris buffer.

8. Take the foam funnel out of the bottom of the gel caster and rinse with water to remove displacing solution. Rinse the gel caster and all tubing with water and mild detergent, followed by a deionized water rinse.

Preparing the DALT Tank for Electrophoresis

NOTE Position the DALT Tank near a sink for easy rinsing and draining.

A magnetically coupled centrifugal pump, mounted on the back of the DALT Tank provides buffer circulation across the heat exchanger tubes and the gels in the center chamber.

IMPORTANT Never lift or move the tank by holding the pump or the tubing at the back of the tank.

The heat exchanger consists of four ceramic tubes below the buffer circulation flutes. It is quite robust, but dropping a heavy, sharp object into the tank could damage it. A cracked heat exchanger can leak coolant into the tank or buffer out of the tank, either of which might be hazardous.

Connecting a Refrigerated Circulating Bath

To maintain a constant buffer temperature, connect a refrigerated circulating bath, such as the MultiTemp III, to the heat exchanger fittings at the back of the DALT Tank. The pump in the bath circulates chilled liquid through the alumina heat exchanger. The pump on the DALT Tank circulates the buffer in the central chamber over the heat exchanger and the gel cassettes, ensuring even gel temperature during the run.

Note For quick and easy connections, install Quick-fit connectors with valves in the line.

1. Prepare two lengths of vinyl or silicone tubing. Slide hose clamps (4 total) onto each end of two lengths of tubing. Attach one end of each length of tubing to the Quick-fit connector attached to the heat exchanger port. Attach the free ends of each length of tubing to the circulator bath ports; one to the inlet and the other to the outlet. Secure the connections to the Quick-fit connectors and the circulating water bath with the hose clamps.
2. Set the circulating bath temperature to 10 °C.

   IMPORTANT The circulator pump must not generate a pressure greater than 0.7 bar (10 psi) above atmospheric pressure.

   If you use a temperature below 10 °C, use a 50/50 water/ethylene glycol solution in the circulating bath. Do not use any organic solvent other than 50/50 ethylene glycol/water as a coolant. Most solvents, including methanol and isopropanol, will craze the plastic parts of the heat exchanger.

   WARNING Do not use automotive antifreeze solution. Ethylene glycol is toxic and may be fatal if ingested, inhaled or absorbed through the skin.

Filling the Tank with SDS Electrophoresis Buffer

Because the DALT Tank has its own circulating pump, you can make the tank buffer within the tank itself. The size and number of the cassettes used determine the amount of SDS electrophoresis buffer needed to cover the cassettes. You can run any number of

gels, up to the tank maximum. The empty slots seal themselves and require no blank cassette. Since the filled tank is too heavy to empty directly, use a siphon pump to remove buffer from the tank. Between runs, rinse the tank with water.

1. Two or three hours before the beginning of a run, fill the tank with the appropriate volume of de-ionized water. Use tape to mark the level of the liquid on the outside of the tank for future runs.

   Always use water near room temperature. Never fill the tank with liquid at a temperature very different from the tank itself.

   IMPORTANT Fill the tank with water before turning the pump on. The pump is not self-priming and can be damaged if run dry.

2. Turn on the pump after the tank is filled.

   At first, the pump blows some bubbles through the circulating flutes in the tank and then establishes a vigorous circulating action in the tank. If the pump doesn't "catch" and no circulation is observed, you have an airlock in the pump. Quickly turn off the pump. Wait a moment for the air to bubble out through the small "bypass" tube that comes up from the pump outlet and enters the back of the tank about halfway up, then restart the pump. You may have to do this a few times. Once the pump is circulating, leave it on.

3. Weigh out the correct SDS electrophoresis buffer mixture. (See page 42.) Add the dry powder directly to the water in the center chamber, distributing it evenly from front to back.

   The pump circulates the contents enough to dissolve the solids in about 1-2 hours. Some solids may lie on the bottom for awhile, but they gradually disappear. You may want to make a number of these buffer packets in 1-liter plastic bottles and have them on hand.

4. Once the buffer is dissolved, use a Phillip's head screwdriver to loosen the white, plastic retaining screws on the exterior of the DALT Tank that hold the barrier combs in place.

   Raise or remove the two barrier combs to mix the total DALT Tank contents with the dissolved contents of the center chamber. (See Figure 14 on page 26.) You may tighten the retaining screws to secure the barrier combs in the raised position while the buffer circulates throughout the tank.

Figure 14. Raising the barrier combs to allow buffer circulation

5. Make a note on a label on the tank lid when you change the buffer.

   Although it is possible to use each tank of buffer for 2-3 runs, by mixing the center and cathode [left] chambers between runs, it is better to change the buffer before every run. Fresh buffer ensures more consistent results.

   When a tank is not going to be used for several days, siphon out the buffer, rinse the tank with water, and allow the tank to sit empty. This prevents growth of bacteria and molds which can occur in used buffer. Such contamination can give rise to "mysterious" protein bands across a whole set of gels.

6. When the buffer is distributed in all chambers, replace the barrier combs in the outer slots to accommodate the 20 × 25 cm cassettes.

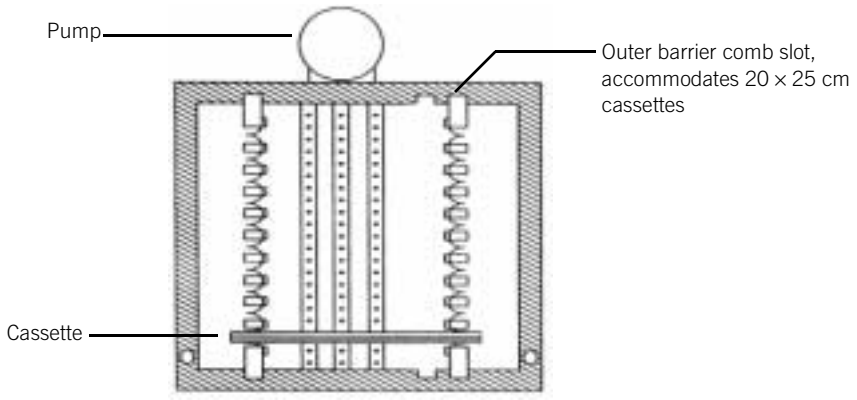


Figure 15. Top view of DALT Tank, with barrier combs in place

When properly positioned, the ends of the silicone rubber flaps face the outer walls of the tank (Figure 15).

Loading and Running Second Dimension Gels

Immobiline DryStrip gels provide a pH gradient (IPG) that is immobilized in an acrylamide gel and supported by a plastic film backing. The Hoefer DALT System can accommodate the entire length of an 18-cm IPG strip, plus markers, and up to 10 gels can be run simultaneously.

To prepare for second dimension electrophoresis, first equilibrate the focused IPG strips in SDS buffer before loading them onto DALT slab gels.

IMPORTANT Prepare the second dimension vertical gel and tank buffer *before* IPG strip equilibration. Prepare agarose sealing solution during IPG strip equilibration.

The exposed edges of the gels tend to dry out over a period of hours. Coordinate both IPG and DALT runs so that the end of the IPG run coincides with the end of unloading the DALT casting unit. Start the DALT polymerization 2.5 hours before the IPG equilibration, to allow up to 2 hours for gel polymerization and 30 minutes for unloading the cassettes from the casting unit.

Equilibrating IPG Strips

The SDS equilibration buffer contains urea, glycerol, SDS, Tris buffer and tracking dye. During equilibration, the sample proteins are saturated with SDS for mobility in the second dimension gel. Urea and glycerol minimize electroendosmosis effects due to the IPG strip. In addition, the equilibration buffer contains DTT to fully reduce the sample prior to the second dimension separation.

Equilibration Buffer (50 mM Tris-Cl) maintains strip pH in a range that allows proper stacking. The pH is typically adjusted to 8.8 to compensate for the acidifying effect of the optional iodoacetamide treatment.

Urea (6 M), together with glycerol, reduces the effects of electroendosmosis by increasing the viscosity of the buffer. Electroendosmosis occurs due to the presence of fixed the charges of the IPG matrix in the electric field and can interfere with protein transfer from the IPG strip to the second dimension gel.

