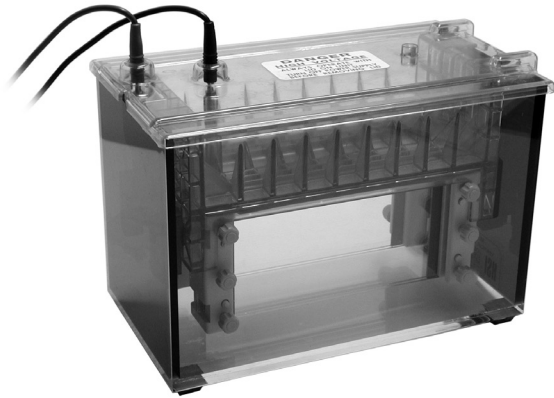


Hofer SE640

Wide-mini Dual Gel Electrophoresis Unit





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Important Information – English

- If this equipment is used in a manner not specified by Hoefer, Inc. the protection provided by the equipment may be impaired.
- This instrument is designed for indoor laboratory use only.
- Only accessories and parts approved or supplied by Hoefer, Inc. may be used for operating, maintaining, and servicing this product.
- Only use a power supply that is CE marked or safety certified by a nationally recognized testing laboratory.
- The safety lid must be in place before connecting the power supply leads to a power supply.
- Turn all power supply controls off and disconnect the power leads before removing the safety lid.
- Circulate only water or 50/50 water/ethylene glycol through the heat exchanger if so equipped. Do not connect the heat exchanger to a water tap or any coolant source where the water pressure is unregulated.
- Never introduce antifreeze or any organic solvent into any part of the instrument. Organic solvents will cause irreparable damage to the unit!
- Do not operate with buffer temperatures above the maximum specified technical specifications. Overheating will cause irreparable damage to the unit!

Důležité Informace – Czech

- Pokud by toto zařízení je použito způsobem, který není podle Hoefer, Inc. ochrana poskytovaná na základě zařízení může být narušena.
- Tento nástroj je určen pro vnitřní použití v laboratoři pouze.
- Pouze příslušenství a části schválené, nebo poskytnuté Hoefer, Inc. mohou být použity pro provoz, údržbu, a údržbě tohoto výrobku.
- zdroj napájení používají jen že je opatřeno označením CE osvědčena nebo bezpečnost vnitrostátně uznanými zkušebními laboratoři.
- Bezpečnosti lid musí být zavedena před připojením napájecí zdroj napájení vede k.
- Turn veškeré napájení kontroly vypnuto a odpojit před odběrem energie vede bezpečnostní víko.

- Rozeslat pouze voda nebo 50/50 voda/ethylenglykolu prostřednictvím výměník tepla je li to vybalena. Nemají připojení výměník tepla s vodními setřepná nebo jakékoli chladicí kapaliny zdroje, kde tlak vody je neregulováno.
- Nikdy zavést prostředek proti zamrznutí nebo jakákoli organická rozpouštědla do jakékoli části z tohoto nástroje. Rozpustidly způsobí nenapravitelné poškození jednotka!
- Nejsou provozována s pufru teplotách nad maximální stanovenou technickými specifikacemi. Přehřátí způsobí nenapravitelné poškození jednotka!

Vigtig Information – Danish

- Hvis dette udstyr bruges i en måde ikke specificeret ved Hoefer, Inc. den beskyttelse, som er blevet forsynet af udstyret kan måske svækkes.
- Dette instrument er designet for indendørs laboratoriumbrug bare.
- Bare tilbehør og del godkendte eller forsynede ved Hoefer, Inc. kan måske bruges for drive, funktionsfejl, og betjening dette produkt.
- bruger Bare en strømforsyning, der er CE markerede eller sikkerhed, som er blevet attesteret af en, som nationalt er blevet anerkendt prøve laboratorium.
- Sikkerhedslåget må være på plads for forbindelse strømforsyningsblyet til en strømforsyning.
- Drejer alle strømforsyningskontroller af og afbryder kraftblyet før fjerning sikkerhedslåget.
- Cirkulerer bare vand eller 50/50 vand/ethylene glykol gennem varmeveksleren i så fald udrustet. Forbind ikke varmeveksleren til en vandhane eller nogen kølemiddelkilde hvor vandtrykket er unreguleret.
- Introducerer Aldrig antifreeze eller noget organisk opløsningsmiddel ind i nogen del af instrumentet. Organiske opløsningsmidler vil forårsage uboelig skade til enheden!
- Driver ikke med stødpudetemperaturer over maksimummet specificerede tekniske specifikationer. Overhedning vil forårsage uboelig skade til enheden!

Belangrijke Informatie – Dutch

- Indien deze uitrusting in een manier wordt gebruikt die niet door Hoefler, Inc. is gespecificeerd de bescherming die door de uitrusting is verzorgd kan worden geschaad.
- Dit instrument is voor binnenlaboratoriumgebruik enkel ontworpen.
- Enkel onderdelen en delen keurden goed of leverden door Hoefler, Inc. kan voor het bedienen worden gebruikt, handhavend en onderhouden van dit product.
- gebruik Enkel een netvoeding die CE is markeerde of veiligheid die door een is gecertificeerd die nationaal is herkend testene laboratorium.
- Het veiligheidsdeksel moet in plaats voor het verbinden van de netvoeding leidt tot een netvoeding zijn.
- Doe alle netvoedingscontroles Uit en koppel los de machtleiding voor het verwijderen van het veiligheidsdeksel.
- Circuleer enkel water of 50/50 water/ethyleen-glycol door de hitte exchanger zo ja uitrust. Verbind de hitte exchanger naar een waterkraan of koelmiddelbron niet waar de waterdruk niet geregulariseerd is.
- Stel Nooit antivriesmiddel of organische oplosmiddelen in deel van het instrument voor. Organische oplosmiddelen zullen onherstelbare schade aan de eenheid veroorzaken!
- Bedien niet met buffertemperaturen boven het maximum specificerde technische specificaties. Oververhittend zal onherstelbare schade aan de eenheid veroorzaken!

Tärkeää Tietoa – Finnish

- Jos tätä varusteita käytetään tavassa ei määritetty Hoefler, Inc. suojele ehkäisty varusteille saattaa olla avuton.
- Tämä väline suunnitellaan sisälaboratoriokäytölle vain.
- Vain lisävarusteet ja osat hyväksyivät tai toimitti Hoefler, Inc. oheen ää voi käyttää käyttämiselle, valvoalle, ja servicing tämä tuote.
- Vain käyttää käyttöjännitettä joka on CE merkitsi tai turvallisuus joka on todistanut aidoksi ohi joka

on kansallisesti tunnustettanut testaaminen laboratorioti.

- Turvallisuuskansi täytyy olla paikallaan ennen yhdistäminen käyttöjännityjyjä käyttöjännitteeseen.
- Kiertää kaikki käyttöjännitevalvonnan ja irrottaa valtalyijyt ennen poistaminen turvallisuuskantta.
- Kiertää vain vesi tai 50/50 vesi/ethyleeni glycol siinä tapauksessa varustetun lämmönvaihtimen läpi. Älä yhdistä lämmönvaihdinta vesinapautukseen eikä jäähdytysnestelähteeseen, missä vesipaine on unregulated.
- Pakkasneste eikä orgaaninen liuotin välineen osassa ei esitele Koskaan. Organiset liuottimet aiheuttavat korvaamattoman vahingon yksikköön!
- Ei käytä puskuria yllä olevia lämpötiloja enintään määritetyillä teknisillä täsmennyksillä. Ylikuumeneminen aiheuttaa korvaamattoman vahingon yksikköön!

Information Importante – French

- Si cet équipement est utilisé dans une manière pas spécifique par Hoefler, Inc. la protection fourni par l'équipement pourrait être diminuée.
- Cet instrument est conçu pour l'usage de laboratoire intérieur seulement.
- Seulement les accessoires et les parties ont approuvé ou ont fourni par Hoefler, Inc. pourrait être utilisé pour fonctionner, maintenir, et entretenir ce produit.
- utilise Seulement une alimentation qui est CET a marqué ou la sécurité certifié par un nationalement reconnu essayant le laboratoire.
- Le couvercle de sécurité doit être à sa place avant connecter l'alimentation mène à une alimentation.
- Tourner tous contrôles d'alimentation de et débrancher les avances de pouvoir avant enlever le couvercle de sécurité.
- Circuler seulement de l'eau ou 50/50 glycol d'eau/éthylène par l'échangeur de chaleur si si équipé. Ne pas connecter l'échangeur de chaleur à un robinet d'eau ou à la source d'agent de refroidissement où la pression d'eau est non régulée.
- Ne Jamais introduire d'antigel ou du dissolvant organique dans n'importe quelle partie de l'instrument. Les dissolvants organiques causeront

des dommages irréparables à l'unité!