Glycerol (30%), together with urea, reduces electroendosmosis and improves transfer of protein from the first to the second dimension.

Dithiothreitol (DTT) preserves the fully reduces state of denatured, unalkylated proteins.

Sodium dodecyl sulfate (SDS) denatures proteins and forms negatively-charged protein-SDS complexes. The amount of SDS bound to protein, and therefore the additional negative charge, is directly proportional to the mass of the protein. Thus electrophoresis of proteins through a sieving gel in the presence of SDS separates proteins on the basis of molecular mass.

Iodoacetamide alkylates sulfhydryl groups on proteins, preventing their re-oxidation during electrophoresis. Protein re-oxidation during electrophoresis can result in streaking and other artifacts. Iodoacetamide also alkylates residual DTT to prevent point streaking and other silver staining artifacts. Iodoacetamide is introduced in an optional second equilibration step.

NOTE The reaction between iodoacetamide and DTT creates hydroiodic acid. When using iodoacetamide, the pH of the equilibration solution is 8.8 to compensate for this acid production.

Tracking dye (bromophenol blue) allows monitoring of electrophoresis.

Equilibration Steps

Use the gel number labels that were polymerized into the separating gel to help orient the IPG strips. Conventionally, the acidic, or pointed, end of the IPG strip is on the left, or label side.

NOTE To avoid protein contamination, wear gloves when handling the IPG strip and hold only the protruding end of the gel backing film when moving the strip.

1. Prepare SDS equilibration buffer (see page 43). Just prior to use, add DTT to the buffer at a concentration of 100 mg DTT per 10 ml SDS equilibration buffer.
2. Place the IPG strips in individual tubes with the support film toward the wall. Screw cap culture tubes (25 × 200 mm) work well.
3. Add 10 ml of the DTT-containing solution to each tube. Cap the tube, or seal it with flexible paraffin film, and place is on its side on a rocker.

Note It takes approximately 10 minutes to prepare Agarose Sealing Solution (page 43). Prepare the solution during IPG equilibration.

4. Equilibrate for 10 – 15 minutes. Do not over-equilibrate, as proteins can diffuse out of the strip during this step.

   An optional second 10-min equilibration with iodoacetamide will alkylate sulfhydryl groups and prevent disulfide reformation. The second equilibration also removes excess DTT which can lead to vertical point streaking when silver staining.

5. **Second Equilibrium (optional)**. Prepare a solution of 25 mg iodoacetamide per 10 ml SDS equilibration buffer. Add 10 ml of solution to each tube containing an IPG strip. Cap the tube, or seal it with flexible paraffin film, and place is on its side on a rocker to equilibrate, for 10 minutes.

   IMPORTANT After equilibration, load IPG strips directly onto the DALT slab gels as soon as possible.

Loading the IPG Strips onto the DALT Slab Gels

Place the DALT gels in the dish rack in alphabetical/numerical order, with respect to the identification label, with the sample application surface of the slab up and the label readable from the front. Be sure to record gel identification numbers.

1. Dip the equilibrated IPG strip in SDS electrophoresis buffer to lubricate it, then place the IPG gel strip onto the DALT gel cassette. (See Figure 16.)

   Position the IPG strip between the plates, touching the surface of the second dimension gel, with the plastic backing against one of the glass plates. For a convenient reference, place the pointed, acidic, end of the IPG strip on the same side as the gel label.

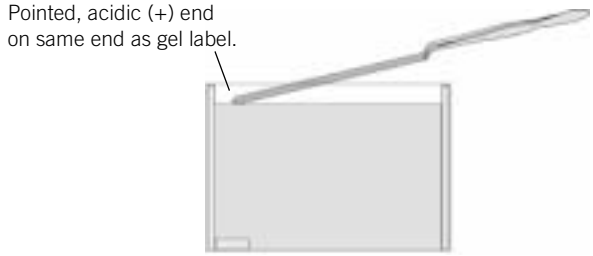


Figure 16. For reference, place the pointed end of the IPG strip above the gel label

   Use a thin plastic spatula or ruler to push against the plastic backing of the IPG strip, *not* the gel itself, and move the strip down into contact with the surface of the second dimension gel. The edge of the strip should just rest on the surface of the slab gel. Avoid trapping air bubbles between the plastic backing and the glass plate or cutting into the SDS gel with the strip. The gel face of the strip should not touch the opposite glass plate.

2. **Optional**: Apply size marker proteins.

   Apply the markers to a paper IEF sample application piece in a volume of 15 – 20 µl. For less volume, cut the sample application piece proportionally. Place the IEF application piece on a glass plate and pipette the marker solution onto it. Apply approximately 50 µl agarose sealing solution to seal markers in the sample application pieces. Pick up the application piece with forceps and apply to the top surface of the gel next to one end of the IPG strip. The markers should contain 200 – 1000 ng of each component for Coomassie staining and about 10 – 50 ng of each component for silver staining.

3. Slowly moving the pipette from left to right across the gel, deliver agarose sealing solution onto the IPG strip to seal it into place. Carefully avoid bubbles when sealing with the agarose. (See Figure 17.) Wait 2 – 5 minutes to allow the agarose to fully solidify before proceeding.

Figure 17. Completely cover the IPG strip with agarose sealing solution

4. Keep a log of run conditions and the identification number of the DALT gel onto which each IPG strip is loaded.

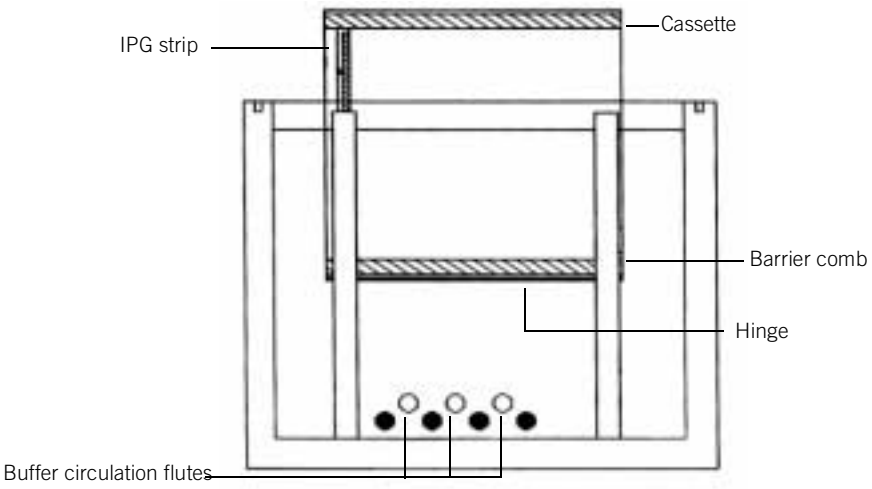
Loading Cassettes into the DALT Tank

1. Carefully load the cassettes after the agarose overlay has fully solidified.

   The cassettes are correctly loaded in running orientation in the DALT Tank slot with the IPG strips vertical along the left, or cathode (-), side and the rubber cassette hinge along the bottom (Figure 18). Dip the hinge side of the cassette into the tank buffer first to lubricate it before inserting it into the flap seals. Use both hands to slide cassettes firmly to the bottom.

   Be careful! The plates slip easily once your hands are immersed in tank buffer. Do not drop the plates into the tank and onto the circulation flutes.

Figure 18. Inserting loaded cassette into DALT Tank

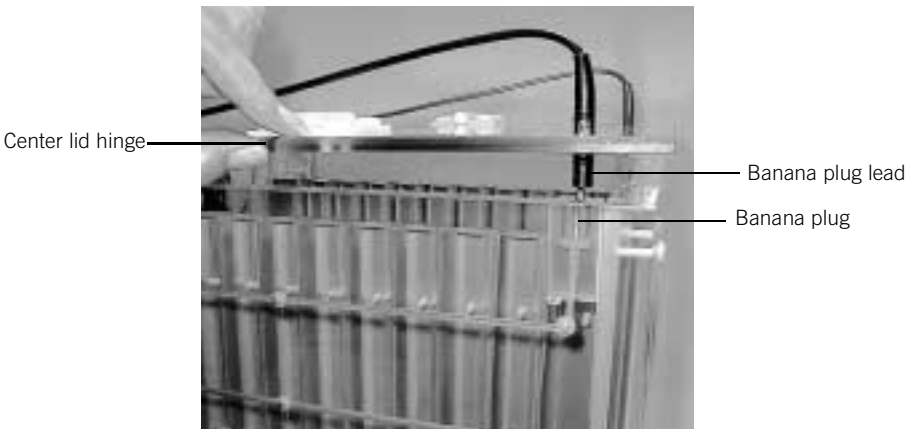


2. Adjust the buffer level after all the cassettes are loaded in position.

   IMPORTANT The tank buffer level should be even with the uppermost spacer of the cassette and neither above the top of the cassette nor below the level of the top edge of the gel. If the level is too low or too high, add SDS electrophoresis buffer or use the siphon pump to correct the buffer level.

3. After the last cassette has been introduced into the tank, close the lid (Figure 19).

Figure 19. Lift the lid slightly at its center hinge to make sure the banana leads make contact with the recessed plugs.



4. Attach the electrical leads to make proper electrical contact with the power supply. Migration proceeds toward the red (+), or right chamber.
5. Turn on the power supply to begin the separation.

Electrophoresis Conditions in the DALT Tank

1. Set the power supply to 100 – 125 V constant voltage for overnight runs or at 40 mA per gel for runs to be completed during the same day.

   A set of ten 20 × 25 cm gels can be done in about 9 hours at maximum settings of 600 mA and 200 V. Run times can vary widely, depending on the acrylamide gradient or concentration, the temperature and pH of the buffers, and the number and size of cassettes. Observe the progress of your first run and set future schedules accordingly.

2. Run the gels until the blue tracking dye reaches the right side of the gel cassette (the "bottom" of the gel) as seen through the front of the DALT Tank.

   The bromophenol blue tracking dye, introduced into the IPG strip from the equilibration buffer or included in the agarose overlay, migrates just ahead of the smallest proteins (Figure 20). For consistency and reproducibility, establish a specific "finish line" distance for the dye front. It may be the actual bottom edge, or a few millimeters before, but when the blue line reaches that point, the run is done.

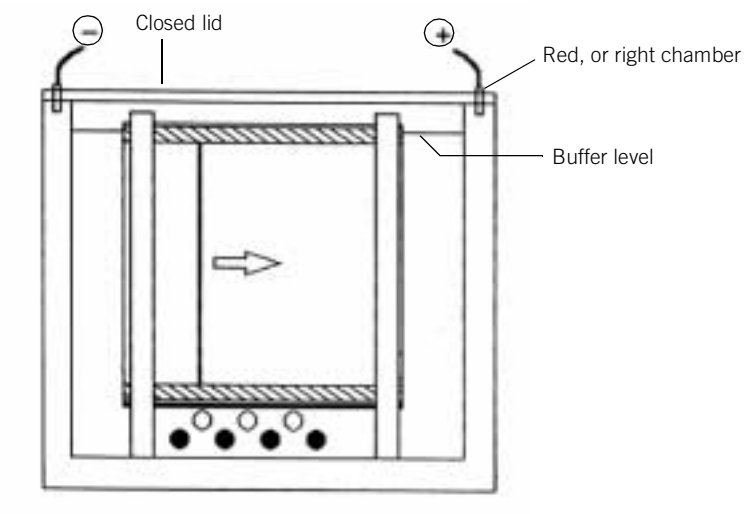


Figure 20. Tracking dye progresses from left to right in the DALT Tank

Unloading the DALT Cassettes

1. When you are ready to remove the cassettes, unplug the buffer circulation pump and the circulating water bath, turn off the power supply and disconnect the power leads. Open the DALT Tank lid only when the power supply is turned off and disconnected.

   NOTE Wear gloves when handling gels that will be silver-stained, since fingerprints will show after staining.

2. Remove the cassettes carefully, one at a time, using both hands.

   The cassettes are slippery because of the presence of SDS. Rubber gloves may improve your grip on the glass. Unload the cassettes into dish racks (ten per rack), that are placed in plastic containers of water, to minimize gel drying.

3. Place a cassette on a flat surface and pry it open very carefully with a plastic wedge such as the Wonder Wedge. Avoid metal wedges or spatulas, which can easily chip the glass.

   Put the stiff wedge end into the top comer of the cassette away from the hinge and twist it gently. Take care not to chip the glass of the cassette. When opening the cassette, make sure that the gel adheres to one of the plates and is not sticking partly to both, to avoid tearing the gel.

   If the gel sticks to the plate with the spacers attached, run a single edge razor blade down between gel and spacers to assure that the gel is not sticking to either spacer.

4. Carefully peel the gel from the glass, lifting it by the bottom (high %T end, if a gradient), and place it in a tray of fixative solution. Use a fixative appropriate for your staining technique.

   In case of a tear in the gel, remove the torn section last, working toward the tear.

Cleaning DALT Cassettes

1. Soak the used cassettes in distilled water if you can't clean them immediately.

   To avoid clogging the sink drain, use a fine, removable sink trap to catch shreds of polymerized acrylamide.

2. To clean the cassettes, thoroughly rinse them in warm water, going over all surfaces with a non-scratching, plastic dish-cleaning pad. The SDS already on the plates is usually sufficient detergent for cleaning.

3. Finally, rinse the cassettes with distilled water and air dry them in the open position on the lab bench or in a drying rack.

4. Store the cassettes in a closed cabinet to keep them free from dust.

The DALT Blotting Kit

The DALT Blotting Kit supports the transfer of proteins from up to five large-format polyacrylamide gels onto a membrane. Gels and membranes are held by a cassette, which is submerged into the transfer tank. Molecules migrate under an electric field to the membrane, where they are bound.

The transfer buffer temperature can be controlled by circulating cooled liquid through the heat exchanger in the base. Coolant is pumped through the alumina heat exchanger located at the base of the unit. Temperature is maintained by buffer circulation through tubes in the base of the tank.

We strongly recommend connecting a constant temperature circulation bath to the heat exchanger to maintain the correct temperature during transfer. A set temperature between 20 and 30 °C is recommended, although lower temperatures can also be used. Never operate the unit for more than one hour under high power conditions (>250 mA) without active cooling.

See page 44 for the recipe for Towbin Buffer. Depending on the membrane type and the manufacturer's recommendations, you may add 0.1% SDS and 10 – 20% methanol.

Preparing the DALT Tank for Transfer

IMPORTANT Gels should be transferred immediately after electrophoresis to avoid sample diffusion. Do not soak gels in fixative. Equilibrate gels with transfer buffer before they are placed in the cassette, if necessary.

1. Configure the unit for active cooling. See "Connecting a Refrigerated Circulating Bath" on page 24.

   Active cooling is optional but strongly recommended. Start the circulating bath at the same time as the transfer. The circulator pump must not generate a pressure greater than 0.7 bar (10 psi) above atmospheric pressure.

2. Loosen the white nylon screws on the exterior of the DALT Electrophoresis Tank and lift out the barrier combs. (See Figure 21.)

Preparing the Transfer Buffer

Because the DALT Tank has its own circulating pump, you can make the tank buffer within the tank itself. In the tank, it may take up to two hours for the buffer solids to dissolve. As an alternative, mix the transfer buffer in a carboy with a magnetic stirbar.

You may reuse the buffer in which you ran the original electrophoresis (25 mM Tris, 192 mM glycine, 0.1% SDS) for the transfer, if you first adjust the SDS and methanol concentrations for optimum binding.

Prepare an additional 4 liters of Towbin Buffer to use for cassette assembly. See page 44 for this recipe.

Figure 21. DALT Electrophoresis Tank, with barrier combs removed

IMPORTANT Fill the tank with water before turning the pump on. The pump is not self-priming and can be damaged if run dry.

1. Fill the tank with 15 liters of water or used electrophoresis buffer.

   Add the appropriate volumes of water and/or methanol to bring up to 22 liters.