- Ne pas fonctionner avec les températures de tampon au-dessus du maximum a spécifié des spécifications techniques. La surchauffe causera des dommages irréparables à l'unité !

Wichtige Informationen – German

- Wenn diese Ausrüstung gewissermaßen nicht angegeben durch Hoefer, Inc. verwendet wird, kann der durch die Ausrüstung zur Verfügung gestellte Schutz verschlechtert werden.
- Dieses Instrument wird für den Innenlaborgebrauch nur dafür entworfen.
- Nur Zusätze und Teile genehmigten oder lieferten durch Hoefer, Inc. kann für das Funktionieren, das Aufrechterhalten, und die Wartung dieses Produktes verwendet werden.
- Verwenden Sie nur eine Energieversorgung, die CE gekennzeichnet oder durch ein national anerkanntes Probelaboratorium bescheinigte Sicherheit ist.
- Der Sicherheitsdeckel muss im Platz vor dem Anschließen der Energieversorgung sein führt zu einer Energieversorgung.
- Alle Energieversorgungssteuerungen abdrehen und die Macht trennen führt vor dem Entfernen des Sicherheitsdeckels.
- Nur Wasser oder 50/50 Glykol des Wassers/ Äthylens durch den Wärmeaustauscher, wenn so ausgestattet, in Umlauf setzen. Verbinden Sie den Wärmeaustauscher mit einem Wasserklaps oder jeder Kühlmittel-Quelle nicht, wo der Wasserdruck unregelt wird.
- Führen Sie nie Frostschutzmittel oder jedes organische Lösungsmittel in jeden Teil des Instrumentes ein. Organische Lösungsmittel werden nicht wiedergutzumachenden Schaden der Einheit verursachen!
- Mit Puffertemperaturen über angegebenen technischen Spezifizierungen des Maximums nicht funktionieren. Die Überhitzung wird nicht wiedergutzumachenden Schaden der Einheit verursachen!

Informazioni Importanti – Italian

- Se quest'apparecchiatura è usata in un modo specificato da Hoefer, Inc. la protezione fornito dall'apparecchiatura potrebbe essere indebolita.
- Questo strumento è disegnato per l'uso di laboratorio interno solo.
- Solo gli accessori e le parti hanno approvato o hanno fornito da Hoefer, Inc. potrebbe essere usato per operare, per mantenere, e per revisionare questo prodotto.
- usa Solo un alimentatore che è CE ha marcato o la sicurezza certificato da un nationalmente riconosciuto testando il laboratorio.
- Il coperchio di sicurezza deve essere nel luogo prima di collegare i piombi di alimentatore a un alimentatore.
- Spagne tutto i controlli di alimentatore e disinserisce i piombi di potere prima di togliere il coperchio di sicurezza.
- Circola solo l'acqua o 50/50 glicole di acqua/etilene attraverso lo scambiatore di calore se così equipaggiato. Non collegare lo scambiatore di calore a un rubinetto di acqua o qualunque fonte di refrigerante dove la pressione di acqua è sregolata.
- Non introduce mai l'antigel o qualunque solvente organico in qualunque parte dello strumento. I solventi organici causeranno il danno irreparabile all'unità!
- Non opera con le temperature di tampone al di sopra del massimo ha specificato le descrizioni tecniche. Il surriscaldamento causerà il danno irreparabile all'unità!

Viktige Informasjon – Norwegian

- Hvis dette utstyret blir brukt i en måte ikke spesifisert ved Hoefer, Inc. beskyttelsen som ha blitt gitt av utstyret kan bli svekket.
- Dette instrumentet er utformet for innendørs laboratoriumbruk bare.
- Bare tilbehør og deler godkjente eller forsynte ved Hoefer, Inc. kan bli brukt for drive, vedlikeholde, og betjene dette produktet.
- bruker Bare en kraftforsyning som er CE merket eller sikkerhet som ha blitt sertifisert av et som nasjonalt ha blitt anerkjent prøver laboratorium.

- Sikkerheten lokket må være på plass før forbindelse kraftforsyningene blyene til en kraftforsyning.
- Vender all kraftforsyningsstyring av og frakopler kreftene blyene før fjerning sikkerheten lokket.
- Sirkulerer bare vann eller 50/50 vann/ethylene glykol gjennom oppvarmingen veksleren i så fall utstyrer. Ikke forbind oppvarmingen veksleren til en vann tapp eller noe kjølemiddelkilde hvor vannet trykket er unregulated.
- Introduserer Aldri antifreeze eller noe organisk løsemiddel inn i noe del av instrumentet. Organiske løsemidler vil forårsake irreparabel skade på enheten !
- Driver med buffertemperaturer over maksimum ikke spesifiserte teknisk spesifikasjoner. Å overoppheting vil forårsake irreparabel skade på enheten !

Wazne Informacje – Polish

- Jeżeli ten sprzęt jest wykorzystywany w sposób nie określone przez Hoefer, Inc. do ochrony przewidzianej przez urządzenie może zostać obniżony.
- Instrument ten jest przeznaczony do użytku w laboratoriach kryty tylko.
- Tylko akcesoriów i części zatwierdzone lub dostarczone przez Hoefer, Inc. mogą być wykorzystane do eksploatacji, utrzymania i obsługi tego produktu.
- korzystać jedynie zasilacza że jest noszące oznakowanie CE lub bezpieczeństwa uwierzytelnione przez uznane na poziomie krajowym laboratorium badawcze.
- Bezpieczeństwo lid musi być w miejsce przed podłączeniem zasilania prowadzi do zasilania.
- Zaś wszystkie źródła zasilania urządzenia sterujące off i odłączyć moc prowadzi przed odbiorem bezpieczeństwa lid.
- Krążą tylko wody lub wody 50/50/ethylene glycol wymiennik ciepła poprzez jeśli tak wyposażone. Nie należy połączyć wymiennik ciepła woda z kranu lub jakimkolwiek chłodziwo źródła, jeżeli ciśnienie wody jest nieuregulowanych.
- Nigdy nie wprowadzać rozpuszczalnika organicznego przeciw zamarzaniu lub jakichkolwiek na dowolną część dokumentu. Rozpuszczalniki organiczne spowoduje nieodwracalne szkody dla jednostki!

- Nie działają w buforze temperatury powyżej maksymalnego określone specyfikacje techniczne. Przegrzania spowoduje nieodwracalne szkody dla jednostki!

Informações Importantes – Portuguese

- Se este equipamento é usado numa maneira não especificada por Hoefer, Inc. que a protecção fornecida pelo equipamento pode ser comprometida.
- Este instrumento é projectado para uso de interior de laboratório só.
- Só acessórios e partes aprovaram ou forneceu por Hoefer, Inc. pode ser usada para operar, manter, e servicing este produto.
- Só usa um estoque de poder que é CE marcou ou segurança registrada por um nacionalmente reconhecido testando laboratório.
- A tampa de segurança deve estar em lugar antes de ligar o estoque de poder leva a um estoque de poder.
- Desliga todos controles de estoque de poder e desconecta os chumbos de poder antes de retirar a tampa de segurança.
- Circulam só água ou 50/50 glicol de água/ethylene pelo exchanger de calor se for assim equiparam. Não ligue o exchanger de calor a uma torneira de água nem qualquer fonte de refrigerante onde a pressão de água é não regulado.
- Nunca introduz anticongelante nem qualquer orgânico solvente em qualquer parte do instrumento. Orgânico solvente causará agressão irreparável à unidade!
- Não opera com temperaturas de buffer acima do máximo especificou especificações técnicas. Superaquecer causará agressão irreparável à unidade!

Información Importante – Spanish

- Si este equipo es utilizado en una manera no especificado por Hoefer, Inc. la protección proporcionado por el equipo puede ser dañada.
- Este instrumento es diseñado para el uso interior del laboratorio sólo.
- Sólo accesorios y partes aprobaron o suministraron

-
- por Hoefler, Inc. puede ser utilizado para operar, para mantener, y para atender a este producto.
- Sólo utiliza una alimentación que es CE marcó o la seguridad certificada por un nacionalmente reconocido probando el laboratorio.
 - La tapa de la seguridad debe estar en el lugar antes de conectar la alimentación lleva a una alimentación.
 - Apaga todos controles de alimentación y desconecta los plomos del poder antes de quitar la tapa de la seguridad.
 - Circula sólo agua o 50/50 glicol de agua/etileno por el intercambiador de calor si ése es el caso equiparon. No conecte el intercambiador de calor a un toque de la agua ni cualquier fuente del líquido refrigerante donde la presión del agua está libre.
 - Nunca introduce anticongelante ni algún solvente orgánico en cualquier parte del instrumento. Los solventes orgánicos causarán daño irreparable a la unidad!
 - No opera con temperaturas de búfer encima del máximo especificó especificaciones técnicas. Recalentar causará daño irreparable a la unidad!

Viktig Information – Swedish

- om denna utrustning används i ett sätt som inte har specificeras av Hoefler, Inc. skyddet tillhandahöll vid utrustningen kan skadas.
- Detta instrument formges för inomhuslaboratorium användning bara.
- Bara medhjälpare och delar godkände eller levererade vid Hoefler, Inc. kan användas för fungera, underhålla, och servicing denna produkt.
- använder bara en kraft tillgång som är CE markerade eller säkerhet intygade vid en nationellt erkänd testande laboratorium.
- Säkerheten locket måste vara på platsen före koppla kraften tillgången blyen till en kraft tillgång.
- Vänder sig alla kraft tillgång kontroller av och kopplar bort kraften blyen före flytta säkerheten locket.
- Cirkulerar bara vatten eller 50/50 vatten/ethylene glycol genom värmen exchanger i så utrustad fall. Inte kopplar värmen exchanger till en vatten kran eller något kylmedel källa där vattnet trycket är

unregulated.

- Inför aldrig kylvätska eller något organiska lösningsmedel in i någon del av instrumentet. Organiskt lösningsmedel ska orsaka irreparable skada till enheten!
- Använd inte med buffert temperaturer över det högsta angivna tekniska specifikationerna. Överhettning skulle orsaka irreparable skador på enheten!

Waste Electrical and Electronic Equipment (WEEE)

English



This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

French



Ce symbole indique que les déchets relatifs à l'équipement électrique et électronique ne doivent pas être jetés comme les ordures ménagères non-triées et doivent être collectés séparément. Contactez un représentant agréé du fabricant pour obtenir des informations sur la mise au rebut de votre équipement.

German



Dieses Symbol kennzeichnet elektrische und elektronische Geräte, die nicht mit dem gewöhnlichen, unsortierten Hausmüll entsorgt werden dürfen, sondern separat behandelt werden müssen. Bitte nehmen Sie Kontakt mit einem autorisierten Beauftragten des Herstellers auf, um Informationen hinsichtlich der Entsorgung Ihres Gerätes zu erhalten.

Italian



Questo simbolo indica che i rifiuti derivanti da apparecchiature elettriche ed elettroniche non devono essere smaltiti come rifiuti municipali indifferenziati e devono invece essere raccolti separatamente. Per informazioni relative alle modalità di smantellamento delle apparecchiature fuori uso, contattare un rappresentante autorizzato del fabbricante.

Spanish



Este símbolo indica que el equipo eléctrico y electrónico no debe tirarse con los desechos domésticos y debe tratarse por separado. Contacte con el representante local del fabricante para obtener más información sobre la forma de desechar el equipo.

Swedish



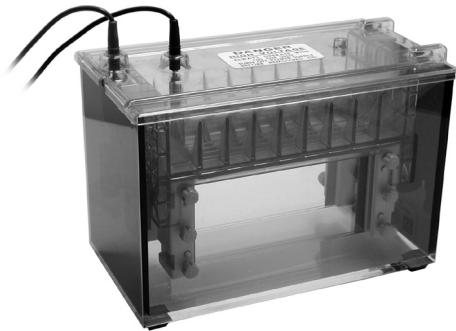
Denna symbol anger att elektriska och elektroniska utrustningar inte får avyttras som osorterat hushållsavfall och måste samlas in separat. Var god kontakta en auktoriserad tillverkarrepresentant för information angående avyttring av utrustningen.

Gel electrophoresis unit function and description

The Hoefer SE600 series vertical slab gel electrophoresis units (SE600, SE640 and SE660) are intended for protein and nucleic acid electrophoresis under commonly used denaturing and non-denaturing conditions. Up to 28 samples can be compared on a single slab gel.

Applications include protein separations, nucleic acid fractionation, and the second-dimension separation of 2-D electrophoresis. First-dimension separation of 2-D protein electrophoresis should be performed on Immobilized pH Gradient Gels. The focused strips are easily transferred to the second-dimension slab gel for size separation.

The SE640 gel plates are 18 cm wide and 8 cm in length. Up to four gels can be run at one time if sandwiches are paired into “club sandwiches”.



Specifications

Gel plate size (w × h):	18 × 8 cm
Gel size:	14 × 8 cm
Maximum watt:	50 W
Maximum volt:	1000 V
Maximum ampere:	500 mA
Maximum temperature:	45 °C
Environmental operating conditions:	Indoor use: 4–40 °C Humidity up to 80% Altitude up to 2000 m Installation category: II Pollution degree: 2
Dimensions (w × h × d):	32 × 22.5 × 14 cm (12.5 × 8.75 × 5.5 in.)
Product certifications:	EN61010-1, UL61010A-1, CSA C22.2 1010.1, CE Certified

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

- used in laboratory locations,
- used as delivered from Hoefer, Inc. except for alterations described in the user manual, and
- connected to other CE-labeled instruments or products recommended or approved by Hoefer, Inc.

Fig 1. Main components of the SE640 series (see Fig 4 for caster components).

Included but not shown:

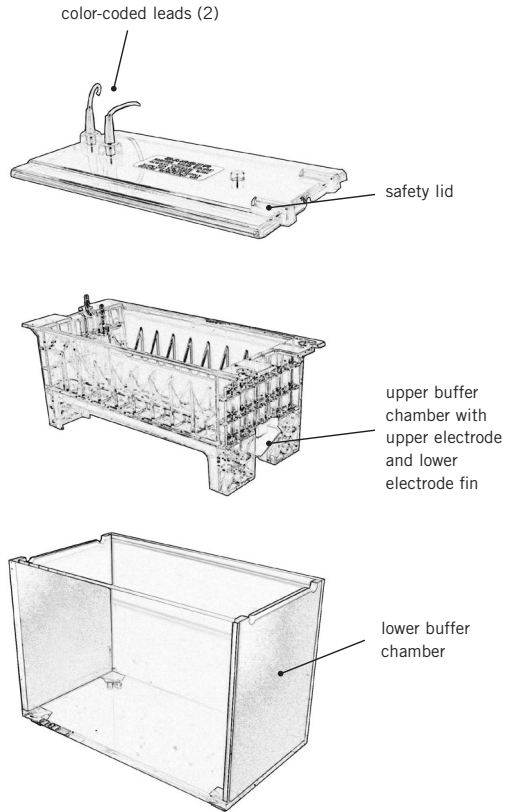
- Gel Seal compound, ¼ oz.
- Spacer-Mate spacer positioning guide
- Glass plates (6)
- Wonder Wedge plate separation tool
- Buffer dam

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

Required but not included:

- Magnetic stirrer
- Power supply with a minimum rating of 300 V, 100 mA (constant A or V)

Note: The ordering section on page 38 lists all accessories and replacement parts.



Note: Before using the first time, disassemble the unit and wash with a dilute solution of a laboratory detergent and rinse thoroughly first with water and then with distilled water.

Unpacking and inventory

Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part is missing, contact your local sales office. Inspect all components for damage that may have occurred while the unit was in transit. If any part appears damaged, contact the carrier immediately. Be sure to keep all packing material for damage claims or to use should it become necessary to return the unit.