2. Turn on the pump after the tank is filled.

   At first, the pump blows some bubbles through the circulating flutes in the tank and then establishes a vigorous circulating action in the tank. If the pump doesn't "catch" and no circulation is observed, you have an airlock in the pump. See the instructions on page 25.

3. If preparing fresh buffer, add the dry buffer mixture.

   NOTE Even if no cooling is required for your system, use the pump to circulate the buffer, to avoid buffer depletion at the electrodes.

Assemble the Transfer Cassette

Note Always wear gloves when handling membranes to avoid contamination with skin proteins.

1. Pre-wet nitrocellulose or nylon membranes with distilled water. Pre-wet PVDF or other hydrophobic membranes in methanol. Then soak all membrane types in transfer buffer for 2–5 minutes.
2. If necessary, equilibrate the gel in transfer buffer for 10–15 minutes.
3. Open the cassette by releasing both latch tabs along the edge opposite the hinges. Place the opened cassette into a tray filled with at least 6 cm of transfer buffer.
4. Assemble the transfer stack so that molecules will migrate toward the membrane. For negatively charged macromolecules, such as proteins coated with SDS, build the stack on the anode (+) half of the cassette.

Note Carefully position the gel. Proteins may begin to transfer immediately. Once transfer has begun, moving the gel will distort results or cause "shadow bands" on the membrane.

Place one 6-mm-thick foam sponge on the opened submersed cassette and press gently until all air is expelled. Place one sheet of blotting paper on the sponge, and then place the membrane on the blotting paper. Place the gel—which contains a sample that has been electrophoretically separated and equilibrated (if required) with transfer buffer—on the membrane. Gently roll a glass pipet or test tube over the gel to expel trapped air between the membrane and gel. Cover the gel with a sheet of blotting paper and an additional 6-mm sponge (see Figure 22) while pressing gently to expel trapped air.

Check that the gel membrane and sponges fit properly within the blotting cassette.

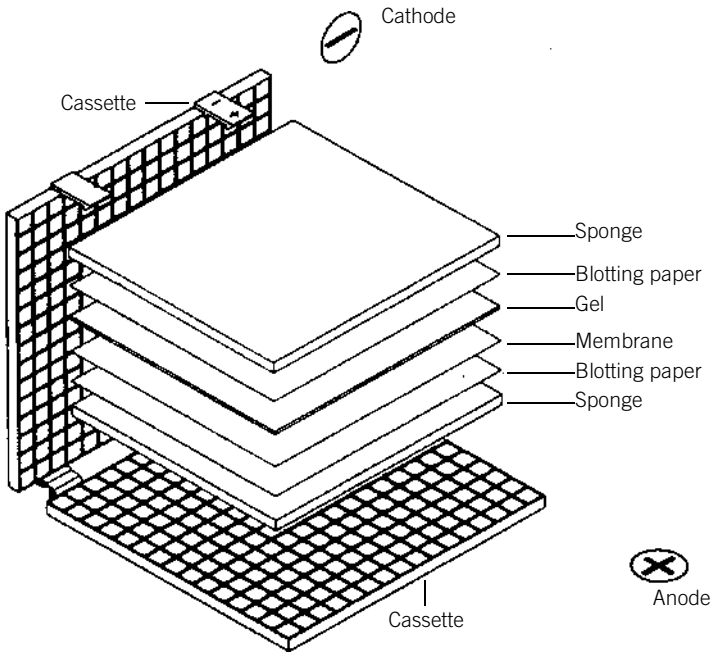


Figure 22. Transfer stack assembly
Orient the stack so that the negatively-charged molecules migrate toward the anode (+).

5. Close the cassette and press lightly to lock the tabs.

   The assembled cassette should hold the gel in firm contact with the membrane without squeezing the gel. If the stack seems loose, add sheets of blotting paper.

Loading the Cassettes

Work quickly when moving the assembled cassette(s) to the tank to avoid draining the sponges. Place the tray holding the cassette(s) near the tank, lift out one cassette at a time, and slide each one into a vertical slot. Do not discard the buffer.

The direction of molecule migration depends on both the characteristics of the sample and the pH of the transfer buffer. If the species of interest is negatively charged in the transfer buffer and the stack is assembled so that the membrane faces the anode (+). Most proteins migrate toward the anode in the Towbin buffer system, independent of the presence of SDS.

1. Place the support rack for the DALT Blotting Kit into the tank, with the rack straddling the heat exchanger flutes in the bottom of the tank and the side engraved "pump" closest to the circulation pump.
2. Lift the cassette sandwich from the tray of buffer and quickly insert it into one of the sets of vertical slots in the submerged cassette-holding rack.

   As many as five gels can be transferred at once in the DALT Blotting Kit. For three or less gels, use the cassette positions nearest the center.
3. Place the cassettes into the unit so that all gels are oriented the same way, with the clip side facing up and all anode (–) sides of the cassettes facing the same side of the transfer unit.
4. After inserting a cassette, tap it a few times to release any air bubbles that may have been introduced in moving from the buffer tray to the tank. A few bubbles left in the sponges should not interfere with the transfer.
5. Check the buffer level to make sure buffer covers the cassettes.

   WARNING The banana plugs corrode if exposed to buffer during transfer. Carefully blot away any buffer that falls into the banana plug recesses.
6. When all transfer cassettes are in the tank, place an empty glass gel cassette horizontally across the tops of the cassettes to anchor them in the tank.

   If you are using only one transfer cassette for blotting, place another empty cassette in a vertical slot to help support the glass cassette anchor.

Connecting the Power Supply

If using a power supply that can be set to either constant current or constant voltage mode, we recommend that it be set to operate in constant current mode. Buffer conductivity increases with temperature. During blotting in an uncooled chamber at constant voltage, Joule heating and rising conductivity may result in dangerous overheating. If a constant voltage power supply must be used, monitor and adjust the voltage to maintain a current at 1000 mA, or less, and voltage should not exceed 100 V.

1. Close the plastic lid on the chamber. The cassettes are labeled to match the leads in the lid.

   To transfer toward the anode, position the lid so that the (+) side of the cassette faces the anode (+), or red lead, and the (–) side of the cassette faces the cathode (–), or black lead.

2. Connect the leads to your power supply.

3. Set the power supply. See Table 1 for typical transfer parameters for proteins.

   Constant current mode is recommended. If constant voltage mode is selected, carefully monitor the current. Increased current increases Joule heating. If the current exceeds 1.0 A, decrease the voltage.

   IMPORTANT The plastics used in the DALT unit may warp at high temperatures. Because electrophoretic transfers are performed at high currents, considerable heat is generated during a run. Do not allow the buffer temperature to exceed approximately 40 °C.

Table 1. Typical Transfer Parameters for Proteins

Buffer*	Towbin*
Current (A)	up to 1
Voltage (V)	approx. 70
Transfer time	approx. 5 hours
Coolant temperature	10 °C

*Empirically determine the parameters for your sample and buffer system.

   If you are planning to do a second transfer or set of transfers in the same buffer, be sure to maintain constant temperature or to cool the buffer to room temperature between the two runs. Do not allow the buffer to overheat and damage the unit.

4. Set the timer.

   Most transfers are complete with 5 hours, but larger molecules or thicker gels may require longer transfer times. Determine the optimum transfer time for your system empirically.

   NOTE For overnight transfers, set the current at 400 mA with active cooling.

Transfer Conditions

The blotting transfer conditions shown in Table 2 are only suggestions. Efficiency of transfer depends on the percentage of gel used for the electrophoresis run, the physical characteristics of the proteins being transferred, and on how many times the transfer buffer has been used.

The conditions were developed using 12% T gels, 193 mM glycine, 25 mM Tris, 20% methanol, 0.1% SDS. Transfers were made onto nitrocellulose. A refrigerated circulating water bath set at 4 °C was used for all transfers.

Table 2. Typical Protein Transfer Conditions

1000 mA for 2 – 3 h	For rapid transfer of proteins, M_r < 70 k
1000 mA for 5 – 6 h	For moderate transfer of proteins, M_r < 100 k
400 mA for 18 h	For most efficient transfer of high and low molecular weight proteins.