Lower buffer chamber

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

Upper buffer chamber

The upper buffer chamber is molded polysulfone, which is chemically resistant to common electrophoretic buffers but not to organic solvents or strong acids and alkali. The upper electrode (cathode) runs along the center ridge, and terminates at a banana plug. The lower electrode (anode) runs along the edge of the electrode fin on the underside, and terminates at a second banana plug.

Safety lid

The banana plug on the upper buffer chamber at the terminus of the cathode wire connects to the black lead. The banana plug on the lower electrode fin at the terminus of the anode wire connects to the red lead. The 4-mm shrouded color-coded leads plug into color-coded jacks in the power supply. Always install the safety lid before use!

Glass plates

The plates are 18 cm wide and 8 cm in length. Three sets of glass plates are included with each unit. Notched divider plates, ordered separately, pair two gel sandwiches to form a “club sandwich” so that up to four gels can be run at one time.

Clamps

Clamps are used to secure the plates and spacers together. The clamp pressure bar, adjusted with screws, distributes pressure evenly.

Casting stand

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

Cams

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

Rubber gaskets

There are two sets of two gaskets: The solid laminated gaskets fit into the bottom of the casting stand and form the seal for casting the gel. The slotted gaskets fit under the upper buffer chamber and form the seal between the upper and lower chambers. The ridges on the upper gasket align the gasket slot to maintain an open channel between the top of the gel and the buffer in the upper chamber.

Spacers

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

Spacer-Mate spacer positioning guide

Aligns spacers for sandwich assembly.

Combs

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

All preparative combs, and 10, 12, 15 and 20-well combs form wells that are 25 mm deep. The 28-well comb forms wells that are only 15 mm deep so that wells do not collapse when the comb is removed. The sample volume held by each well depends on the gel thickness, well depth and the number of wells per comb. Table 1 on page 16 lists sample volumes of each well for all combs.

Wonder Wedge plate separator tool

Used to disassemble gel sandwiches and to gauge spacer and comb thickness.

Operating instructions

Gel casting and electrophoresis procedures follow. Included are instructions for polyacrylamide gels (used with continuous or discontinuous buffer systems), and gradient gels.

The gels required for the SE640 must be self-cast. The Dual Gel Caster (included) holds two gel sandwiches.

Prepare the gel sandwich

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

Construct the gel sandwich and insert into caster

①

Prepare the caster and clamps

Place the spirit level into the caster center and adjust the leveling feet. Loosen all clamp screws and make space for the sandwich by sliding the pressure plates toward the screws.

②

Construct each gel sandwich

For each sandwich, choose two perfectly clean unchipped glass plates and two spacers. Lay one plate on a flat surface, lay the Spacer Mate spacer positioning guide onto the plate (wide side at the top of the plate), place a spacer along each edge, and lay the second glass plate on top.

③

Secure the sandwich with clamps

Slide one clamp at a time along the sandwich sides. Finger tighten one screw on each clamp, set the sandwich upright on a flat surface, and loosen the screw to align the stack. Take great care in aligning to ensure a seal. Finger tighten all screws. Remove the Spacer-Mate.

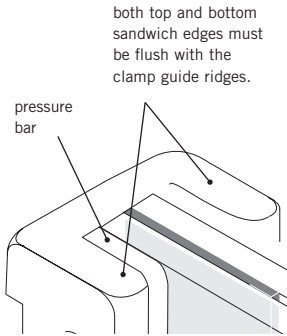


Fig 2. Sandwich assembly.
Inspect glass plates for nicks. Use only unchipped plates to prevent leaking.

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

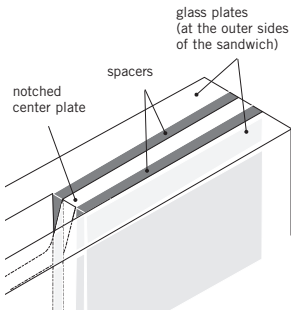


Fig 3. Club sandwich assembly.
Side clamps will accommodate two spacers up to 1.5 mm thick.

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

Club sandwich

A notched center divider plate (ordered separately) pairs two sandwiches to double the number of gels that can be cast and run.

Assemble a club sandwich in the same manner as a regular sandwich, except before placing the top glass plate, lay the divider plate and a second set of spacers on the stack. Place the notch so that it will be at the top of the gels. It is essential that the spacers and plates align perfectly in order to create a seal.

4

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

Optional: Apply a light film of Gel Seal compound only on the bottom corner surfaces created by the spacers and plates if your sandwiches continue to leak after several attempts of alignment.



5

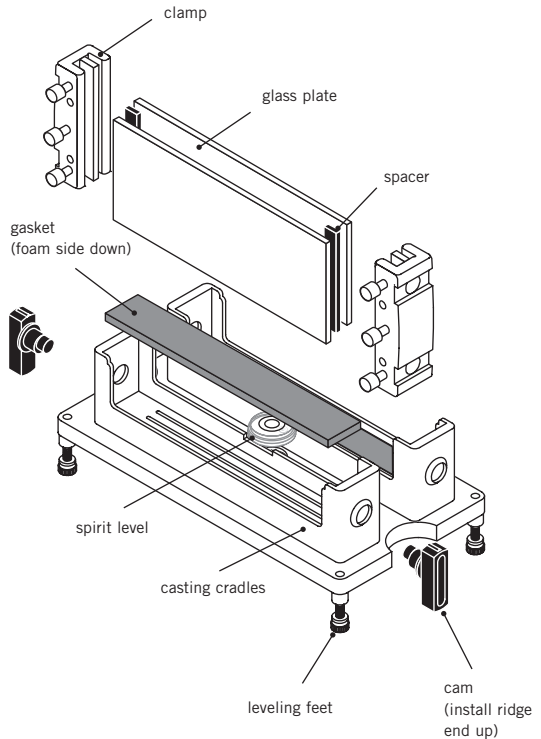
Place the laminated gasket into the casting cradle (See Fig 4) with the *foam side down*. Place the clamp assembly in the casting cradle, screw side facing out.

6

Insert a cam into the hole on each side of the casting tray with the ridge (short end) pointing up. Seal the gel sandwich against the casting gasket by turning both cams as far as needed, usually 90° to 150°, up to 180°. The cam action presses the plates down into the gasket to seal the bottom of the sandwich. The seal is complete once the glass edge appears darker and nearly transparent against the gasket. Do not turn the cam past this point.

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

Fig 4. Caster components and setup.



Acrylamide gels

1

Prepare the monomer solution and pour the gel

See Appendix A for SDS-PAGE recipes. Prepare the required amount of monomer solution. De-aerate and add the initiator (ammonium persulfate, APS) and catalyst (TEMED) just prior to pouring the gel. Pipet the solution into one corner of the sandwich, taking care not to introduce any air bubbles. See below for the appropriate solution level according to the application.

No stacking gel (Continuous system)

Fill solution to just below the top of the upper plate edge. If bubbles are trapped, remove with a pipette or syringe. Introduce a comb (at a slight angle) into each sandwich, taking care not to trap air bubbles under the teeth.

Club sandwich

Pipette the solution into both sandwiches, filling each to the same level below the notched edge.

Stacking gel

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

2-D electrophoresis (Discontinuous protein system)

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

2

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

3

Allow the gel to polymerize for a minimum of one hour.

Stacking gel preparation

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

1

Remove the overlay by rinsing the top of the gel several times with distilled water. Invert the caster to drain. To ensure a seamless contact between the resolving and stacking gels, remove residual liquid by blotting one corner with a lint-free tissue.

2

Prepare the required amount of stacking gel monomer solution, deaerate it, and add catalyst (APS) and initiator (TEMED). Pour the stacking gel onto the resolving gel with a disposable or Pasteur pipette to a level about 2 mm from the top of the plate.

3

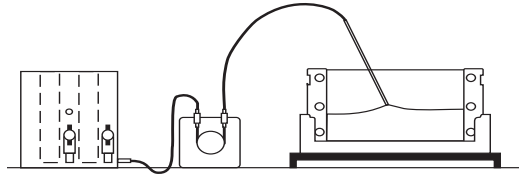
Introduce a comb (at a slight angle) into the sandwich, taking care not to trap air under the teeth. Allow a minimum of one hour for the gel to polymerize.

Gradient gels

Both linear and exponential gradient gels can be poured in the dual gel caster. We recommend using a Hoefer SG Series Gradient Maker. Gradient gels are poured with a cannula from the top of the dual gel caster (see Fig 5). A stacking gel is then poured over the gradient gel.