Completing the Transfer

1. When the transfer is complete, turn the voltage and current settings to zero and turn off the power supply. Disconnect the leads from the power supply jacks.
2. Open the lid and lift out the cassettes.
3. Open each cassette carefully and remove the gels. Lift the membranes with blunt forceps.

   Discard the blotter paper. Rinse the sponges with ddH_2O and reuse them indefinitely.
4. With a soft-lead pencil, label each membrane and indicate the sample side.
5. Rinse the unit immediately after use.

Note Transfer buffer may be re-used two to three times. Store buffer in a separate container and cool to 10 °C before reuse.

Factors Affecting the Transfer

Sample characteristics, membrane type, gel pore size, and the transfer buffer used affect the efficiency of macromolecule transfers. The most widely used buffer system for transferring proteins is that of Towbin, et al. Conditions required for efficient elution may not coincide with optimal conditions for binding. To find the optimum conditions for transferring your sample, balance these effects:

- If the sample elution rate is slow, a longer transfer period may be required. In our experience, high current transfers for short periods of time are superior to low voltage transfers for longer periods.
- If sample binding is inadequate, try different buffer conditions, methanol or SDS concentration. For a comprehensive review, see Gershoni and Palade (1983).

If the transfer buffer system is different from the electrophoresis buffer system, the gel should be equilibrated briefly with the transfer buffer before the transfer to assure swelling or shrinking occurs before the gel contacts the transfer membrane. If this step is skipped, band distortion or loss of resolution could result.

Recipes

DALT Acrylamide Stock (30.8 %T)

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

	Final Conc.	Amount
Acrylamide (best affordable grade, MW 71.08)	30%	900 g
Bis (N, N' methylenebis-acrylamide, purest grade, FW 154.17)	0.8%	24 g
Water (purest available)		up to 3000 ml

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

1.5 M Tris-Cl, pH 8.8

	Final Conc.	Amount
Tris (MW 121.14)	1.5 M	545 g
6N HCl to pH 8.8		about 150 ml
Water		to 3000 ml

Adjust to pH 8.8, store at 4 °C.

10% SDS

	Final Conc.	Amount
Sodium dodecylsulfate (MW 288.38)	10%	10 g
Water		up to 100 ml

Store at room temperature.

10% Ammonium Persulfate

	Final Conc.	Amount
Ammonium persulfate (MW 228.2)	10%	2 g
Water		up to 20 ml

Make fresh.

10% TEMED

	Final Conc.	Amount
TEMED (v/v, MW 116.2)	10%	0.5 ml
Water		4.5 ml

Prepare fresh, in glass vessel.

Displacing Solution

(0.375 M Tris-Cl, pH 8.8, 50% glycerol, bromophenol blue, 200 ml)

	Amount
Tris-Cl (1.5M, pH 8.8)	50 ml
Glycerol	100 ml
Bromophenol blue	2 mg
Water	50 ml

Prepare fresh. Stored solution may support microbial growth.

Water-saturated *n*-Butanol

	Amount
n-butanol	50 ml
Double-distilled 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 Tris-Cl, pH 8.8, 0.1% SDS, 2.0 liters)

	Final Conc.	Amount
1.5 M Tris-Cl, pH 8.8		500 ml
10% SDS	0.1%	20 ml
Double distilled H_2O		to 2000 ml

Store at 4 °C.

SDS Electrophoresis Buffer

(25 mM Tris, 192 mM glycine, 0.1% SDS, approximate pH 8.3, 20 liters)

	Final Conc.	Amount
Tris (MW 121.14)	25 mM	60.5 g
Glycine (MW 75.07)	192 mM	288.0 g
SDS (FW 288.38)	0.1% (w/v)	20.0 g
Double distilled H_2O		to 20 liters

Do not adjust the pH of this solution.

SDS Equilibration Buffer

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

	Final Conc.	Amount
Tris-Cl, pH 8.8 (1.5 M solution)	50 mM	6.67 ml
Urea (FW 60.06)	6 M	72.07 g
Glycerol (87% v/v, MW 92.09)	30% (v/v)	69 ml
SDS (FW 288.38)	2% (w/v)	4.0 g
Bromophenol blue	trace	a few grains
Double distilled H_2O		to 200 ml

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

Agarose Sealing Solution

(25 mM Tris, 192 mM glycine, 0.1% SDS, 0.5% agarose, approximate pH 8.3, 25 ml)

	Final Conc.	Amount
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 dissolved, about 1 minute. Do not allow the solution to boil over.
Allow the agarose to cool slightly before using. Do not adjust pH.
The volume of sealing solution prepared from this recipe is sufficient for a complete set of second-dimension DALT gels.

Towbin Buffer for DALT Blotting Tank

(25 mM Tris, 192 mM glycine, approximate pH 8.3, 22 liters [optional 20% v/v methanol])

	Final Conc.	Amount
Tris (FW 121.14)	25 mM	66.6 g
Glycine (FW 75.07)	192 mM	317.1 g

Dissolve in 15 liters distilled water and mix in the DALT Tank itself by turning on the circulation motor. Remove barrier combs. Add methanol as required. Alternatively, mix in a carboy with a magnetic stirbar.*

Optional: Adding 0.1% SDS can improve transfer efficiency but may decrease binding to the membrane.

The pH of this buffer may vary from 8.2 - 8.4. Do not adjust the pH. Adjusting the pH alters the conductivity of the buffer.

* Depending on the membrane type, adding 10 – 20% methanol can improve protein binding and reduce gel swelling during transfer. Buffers containing methanol may deteriorate if stored for long periods.

Towbin Buffer for Transfer Cassette Assembly

(25 mM Tris, 192 mM glycine, approximate pH 8.3, 4 liters [optional 20% v/v methanol])

	Final Conc.	Amount
Tris (FW 121.14)	25 mM	12.11 g
Glycine (FW 75.07)	192 mM	57.65 g

Dissolve in 3 liters distilled water and mix in a carboy with a magnetic stirbar. Add methanol as required. Bring to 4 liters with water.*

The pH of this buffer may vary from 8.2 - 8.4. Do not adjust the pH. Adjusting the pH alters the conductivity of the buffer.

* If recommended by the membrane manufacturer, you may add 10 – 20% methanol.

Preparation of Creatine Kinase (CK) Charge Standards

Use CK charge standards to evaluate first-dimension isoelectric focusing and second dimension separation.

1. Dissolve 5 mg of rabbit muscle creatine phosphokinase (Sigma) in 1 ml of a solution of 8 M urea and 1% mercaptoethanol, to give a CK concentration of 5 mg/ml.
2. Aliquot the above mixture into 7 tubes.
3. Heat six of the tubes for 4, 6, 8, 10, 12, and 15 minutes at 95 °C in a heating block, or in a boiling water bath. After heating, place tubes in an ice bucket.
4. Mix the contents of the 7 tubes together and aliquot 50 µL of the pool into small microcentrifuge tubes for storage at -70 °C.
5. Thaw out a tube for each experiment and load 2 µL CK mix per IPG strip.

   During electrophoresis, the CK charge standards produce a carbamylation chain of spots, separated according to pI.

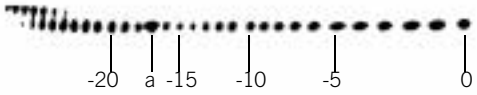


Figure 23. An example of electrophoresis of CK charge standards for testing pI separation

Homogeneous Gel Solutions

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 these volumes for your lab because of differences in temperature and reagent quality. Perform a small-scale test before first using a new composition to check that your solution polymerizes in about 10 minutes.

1000 ml. Amounts shown in ml.

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	233	267	300	333	367	400	433	467	500	533	567	600	633
1.5M Tris-Cl, pH 8.8	250	250	250	250	250	250	250	250	250	250	250	250	250
Water	494	461	428	395	362	329	295	262	229	196	162	129	96
10% SDS	10	10	10	10	10	10	10	10	10	10	10	10	10
10% APS	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
10% TEMED	2.45	2.14	1.90	1.71	1.55	1.43	1.32	1.22	1.14	1.07	1.01	0.95	0.90

25 gels, 1.0 mm

1500 ml. Amounts shown in ml.