Fig 5. Pouring a gradient gel.

The gel solution may be introduced into the gel sandwich through a pipette tip at a rate that maintains a continuous stream.



Pouring a linear gradient gel

1

Assemble sandwich(es) into the dual gel caster as described on page 8.

2

Set up the monomer solution flow path

Run a length of Tygon™ tubing through a peristaltic pump. Attach one end of the tubing to the gradient maker outlet port and the other end to a 9 cm cannula. (The OD of the cannula must be less than the spacer thickness.) Place the cannula so that it rests at the bottom of the sandwich, midway between the spacers.

3

Prepare the monomer solution

Calculate the volume of monomer solution needed. Divide the total volume in half and prepare this volume of both the higher- and lower-percentage acrylamide solutions.

Optional: Adjust the higher-percentage acrylamide solution to 15% (w/v) sucrose or 25% (v/v) glycerol to improve layering.

4

Pour the “light” solution into the reservoir chamber (the chamber furthest from the inlet). Open the stopcock long enough to displace air between the chambers and then close. Pour the “heavy” solution into the mixing chamber and place a stirring bar into this chamber. Place the gradient maker onto a magnetic stirrer and begin stirring at a rate that mixes well but does not introduce bubbles into the solution.

5

Mix the gradient and pump the solution into the sandwich

While the solution is stirring, begin pumping from the mixing chamber and open the stopcock to the reservoir chamber. Raise the cannula as liquid enters the sandwich, keeping the tip at the gel surface. Prepare more gels as required.

6

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

7

Allow the gels to polymerize for a minimum of one hour. After polymerization, pour off the overlay and rinse the gel surface several times with distilled water.

8

Prepare the stacking gel monomer solution, pour the stacking gel and introduce a comb (at a slight angle) into the sandwich, taking care not to trap air under the teeth. Allow a minimum of one hour for the gel to polymerize.

Note: With Coomassie™ Blue it is possible to detect 1 µg of protein in a single band. With the more sensitive silver stains, it is possible to detect as little as 10 ng of protein.

Sample preparation and loading

The sample can be loaded either while the sandwich is in the caster or after the upper buffer chamber is attached. When loading samples while using divider plates, the samples must be loaded without the upper buffer chamber in place.

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

1

Prepare the wells

Remove the comb by gently rocking it side to side and then lifting it straight up to avoid damaging the well walls. Carefully rinse each well with distilled water to remove unpolymerized acrylamide and then drain by inverting the gel sandwich (or caster). Fill each well with electrophoresis buffer.

2

Prepare the sample

Increase liquid sample density with 10% glycerol or sucrose. Add a tracking dye such as phenol red, bromophenol blue, or pyronin Y.

For SDS protein gels, use 2X treatment buffer to denature both liquid and dry samples in a test tube.

To **liquid protein solutions**, add an equal volume of 2X buffer.

To **dry protein samples**, add equal volumes of 2X sample buffer and high purity water to achieve the desired concentration.



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

3

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

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

4

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

Table 1. Sample volume for standard comb sizes

no. of wells	comb thickness (mm)		
	0.75	1.0	1.5
10	6.2	8.3	12.4
12	5.8	7.7	11.5
15	4.3	5.7	8.6
20	3.1	4.1	6.2
28	2.1	2.7	4.1
1 prep/1 ref	90/4	121/6	183/9
1 prep/2 ref	85/4	113/6	171/9



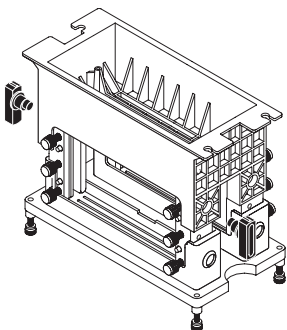


Fig 6. Attaching gel sandwiches to the upper buffer chamber.

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

- A. Remove cams from the lower cam holes. Place the upper chamber onto the sandwiches and then insert the cams into the upper cam holes, ridge (short end) pointing down.
- B. The final cam position (not shown) must be vertical so that the assembly fits into the lower buffer chamber.

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

Final assembly

Upper buffer chamber

1

Rinse both buffer chambers with water and distilled water thoroughly before each use.

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

2

If running only one gel:

Block the second upper buffer chamber slot by installing the acrylic buffer dam included with the unit. Fit clamps onto the dam, taking care to align the clamp ends and dam edges. Install the “dummy” gel, screws facing out, in the second cradle in the dual gel caster.

3

Attach the gel sandwich to the upper buffer chamber.

Turn the upper buffer chamber upside down and place a slotted gasket into both sandwich holder recesses. Both the slot in the gasket and the slot in the recess must align. Both slotted gaskets must be used even if running only one gel sandwich. Grooves along each slot help keep the gasket in place. Additionally, a small amount of Gel Seal can be applied at each end of the gasket before install to help hold the gasket against the upper buffer chamber.

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

4

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

Lower buffer chamber

1

Place a magnetic spin bar into the lower buffer chamber and place the unit on a magnetic stirrer. Fill the lower chamber with a minimum of 2.1 liters of buffer.

Optional: Prechill the buffer.

2

Fit the upper buffer chamber assembly into the lower buffer chamber. Use a steady hand to avoid disturbing the samples: Grasp the assembly in the casting stand by the upper buffer chamber and carefully lower it into the lower buffer chamber.

3

Inspect the installation and adjust the buffer levels.

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

Lower chamber. The lower buffer level chamber requires a minimum of 2.1 liters and a maximum of 2.8 liters of buffer; enough to cover the wire on the lower electrode fin, but maintaining a 1.5 cm clearance from the underside of the upper buffer chamber.

4

Place the safety lid on the unit.

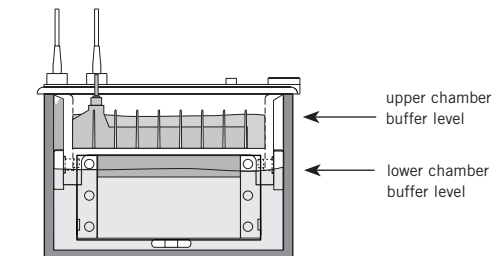
5

Plug the color-coded leads into the jacks of an approved power supply. Plug the red lead into the red output jack and the black lead into the black output jack. In most systems, the red lead, which is connected to the bottom electrode, is the anode (+), and the black lead, connected to the top electrode, is the cathode (-).

Important assembly notes:

- Do not fill the upper or lower chamber above the recommended levels illustrated in Fig 7. Remove buffer in contact with the electrode posts.
- Pour buffer slowly and away from the slots in the upper buffer chamber to avoid disturbing the samples.

Fig 7. Buffer chamber levels.



Separating the sample

Electrophoresis parameters for discontinuous polyacrylamide gels

Note: All SE600 series models use 18-cm wide plates. The gel thickness determines the cross section (and current requirement). The length of the plate determines the running time.

Table 2: Laemmli buffer system starting point guidelines

Gel thickness*	1.5 mm
Current per gel [†]	25 mA constant current
Starting voltage [‡]	80–90 V
Final voltage	220–400 V

*Thicker or thinner gels require proportionally more or less current. For example, a 0.75 mm gel, which is half as thick as a 1.5 mm gel, requires half as much current, or 12.5 mA.

[†]The current must be multiplied by the number of gels. For instance, if two club sandwiches are installed, the four gels require four times as much current. The current can be increased for faster runs though overheating will eventually become a problem, and it can be decreased for slower overnight runs.

[‡]At 25 mA per gel.

Gels may be run at either constant current or constant voltage settings. A constant current setting is traditionally used with a discontinuous buffer system so that the rate of electrophoretic migration remains unchanged throughout the run. Under these conditions, voltage increases as the run proceeds. A lower current setting is recommended for higher resolution. The optimal current level must be determined empirically; the main factors that must be balanced include the gel concentration and migration speed, and the resulting Joule heating and band distortion. Table 2 lists starting point guidelines and adjustments for gel thickness, number of gels, and migration rate.

Current

Current acts on the total cross-section area of all the gels because the gels are connected in parallel in the electrical circuit. Thus the current setting for one gel must be multiplied by the number of gels of the same gel thickness that are run simultaneously. For a gel 1.5 mm thick, we suggest a starting current setting of 25 mA. (Two 1.5 mm gels = 50 mA.)