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	350	400	450	500	550	600	650	700	750	800	850	900	950
1.5M Tris-Cl, pH 8.8	375	375	375	375	375	375	375	375	375	375	375	375	375
Water	741	692	642	592	5443	493	443	393	343	293	243	194	144
10% SDS	15	15	15	15	15	15	15	15	15	15	15	15	15
10% APS	15.00	15.00	15.00	15.50	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
10% TEMED	3.68	3.21	2.85	2.57	2.33	2.15	1.98	1.83	1.71	1.61	1.52	1.43	1.35

12 gels, 1.0 mm

800 ml. Amounts shown in ml.

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acrylamide Stock	187	213	240	267	293	320	347	373	400	427	453	480	507
1.5M Tris-Cl, pH 8.8	200	200	200	200	200	200	200	200	200	200	200	200	200
Water	395	369	342	316	289	263	236	210	183	156	130	103	77
10% SDS	8	8	8	8	8	8	8	8	8	8	8	8	8
10% APS	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
10% TEMED	1.96	1.71	1.52	1.37	1.24	1.14	1.06	0.98	0.91	0.86	0.81	0.76	0.72

23 gels, 1.5 mm

1800 ml. Amounts shown in ml.

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acrylamide Stock	420	480	540	600	660	720	780	840	900	960	1020	1080	1140
1.5M Tris-Cl, pH 8.8	450	450	450	450	450	450	450	450	450	450	450	450	450
Water	890	830	771	711	651	591	532	472	412	352	292	232	172
10% SDS	18	18	18	18	18	18	18	18	18	18	18	18	18
10% APS	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
10% TEMED	4.41	3.85	3.42	3.08	2.79	2.57	2.38	2.20	2.05	1.93	1.82	1.71	1.62

12 gels, 1.5 mm

1300 ml. Amounts shown in ml.

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acrylamide Stock	257	293	330	367	403	440	447	513	550	587	623	660	697
1.5M Tris-Cl, pH 8.8	275	275	275	275	275	275	275	275	275	275	275	275	275
Water	544	507	471	434	398	361	325	288	252	215	179	142	105
10% SDS	11	11	11	11	11	11	11	11	11	11	11	11	11
10% APS	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
10% TEMED	2.70	2.35	2.09	1.88	1.71	1.557	1.45	1.34	1.25	1.18	1.11	1.05	0.99

Gradient Gel Solutions

The concentrations of APS and TEMED in gradient solutions vary to assure top-down polymerization. We suggest: for light solutions, 0.025 – 0.09% (v/v) TEMED and 0.1% (w/v) APS; for heavy solutions 0.0071 – 0.0028% (v/v) TEMED and 0.05% (w/v) APS. The amounts of TEMED and APS suggested here are based on our experience. You may want to change these volumes for your lab because of differences in temperature and reagent quality.

Perform a small-scale test before first using a new composition to check that your light solution polymerizes in about 10 minutes and your heavy solution in about 20 minutes. Vary the TEMED concentration to control the rate of polymerization.

Note that the light mix is always assumed to have a lower acrylamide concentration than the heavy mix; the gradient increases in %T from top to bottom. These solutions can be degassed, though this is not usually necessary.

Light Solution, 1000 ml. Amounts shown in ml.

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	233	267	300	333	367	400	433	467	500	533	567	600	633
1.5M Tris-Cl, pH 8.8	250	250	250	250	250	250	250	250	250	250	250	250	250
Water	494	461	428	395	362	329	295	262	229	196	162	129	96
10% SDS	10	10	10	10	10	10	10	10	10	10	10	10	10
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0
10% APS	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
10% TEMED	2.45	2.14	1.90	1.71	1.55	1.43	1.32	1.22	1.14	1.07	1.01	0.95	0.90

Heavy Solution, 1000 ml Amounts shown in ml.

Final %T	8	9	10	11	12	13	14	15	16	17	18	19	20
Acryl. Stock	267	300	333	367	400	433	467	500	533	567	600	633	667
1.5M Tris-Cl, pH 8.8	250	250	250	250	250	250	250	250	250	250	250	250	250
Water	400	366	333	300	267	233	200	167	133	100	67	33	0
10% SDS	10	10	10	10	10	10	10	10	10	10	10	10	10
Glycerol	68	68	68	68	68	68	68	68	68	68	68	68	68
10% APS	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
10% TEMED	0.71	0.64	0.57	0.52	0.47	0.44	0.41	0.38	0.35	0.34	0.32	0.30	0.28

For 25 gels, 1.0 mm

750 ml each Heavy and Light, **by volume** in ml.

Light Solution

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	175	200	225	250	275	300	325	350	375	400	425	450	475
1.5M Tris-Cl, pH 8.8	188	188	188	188	188	188	188	188	188	188	188	188	188
Water	371	346	321	296	271	246	222	197	172	147	122	97	72
10% SDS	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0
10% APS	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
10% TEMED	1.84	1.61	1.43	1.28	1.16	11.07	0.99	0.92	0.86	00.80	0.76	0.71	0.68

Heavy Solution

Final %T	8	9	10	11	12	13	14	15	16	17	18	19	20
Acryl. Stock	200	225	250	275	300	325	350	375	400	425	450	475	500
1.5M Tris-Cl, pH 8.8	188	188	188	188	188	188	188	188	188	188	188	188	188
Water	300	275	250	225	200	175	150	125	100	75	50	25	0
10% SDS	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Glycerol	51	51	51	51	51	51	51	51	51	51	51	51	51
10% APS	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
10% TEMED	0.53	0.48	0..43	0.39	0.35	0.33	0.31	0..29	0.26	0.26	0.24	0.23	0.21

For 12 gels, 1.0 mm

400 ml each Heavy and Light, **by volume** in ml.

Light Solution

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	93	107	120	133	147	160	173	187	200	213	227	24	253
1.5M Tris-Cl, pH 8.8	100	100	100	100	100	100	100	100	100	100	100	100	100
Water	198	184	171	158	145	131	118	105	92	78	65	52	38
10% SDS	4	4	4	4	4	4	4	4	4	4	4	4	4
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0
10% APS	4	4	4	4	4	4	4	4	4	4	4	4	4
10% TEMED	0.98	0.86	0.76	0.68	0.62	0.57	0.53	0.49	0.46	0.43	0.40	0.38	0.36

Heavy Solution

Final %T	8	9	10	11	12	13	14	15	16	17	18	19	20
Acryl. Stock	107	120	133	147	160	173	187	200	213	227	240	253	267
1.5M Tris-Cl, pH 8.8	100	100	100	100	100	100	100	100	100	100	100	100	100
Water	160	147	133	120	107	93	80	67	53	40	27	13	0
10% SDS	4	4	4	4	4	4	4	4	4	4	4	4	4
Glycerol	27	27	27	27	27	27	27	27	27	27	27	27	27
10% APS	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
10% TEMED	0.28	0.26	0.23	0.21	0.19	0.18	0.16	0.15	0.14	0.14	0.13	0.12	0.11

For 23 gels, 1.5 mm

900 ml each Heavy and Light, **by volume** in ml:

Light Solution

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	210	240	270	300	330	360	390	420	450	480	510	540	570
1.5M Tris-Cl, pH 8.8	225	225	225	225	225	225	225	225	225	225	225	225	225
Water	445	415	385	355	326	296	266	236	206	176	146	116	86
10% SDS	9	9	9	9	9	9	9	9	9	9	9	9	9
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0
10% APS	9	9	9	9	9	9	9	9	9	9	9	9	9
10% TEMED	2.21	1.93	1.71	1.54	1.40	1.29	1.19	1.10	1.03	0.96	0.91	0.86	0.81

Heavy Solution

Final %T	8	9	10	11	12	13	14	15	16	17	18	19	20
Acryl. Stock	240	270	300	330	360	390	420	450	480	510	540	570	600
1.5M Tris-Cl, pH 8.8	225	225	225	225	225	225	225	225	225	225	225	225	225
Water	360	330	300	270	240	210	180	150	120	90	60	30	0
10% SDS	9	9	9	9	9	9	9	9	9	9	9	9	9
Glycerol	61	61	61	61	61	61	61	61	61	61	61	61	61
10% APS	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
10% TEMED	0.64	0.58	0.51	0.47	0.42	0.40	0.37	0.34	0.32	0.31	00.29	0.27	0.25

For 12 gels, 1.5 mm

550 ml each Heavy and Light, **by volume** in ml.

Light Solution

Final %T	7	8	9	10	11	12	13	14	15	16	17	18	19
Acryl. Stock	128	147	165	183	202	220	238	257	275	293	312	330	348
1.5M Tris-Cl, pH 8.8	138	138	138	138	138	138	138	138	138	138	138	138	138
Water	272	254	235	217	199	181	162	144	126	108	89	71	53
10% SDS	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0
10% APS	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
10% TEMED	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35

Heavy Solution

Final %T	8	9	10	11	12	13	14	15	16	17	18	19	20
Acryl. Stock	147	165	183	202	220	238	257	275	293	312	330	348	367
1.5M Tris-Cl, pH 8.8	138	138	138	138	138	138	138	138	138	138	138	138	138
Water	220	201	183	165	147	128	110	92	73	55	37	18	0
10% SDS	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Glycerol	37	37	37	37	37	37	37	37	37	37	37	37	37
10% APS	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75
10% TEMED	0.393	0.35	0.31	0.29	0.26	0.24	0.23	0.21	0.19	0.19	0.18	0.17	0.15

Gel Identification Numbers

For positive identification of gels, label each slab by incorporating a small label printed on thin filter paper in the bottom corner of the gel. Use a carbon typewriter ribbon, photocopier or laser printer to make these labels, since many liquid-based inks are electrophoresed off paper during an SDS electrophoresis run.

A variety of numbering schemes are possible. In our experience the easiest uses three parts as follows:

- An upper-case letter to identify the investigator or an extended gel series.
- A two- or three-digit serial number to identify the slab gel batch.
- A lower-case letter to identify a gel in the batch. Since a maximum of 22 gels can be made in a batch, use the letters a-v.

The resulting numbers, in the format A63a, A63b...., etc., provide a useful system for keeping track of and cross-indexing experiments and gel production.

Care and Maintenance

Cleaning

- Do not autoclave or heat any part above 45 °C.
- Do not expose the unit or its parts to organic solvents.
- If using radioactive reagents, decontaminate the unit with a cleaning agent such as CONTRAD 70 or Decon 90 from Decon Laboratories, Inc.

Rinse the DALT Tank, cassettes and sponges with distilled water immediately after each use. Allow the unit to air dry completely. Periodically wash with a dilute solution of a mild detergent.

Clean gaskets with mild detergent and rinse with distilled water. Allow to air dry.

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

Removing the Electrode Panels from the DALT Tank

You can remove each electrode panel for more thorough cleaning, or to replace damaged electrodes.

1. Use a Phillip's head screwdriver to loosen the white plastic retaining screws on the exterior of the DALT Tank. You do not need to remove the screws.
2. Lift and slide the panel out.

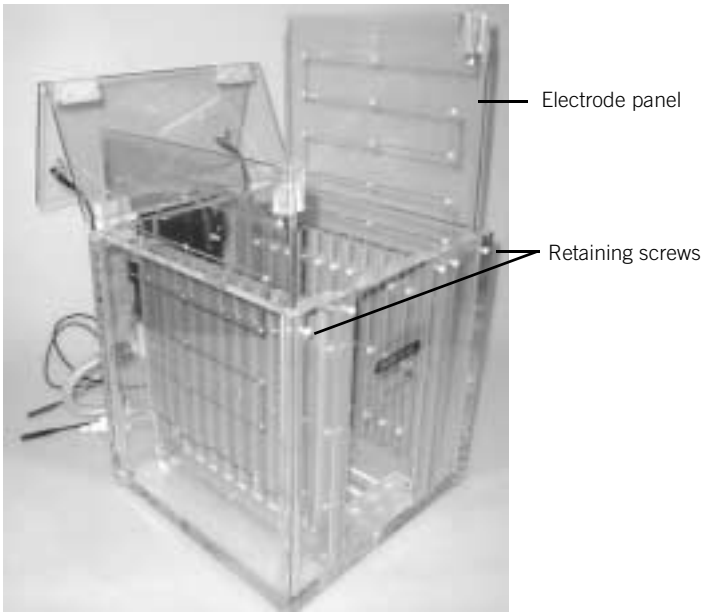


Figure 24. Removing the electrode panels

Handle the electrode panel carefully to avoid stretching or breaking the platinum wire.

Troubleshooting

Gel Casting

Gel caster leaks

- Apply a light film of GelSeal to the foam gasket each time the unit is used.
- Check the foam gasket for cracks or nicks and replace if necessary.
- If the stack is too tall, the front plate may not seat firmly against the gasket. Remove filler plates or cassettes until the gasket seals.

Incomplete gel polymerization

- Use only recent stock of the highest quality reagents.
- If the dry ammonium persulfate does not crackle when water is added to it, replace with fresh stock.
- Solutions with extreme pH values (especially acidic) may not polymerize.
- Remove oxygen from the gel environment. Degas the monomer solution 5 minutes before pouring and then overlay the gel surface with water-saturated n-butanol.
- Adjust the gel solution temperature to a minimum of 20 °C, especially for low% T gels.
- Increase both APS and TEMED by 30 – 50%.
- Make up fresh APS daily.

Gel is too soft, too brittle or white

- Adjust crosslinker concentration. Crosslinker should be at 2.6% C for standard SDS gels where %C = (g bis × 100) ÷ (g monomer + g bis)

Gel contains swirls

- If gel polymerized in <10 min, too much catalyst. Reduce concentration of ammonium persulfate and TEMED by 25%.
- If gel polymerized in >50 min, not enough catalyst. Increase concentration of ammonium persulfate and TEMED by 50%.
- Make up fresh acrylamide stock solution.

Dye front curves up (smiles) at the edges.

- Fill the lower buffer chamber to the level appropriate for the run.
- Circulate coolant.
- Pre-chill the buffer.
- Decrease the current or voltage setting.

Vertical protein streaks

- IPG Strip not properly placed on gel surface. Avoid gouging separating gel while loading strips.
- Perform iodoacetamide treatment.
- Make sure IPG strip uniformly contacts the gel surface along the entire length of the strip.

Gels cast simultaneously are different sizes.

- Wait one minute before overlaying each gel so that the solution “settles.” Use the same amount of overlay on each separation gel. Add the overlay as rapidly as possible.
- Make sure the cassettes and foam sponges are not clogged with polyacrylamide.

Gradient gels-uneven layering

- Add sucrose (15% final concentration) or glycerol (25% final concentration) to the high-percent monomer solution.
- Add a small amount of bromophenol blue to the heavy solution to track the gradient formation. Excessive amounts of bromophenol blue inhibit polymerization.

Power Supply detects current leak

- Cracked or broken heat exchangers. Call your Amersham Biosciences Service representative.

Spots are skewed or distorted

- Gels run too fast-uneven migration.
- Overlay the running gel with water-saturated *n*-butanol before polymerization begins to avoid forming an uneven gel surface.
- Uneven gel polymerization or gradient formation.

Heavy background after silver staining

- Use reagents specified as electrophoresis purity.
- Use only double-distilled water.

Unusually slow or fast run

- Check for leaks; all plates and spacers must be clean, dry and free of grease.
- Make sure buffer level is not above the level of the upper spacer.
- If the required pH of a solution is exceeded, do not back-titrate. Prepare fresh buffer.
- Check recipes, gel concentrations, and buffer dilutions. (For instance, do not use Tris·HCl in place of Tris for the SDS electrophoresis buffer.)
- Dispose of older acrylamide solutions and use only stock of the highest quality.
- Only use freshly deionized urea.
- To increase or decrease the migration rate, adjust the voltage or current by 25–50%

Protein spots are diffuse or broader than usual

- Use only high-quality acrylamide and bis.
- Ensure that polymerization is complete.
- Fully equilibrate IPG strips before second dimension.
- Incomplete IPG focusing.
- Make sure the IPG strip rests on the gel surface without gouging or separating the gel.