Voltage

The starting voltage for a 1.5 mm slab gel connected to a power supply set to 25 mA is usually 80 to 90 V (using the SE600 with a Laemmli discontinuous buffer system for SDS gels). The final voltage can typically range from 220 to 400 V, depending on the length of the gel. (See Table 2.)

Time

A run is complete when the tracking dye reaches the bottom of the gel. A 1.5-mm thick Laemmli SDS gel, run at 25 mA/gel without cooling, usually requires 2.5 hours.

Electrophoresis parameters for DNA/acrylamide gels

DNA gels are usually run at a constant voltage setting, and since buffer systems are continuous, both current and voltage readings remain constant throughout the run. Running conditions are expressed in units of V/cm. Published running conditions vary widely, but voltages in the range of 1 to 3 V/cm are common for overnight runs.

Record each run

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

Check band progress after 5 minutes, and again after an hour, keeping an eye on the migration rate of the tracking dye. The run is complete when the tracking dye reaches the bottom of the gel. Watch the buffer level and, if necessary, replenish it as required to keep the top electrode submerged. (A small volume of buffer may leak past a nicked plate or gasket, or buffer may pass through the gel.)



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

After electrophoresis

①

Once the tracking dye reaches the bottom of the gel, turn off the power supply, disconnect the leads and remove the safety lid. (Lift straight up to avoid bending the banana plugs.)

②

Pull out the upper buffer chamber assembly. Pour the buffer into a sink. Install the assembly in the dual gel caster and then release the sandwiches by turning and removing the cams.

③

Unscrew the clamps from the sandwiches and remove. Gently loosen and then slide away both spacers. Use the Hoefer Wonder Wedge plate separator tool to separate the plates.

④

Carefully lift the glass plate with the gel attached. *Handle the gel with care to avoid damaging it.* Invert the plate and position the gel low over the staining tray. Pry one corner of the gel away from the glass and allow it to drop into the tray, or, if the gel is thick enough to handle, lift it and place it into the tray. To avoid splashing, add staining or fixative solution to the tray after the gel is transferred.

⑤

Clean the unit as described in the next section.

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



Caution! Always unplug unit from electrical supply before cleaning or drying the unit.

Care and maintenance

Cleaning

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

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

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

Customer service information



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

Note: A Return Authorization (RA) number must be obtained from Hoefler, Inc. before returning any item to Hoefler, Inc.

Technical service and repair

Hoefler, Inc. offers complete technical support for all of 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 Hoefler, Inc. representative.

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Troubleshooting

problem	possible cause	remedy
Gel sandwich leaks while casting	Dirty or damaged components	Plates, spacers, and the gasket must be completely clean. Wash if necessary. Replace chipped plates (especially if chipped near the spacers). Check the caster gasket for cuts or cracks and replace if necessary.
	Mis-aligned parts	Check plate and spacer alignment, realign if necessary.
	Over-clamping	Turn cam only as far as necessary to create a seal (usually 90–150°, but up to 180°). On each spacer apply a light film of Gel Seal compound to the bottom outside corner only. Do not use silicone grease.
Sample wells damaged or irregular	Air bubbles	Remove air bubbles before inserting combs. Slide comb into solution at an angle. If comb must be removed, add more monomer solution before reinserting the comb.
	Incomplete or delayed polymerization	Allow acrylamide gels to set for a minimum of 1 h.
	Debris in wells	Rinse out unpolymerized gel with sample buffer.
	Comb removal	Remove the comb at a slight angle and very slowly to prevent damaging the gel. Agarose gels: Lower the comb no more than 1 cm into the gel.
Incomplete gel polymerization	Chemicals	Use only recent stocks of the highest-quality reagents. If the dry ammonium persulfate does not crackle when added to water, replace with fresh stock. Increase TEMED or APS concentration, or both.
	pH	Solutions with extreme pH values (especially acidic) may not polymerize.
	Oxygen	Remove oxygen from the gel environment: Degas the monomer solution 5–10 min before pouring and then overlay the gel surface with water-saturated n-butanol.
	Temperature	Adjust the gel solution temperature to a minimum of 20 °C, especially for low %T gels.

problem	possible cause	remedy
Upper buffer chamber leaks	Mis-aligned parts	Check that the glass plates, spacers, and clamps are aligned and fit snugly into the upper chamber gasket. Check that both gaskets are centered and that the positioning ridges fit inside the grooves.
	Dirty or damaged components	Check that the gasket is not damaged or pinched. Replace if necessary. Check that the upper buffer chamber is not warped from prior exposure to excessive heat.
Power supply detects current leak	Electrical path to outside ground/earth	Add more silicone grease to seal heat exchanger grommets. Check for leaks or cracks in the heat exchanger. Replace worn grommets.
Dye front curves up (smiles) at edges	Uneven heat distribution	Fill the lower buffer chamber to the level appropriate for at edges the run. (See Fig 7, page 19). Use magnetic stirrer and stir bar to keep buffer well mixed.
	Excessive heat	Circulate ext. coolant. Decrease the current or voltage setting. Prechill the buffer. Run the gel in the cold room.
Protein streaks vertically	Particulates in sample	Centrifuge or filter sample before loading to remove particulates.
	Overloading	Load less sample.
	Degradation	Add protease inhibitor such as PMSF.
Unusually slow (or fast) run	Current leakage around gel	Check for leaks; all plates and spacers must be aligned and free of grease and cracks. If used, the buffer dam must be secure.
	Sample or reagent preparation	If the required pH of a solution is overshot, do not back-titrate. Discard and prepare fresh buffer. Check recipes, gel concentrations, and buffer dilution. (For instance, do not use Tris-HCl instead of Tris for Laemmli tank buffer.) Decrease the salt concentration of samples.
	Reagent quality	Dispose of older acrylamide solutions and use only stock of the highest quality. Use only freshly deionized urea.
	Voltage or current settings	To increase or decrease the migration rate, adjust the voltage or current by 25–50%.

problem	possible cause	remedy
Bands are skewed or distorted	Incomplete gel preparation and polymerization	Degas the stacking-gel solution and avoid trapping air bubbles under the comb teeth.
	Irregular interface between stacking and running gels	Overlay the running gel with water-saturated butanol before polymerization begins, to avoid forming an uneven gel surface.
	Sample preparation	Dialyze or desalt the sample.
Stained sample collects:		
<i>Near the buffer front</i>	Gel concentration	Molecules are not sufficiently restricted by the resolving gel pore size: increase the %T.
	Degradation	Proteins may be degraded by endogenous proteases: use protease inhibitors during the isolation step.
<i>Near the top of the gel when the buffer front has reached the bottom</i>	Gel concentration	The gel pore size is too small: decrease the %T of the resolving (or stacking) gel.
	Precipitation	The protein has precipitated. Heat the sample at a lower temperature (70 °C or less) for 1–2 min.
<i>At both top and bottom of the gel</i>	Gel concentration	The molecular weight range of the sample requires an acrylamide concentration gradient to resolve the full range of protein sizes.
Tracking dye doesn't sharpen into a concentrated zone in the stacking gel	Poor stacking	Pour a taller stacking gel. (For best results, allow a stacking-gel height of 2.5 times the height of the sample in the well.)
	Reagent quality	Dispose of outdated acrylamide solutions and use only the highest grade of acrylamide.
	Sample preparation	When preparing samples, avoid using solutions with high salt concentrations.

problem	possible cause	remedy
Poor band resolution	Running conditions	Begin electrophoresis as soon as the sample is loaded to prevent low molecular weight species from diffusing. Conduct the separation at a lower current or voltage setting to reduce Joule heating.
	Reagent quality	Use only the highest-quality reagents.
	Poor stacking	Use only gels that were recently prepared. Add a stacking gel or increase height of the stacking gel. Prepare the resolving-gel surface by first rinsing it with stacking-gel monomer before pouring the stacking gel to ensure continuity between the gels. Check pH values of the resolving- and stacking-gel solutions. Do not back-titrate buffers.
	Incomplete gel polymerization	Allow gel to polymerize fully.
	Sample preparation	Store sample on ice before it is denatured. Dialyze or desalt the sample. Heat samples in SDS sample buffer for no more than 1–2 min at 100 °C to improve dissociation of subunits. Store on ice after heating. Adjust the sample volume or concentration. Add more mercaptoethanol or dithiothreitol; check sample treatment. Add protease inhibitors such as PMSF if necessary to prevent proteolytic degradation of sample. Increase glycerol or sucrose to increase sample density. Store samples to be frozen in aliquots to avoid repeated freeze-thawing. Store at -40 to -80 °C.