Stained Sample Collects:

Near the buffer front

- Molecules are not sufficiently restricted by the resolving gel pore size; increase the %T.
- Proteins may be degraded by endogenous proteases; use protease inhibitors during sample separation.
- Adjust the pH of 1.5 M Tris-Cl to pH 8.8. Samples migrate faster when pH <8.8.

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

- The gel pore size is too small. Decrease the %T of the resolving (or stacking) gel.
- Adjust the pH of 1.5 M Tris-Cl to pH 8.8. Samples migrate slower when pH >8.8

At each end of the gel

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

Poor Spot Resolution

- Allow gel to polymerize fully.
- Begin electrophoresis as soon as the IPG strips are loaded to prevent low molecular weight species from diffusing.
- Conduct the separation at a lower current or voltage setting.
- Reduce the temperature setting

Reagent quality and gel preparation

- Use only the highest quality reagents.
- Only use gels that were recently prepared.
- Check pH values of the stacking gel solutions. Do not back-titrate buffers.

Sample preparation

- Store IPG strips at –40 °C or below.
- Add a protease inhibitor, such as phenylmethylsulfonyl fluoride (PMSF), if necessary, to prevent proteolytic degradation of sample.

Insufficient equilibration

- Extend equilibration to 15 minutes.
- Add DTT and iodoacetamide fresh before use.

Tracking Dye Doesn't Sharpen into a Concentrated Zone in the Stacking Gel

- Dispose of outdated acrylamide solutions and use only the highest grade of acrylamide.
- Buffer reused too many times. Prepare fresh each time, for best results.

Incomplete Transfer

Blank areas on the membrane

- Remove all trapped air pockets in the transfer stack assembly. Assemble the stack while it is submerged in transfer buffer. Gently press on each sponge as it is added to the stack. Roll a glass pipette or test tube over the membranes and gel to eliminate all bubbles.
- Process only one strip or membrane in each tray or cassette to prevent overlapping.
- Use buffer with a lower ionic strength.
- Check electrode continuity. During the transfer, a continuous stream of gas is released along the entire length of the electrodes. If bubbles do not form along the entire length of the electrode, replace the electrode.

Grid pattern on membrane

- Add sheets of blotting paper to increase the clearance between the cassette panel and the gel. Take care not to overstuff the cassette. The gel should be held firmly and evenly between the sponges, but not so tightly that it is squeezed.

Molecules do not migrate out of gel

- Increase the field strength.
- Increase, or double, the transfer period.
- Do not use staining or fixing agents on the gel before transfer.
- Use a thinner gel.
- Reduce the gel acrylamide concentration.
- Check that the buffer pH is close to the intended pH. Most buffers should not be titrated. Make fresh buffer.
- Use 3.5 mM SDS (0.1%) in the transfer buffer.
- Use reagent-grade chemicals.
- Increase the net charge on the protein by changing to a transfer buffer with a different pH. Lower pH (<6) increases the positive charge on proteins. Higher pH (>6) increases the negative charge on proteins.

Diffuse band patterns

- Transfer immediately after electrophoretic separation. If equilibrating before the transfer, shorten or eliminate the equilibration time or move the gel to the cold room during equilibration.
- If transfer buffer contains methanol (≥ 10%), equilibrate the gel before in transfer buffer for 30 minutes to allow it to shrink before assembling the stack. Because methanol causes the gel to shrink slightly, large molecules may migrate more slowly.
- Make sure the gel is held firmly against the membrane and that it does not shift once contact is made.
- If excess heating occurs during the transfer, lower the temperature setting of the circulating cooler.
- Check that the preferred binding surface of the membrane, if any, contacts the gel.

Inefficient binding to membrane

Chemical parameters

- Prepare protein transfer buffer **without** SDS.
- Verify the optimal amount of methanol required for the membrane type and check the buffer solution. Add 10 - 20% methanol to the transfer buffer to enhance binding to nitrocellulose.

Membrane parameters

- Wear gloves when handling membranes.
- Store membranes at ambient temperature out of direct sunlight to keep the membranes activated.
- Use a membrane with a smaller pore size (0.10 - 0.20 μm) if proteins pass through the membrane, or use a different membrane type.
- If you suspect one protein is moving in the opposite direction from the majority of the proteins, place a membrane both over and under the gel. Check both membranes for proteins.
- Check if too much sample is available for the binding surface area by applying two membranes instead of one. If “blow through” occurs, reduce the sample load.

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Customer Service Information

Technical Service and Repair

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

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

Ordering information

	Qty.	Code No.
Hoefer DALT Multiple Electrophoresis Tank with buffer circulation pump		
115 V~	1	80-6068-79
230 V~	1	80-6068-98
DALT Multiple Gel Caster with filler blocks and separator sheets. Cassettes not included.	1	80-6330-61
DALT Gel Cassette		
for 1.0 mm thick gel, 25 × 20 cm	1	80-6067-27
for 1.5 mm thick gel, 25 × 20 cm	1	80-6067-46
Separator sheets, 30/pkg	1 pkg	80-6436-63
Filler block set: four 25-mm, one 12-mm, one 6-mm, one 3-mm	1 set	80-6436-82
Knobs, 4/pkg	1 pkg	80-6437-58
Sponge funnel, 8 pieces	1 pkg	80-6437-01
Acrylic feed tube, 4.6 mm o.d, 178 mm	1	80-6437-20
Foam sealing gasket	1	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	1 pkg	80-6437-39
DALT Gradient Maker with peristaltic pump		
115 VAC	1	80-6067-65
230 VAC	1	80-6067-84
Gradient maker gasket/divider	1	80-6068-41
Gasket adjuster rod	1	80-6068-60
Knobs, 4/pkg	1 pkg	80-6437-58
Plastic feed tubing (6 feet)	1	80-6068-03
Bow-tie mixer kit	1	80-6068-22
Acrylic feed tube, 4.6 mm o.d., 178 mm	1	80-6437-20

	Qty.	Code No.
DALT Blotting Kit with rack, 5 transfer cassettes and sponges, blotting paper (50 pc)	1	80-6069-17
DALT Transfer Cassette, with 2 sponges	1	80-6069-55
Sponges	2	80-6069-74
DALT Blotting Paper, 24 × 20 cm (50/pkg)	1 pkg	80-6069-93
Replacement Parts		
GelSeal, 1/4 oz. tube	1	80-6421-43
Wonder Wedge	1	80-6127-88
Knobs, for Gel Caster or Gradient Maker, 4/pkg	1 pkg	80-6437-58
Quick-fit connectors, female, to fit 9 mm i.d. tubing	2	80-6115-15
Quick-fit connectors, male, to fit 9 mm i.d. tubing	2	80-6115-53
Accessories		
MultiTemp III Thermostatic Circulator includes insulation tubing for cooling		
115 VAC	1	18-1102-77
230 VAC	1	18-1102-78
Hoefer EPS 2A200 Power Supply, 200V, 2000 mA	1	80-6406-99
EPS 601 Power Supply, 600 V, 400 mA	1	18-1130-02
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%)	1000 ml	17-1325-01
Acrylamide IEF (acrylic acid <0.002%)	1 kg	17-1300-02
Acrylamide IEF 40% solution	1000 ml	17-1301-01
N,N',-Methylene bisacrylamide	25 g	17-1304-01
N,N',-Methylene bisacrylamide 2% solution	1000 ml	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
Molecular Weight Markers		
MW Range 2,512 – 16,949, 2 mg/vial	1 vial	80-1129-83
MW Range 14,400 – 94,000, 575 µg/vial	10 vials	17-0446-01
MW Range 53,000 – 212,000, 175 µg/vial	10 vials	17-0615-01
2-D Technical Manual		
2-D Electrophoresis: Using Immobilized pH Gradients	1	80-6429-60

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