Appendix A: Laemmli system gels

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

Polyacrylamide gel composition is indicated by two different percentages:

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

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

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

Final concentrations

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

*To achieve any other desired final concentration, adjust the acrylamide stock and water volumes. Volumes for different concentrations are listed on page 34.

†Tetramethylethylenediamine



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

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

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

Solutions

1. Acrylamide stock solution

(30.8% T 2.6% C Bis, 200 ml)

Acrylamide (FW 71.08)	30% w/v	60 g
Bis* (FW 154.2)	0.8% w/v	1.6 g
Deionized H ₂ O		to 200.0 ml

Store at 4 °C away from light.

*N,N' Methylenebisacrylamide

2. 1.5 M TrisCl, pH 8.8

(4X Resolving gel buffer, 1 liter)

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

3. 0.5 M TrisCl, pH 6.8

(4X Stacking gel buffer, 500 ml)

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

4. 10% SDS solution

(100 ml)

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

5. 10% APS

(Initiator, 1 ml)

Ammonium persulfate (APS) (FW 228.2)	0.44 mM	0.1 g
Deionized H ₂ O		to 1.0 ml

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

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

(Resolving gel overlay, 100 ml)

1.5 M Tris-Cl, pH 8.8 (Soln. #2)	0.375 M	25.0 ml
10% SDS (Soln. #4)	3.5 mM	1.0 ml
Deionized H ₂ O		to 100.0 ml

—OR—

Water-saturated *n*-butanol

Shake *n*-butanol and deionized H₂O in a separatory funnel. Remove the aqueous (lower) phase. Repeat this procedure several times. Use the upper phase.

—OR—

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

7. 2X Sample treatment buffer

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

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

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

—OR—

6X Sample treatment buffer

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

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

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

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

(Electrophoresis buffer, 5.0 liters)

Tris (FW 121.1)	0.025 M	15.1 g
Glycine (FW 75.07)	0.192 M	72.0 g
SDS (FW 288.4)	3.5 mM	5.0 g
Deionized H ₂ O		to 5.0 liters

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

9. Coomassie stain solutions

Coomassie stain solution

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

Coomassie Blue R-250 (FW 826)	0.3 mM	0.5 g
Methanol (Stir until dissolved)	40% v/v	800.0 ml
Glacial acetic acid (99%)	7% v/v	140.0 ml
Deionized H ₂ O		to 2.0 liters

Destaining solution I

(40% methanol, 7% acetic acid, 1 liter)

Methanol	40% v/v	400.0 ml
Glacial acetic acid (99%)	7% v/v	70.0 ml
Deionized H ₂ O		to 1.0 liter

Destaining solution II

(7% acetic acid, 5% methanol)

Methanol	5% v/v	50.0 ml
Glacial acetic acid (99%)	7% v/v	70.0 ml
Deionized H ₂ O		to 1.0 liter

Gel recipes

The Laemmli gel recipes are for 30 ml of a single concentration solution (enough for two 1.5 mm, 18 × 8 cm gels). Tabulated are ingredients and volumes for relatively large pore gels (7.5–10% T range) as well as smaller pore gels (12.5–15% T range). A 4% stacking gel is common. The linear gradient recipe is for 100 ml of solution. The total volume needed depends on the number of gels cast and the gel thickness; adjust as necessary. All gels are crosslinked with 2.6% C.

Laemmli gel

(per 30 ml resolving gel solution, 5 ml stacking gel solution)

	Separating gel				Stacking gel
	7.5%	10%	12.5%	15%	4%
Acrylamide stock (Solution #1)	7.5 ml	10.0 ml	12.5 ml	15.0 ml	0.67 ml
1.5 M TrisCl, pH 8.8 (Soln. #2)	7.5 ml	7.5 ml	7.5 ml	7.5 ml	—
0.5 M TrisCl, pH 6.8 (Soln. #3)	—	—	—	—	1.25 ml
10% SDS (Solution #4)	0.3 ml	0.3 ml	0.3 ml	0.3 ml	0.05 ml
Deionized H ₂ O	14.6 ml	12.1 ml	9.6 ml	7.1 ml	3.00 ml
10% APS (Solution #5)	150 µl	150 µl	150 µl	150 µl	25 µl
TEMED	10 µl	10 µl	10 µl	10 µl	2.5 µl
Final Volume	30.0 ml	30.0 ml	30.0 ml	30.0 ml	5.0 ml

For linear gradient gels, use equal volumes of low % and high % acrylamide solutions in the 2 chambers of the gradient maker. Less APS is added to extend polymerization time, and less still is added to the higher %T solution to allow polymerization to occur from the top down. In our experience with the concentrations in the 10–20% gradient example below, gels can be poured at a flow rate of 5–10 ml/min.

Linear gradient gel

(per 100 ml of solution)

	10% T	20% T
Acrylamide stock (Solution #1)	33.30 ml	66.70 ml
Sucrose	—	15.00 g
1.5 M TrisCl, pH 8.8 (Soln, #2)	25.00 ml	25.00 ml
10% SDS (Solution #4)	1.00 ml	1.00 ml
Deionized H ₂ O	a 100.00 ml	a 100.00 ml
10% APS (Solution #5)	0.300 ml	0.060 ml
TEMED	0.036 ml	0.036 ml

Appendix B: Bibliography

General

- Gallagher, S. R., and Smith, J. A., Electrophoretic separation of proteins. In *Current Protocols in Molecular Biology*. (Ausubel, F. A., eds.), OSC 10.2.1–10.2.21 (1991).
- Hames, B. D., and Rickwood, D., *Gel Electrophoresis of Proteins: A Practical Approach*: Second edition, City IRL Press (1990).
- Sambrook, J, Fritsch, E. F., and Maniatis, T., *Standard Form-aldehyde Protocol. Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1990).
- Sasse, J., and Gallagher, S. R., Staining proteins in gels. *Current Protocols in Molecular Biology*. (Ausubel, F. A., et al., eds.), OSC 10.6.1–10.6.8 (1991).

Non-denaturing gel systems

- Reisfeld, R. A., et al., Acidic buffer system for resolution of cationic proteins. *Nature*. **195**, 281 (1962).
- McLellan, T. Electrophoresis buffers for polyacrylamide gels at various pH values. *Anal. Biochem.* **126**, 94 (1982).
- Hedrick, J. L. and Smith, A. J., Size and charge isomer separation and estimation of molecular weights of proteins by discontinuous gel electrophoresis. *Arch. Biochem. Biophys.* **126**, 155 (1968).

Denaturing gel systems

- Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T. *Nature*. **227**, 680–685 (1970).
- Matsudaira, P. T. and Burgess, D. R., SDS microslab linear gradient polyacrylamide gel electrophoresis. *Anal. Biochem.* **87**, 386–396 (1978).
- Schreier, M. H., Erni, B., and Staehelin, T., Initiation of mammalian protein synthesis. I. Purification and characterization of seven initiation factors. *J. Mol. Biol.* Nov; **116**(4):727–753 (1977).
- Shapiro, A. L., and Maizel J. V. Jr., Molecular weight estimation of polypeptides by SDS-polyacrylamide gel electrophoresis: further data concerning resolving power and general considerations. *Anal. Biochem.* Jun; **29**(3):505–514 (1969).
- Schaeffer, H. and Von Jagow, G., Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **166**, 368–379 (1987).
- Weber, K., and Osborn, M., The reliability of molecular weight determinators by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **224**, 4406–4412 (1969).

Two-dimensional electrophoresis

- Adams, L. D. and Gallagher, S. R., Two-Dimensional Gel Electrophoresis Using the O'Farrell System. *Current Protocols in Molecular Biology*, (Ausubel, F. A., *et al.*, eds.), OSC pp. 10.4.1–10.4.13 (1992).
- Anderson, N. G., Anderson, N. L., and Tollaksen, S. L., Proteins of human urine. I. Concentration and analysis by two-dimensional electrophoresis. *Clin. Chem.* Jul; **25**(7): 1199–2210 (1979).
- Anderson, Leigh and Anderson, Norman G., High resolution two-dimensional electrophoresis of human plasma proteins. *Proc. Natl. Acad. Sci. USA.* **74**:5421–5425 (1977).
- Anderson, L. *Two-Dimensional Electrophoresis, Operation of the ISO-DALT® System*, Second Edition. Large Scale Biology Press (1991).
- Bravo, R., Schafer, R., Willecke, K., MacDonald-Bravo, H., Fey S. J., and Celis J. E., More than one-third of the discernible mouse polypeptides are not expressed in a Chinese hamster-mouse embryo fibroblast hybrid that retains all mouse chromosomes. *Proc. Natl. Acad. Sci. USA.* Apr; **79**(7):2281–2285 (1982).
- Hurkman, W. J., and Tanaka, C. K., Solubilization of Plant Membrane Proteins for Analysis by Two-Dimensional Gel Electrophoresis. *Plant Physiology.* **81**:802–806 (1986).
- Mets, L. J. and Bogorad, L. Two-dimensional polyacrylamide gel electrophoresis: an improved method for ribosomal proteins. *Anal Biochem.* Jan; **57**(1):200–210 (1974).
- O'Farrell, P. H., High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* May 25; **250**(10):4007–4021 (1975).
- Bjellqvist, B., *et al.*, Isoelectric focusing in immobilized pH gradients: principle, methodology and some applications. *J. Biochem. Biophys. Methods* **6**, 317–339 (1982).
- Görg, A, *et al.*, The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* **9**, 531–546 (1988).
- Görg, A. Two-dimensional electrophoresis with immobilized pH gradients: current state. *Biochem. Soc. Trans.* **21**, 130–132 (1993).
- Bjellqvist, B., *et al.*, Micropreparative two-dimensional electrophoresis allowing the separation of samples containing milligram amounts of proteins. *Electrophoresis* **14**, 1375–1378 (1993).
- Blomberg, A., *et al.*, Interlaboratory reproducibility of yeast protein patterns analyzed by immobilized pH gradient two-dimensional gel electrophoresis. *Electrophoresis* **16**, 1935–1945 (1995).

Ordering information

product	quantity	code no.
SE640 Dual Vertical Unit, basic. Includes: 3 sets of glass plates, four 8 cm clamp assemblies, 6 cams, dual gel casting stand with leveling base and level, buffer dam, Spacer-Mate alignment template and Wonder Wedge plate separation tool. (Order 2 combs and 2 sets of 8 cm spacers separately.)	1	SE640
SE640 Dual Vertical Unit, complete. Includes: basic unit plus two 15-well combs and 2 sets of 8 cm spacers 1.5-mm thick.	1	SE640-15-1.5

Replacement parts

Wonder Wedge gel plate separation tool	1	SE1514
Slotted silicone rubber gaskets for upper buffer chamber	2	SE6008B
Laminated silicone rubber gaskets for casting stand	2	SE6009
Buffer dam	1	SE6432
Upper buffer chamber with electrode fin	1	SE6454
Lid with high voltage leads	1	SE6056
High-voltage safety lead set	1	SE6056-HV
Lower buffer chamber	1	SE6450
Replacement electrode fin for SE6454	1	SE6870
Banana plug, gold, with 2 washers	1	SE6067
Spirit level	1	SER11
Gel Seal, ¼ oz. tube	1	SE6070

product	quantity	code no.
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Gel Caster for 1 or 2 gels:

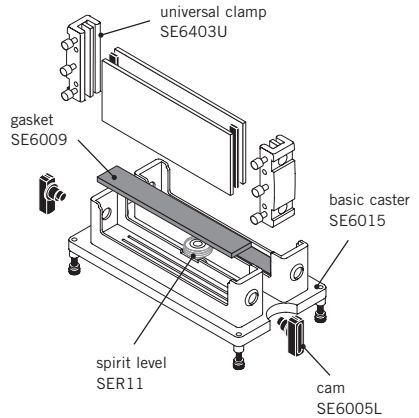
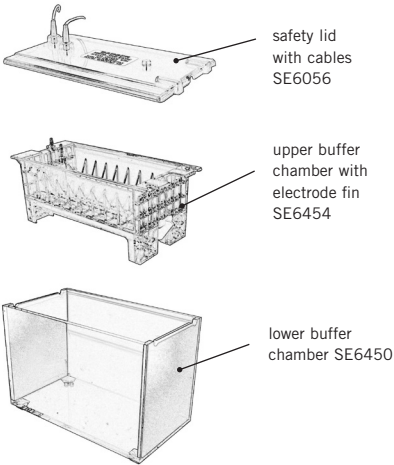
Dual Gel Caster, 1–2 gels, 18 cm wide. Includes: 2 blank gaskets. (One included with each SE640 unit)	1	SE6015
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Clamps and Cams

Replacement thumbscrews for clamps	12	SE6003U-2
Cams, black, for clamps with cam holes	4	SE6005L
Clamp assemblies, 8 cm	2	SE6403U

Glass Plates 18 × 8 cm

Glass plates	2	SE6402
Glass plates, low fluorescence	2	SE6402LF
Glass plate, club sandwich divider, notched	1	SE6402D



Combs

number of wells	thickness (mm)	width (mm)	quantity	code number
10	0.75	8.3	1	SE511-10-.75
10	1.00	8.3	1	SE511-10-1.0
10	1.50	8.3	1	SE511-10-1.5
12	0.75	7.6	1	SE511-12-.75
12	1.00	7.6	1	SE511-12-1.0
12	1.50	7.6	1	SE511-12-1.5
15	0.75	5.7	1	SE511-15-.75
15	1.00	5.7	1	SE511-15-1.0
15	1.50	5.7	1	SE511-15-1.5
20	0.75	4.1	1	SE511-20-.75
20	1.00	4.1	1	SE511-20-1.0
20	1.50	4.1	1	SE511-20-1.5
28 ^a	0.75	2.7	1	SE511-28-.75
28 ^a	1.00	2.7	1	SE511-28-1.0
28 ^a	1.50	2.7	1	SE511-28-1.5

^aComb depth 15 mm; all others 25 mm.

Preparative combs

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

no. of wells prep/ref	thickness (mm)	width (mm) prep/ref	quantity	code number
1/1	0.75	121/6	1	SE511-R-.75
1/1	1	121/6	1	SE511-R-1.0
1/1	1.50	121/6	1	SE511-R-1.5
1/2	0.75	113/6	1	SE511-DR-.75
1/2	1	113/6	1	SE511-DR-1.0
1/2	1.50	113/6	1	SE511-DR-1.5

Adjustable comb back 1 SE511-BKA

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

Spacers

thickness (mm)	length	width (mm)	quantity	code no.
0.75	8	2	2	SE6419-2-.75
1.00	8	2	2	SE6419-2-1.0
1.50	8	2	2	SE6419-2-1.5

Companion products

Hofer SE100 Plate Mate washing and storage unit	1	SE100
Hofer TE62 Tank Transfer Unit	1	TE62
Hofer TE70XP Semi-Dry Transfer Unit	1	TE70XP

Hofer reagents for gel casting and buffers

Acrylamide, MB grade	1 kg	GR141-1
bis-Acrylamide, MB grade	100 g	GR142-100
TEMED	25 g	GR151-25
Ammonium persulfate, ACS reagent grade	10 g	GR152-10
Tris-Glycine-SDS Buffer, 10X Solution, MB grade	1 L	GR149-1
Tris, reagent grade	1 kg	GR132-1
Glycine	1 kg	GR125-1
Sodium Dodecyl Sulfate (SDS)	500 g	GR126-500
Sodium Dodecyl Sulfate (SDS), 10% Solution	1 L	GR155-1

Hofer reagents for sample loading and gel staining

Dithiothreitol (DTT), MB grade	5 g	GR122-5
EDTA, 0.5 M Solution, MB grade	100 ml	GR123-100
Bromophenol Blue, sodium salt, ACS reagent grade	10 g	GR120-10
Glycerol, MB grade	1 L	GR124-1
Protein determination reagent, 500 assays	500 ml	GR133-500
Coomassie Brilliant Blue G-250	25 g	GR134-25
Coomassie Brilliant Blue R-250	25 g	GR135-25



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