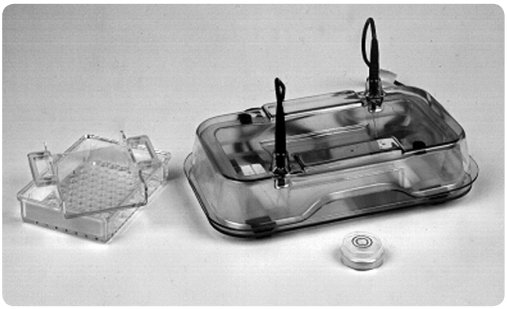
user manual
nucleic acid electrophoresis

Hoefer HE33

Mini submarine electrophoresis unit



um HE33-IM

Hoefer®

Page finder

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!

Duležité 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 schválen, nebo poskytnutých Hoefer, Inc. mohou být použity pro provoz, údržbu, a údržbě tohoto výrobku.
- zdroj napájení používají jen že je opatřen označením CE osvědčena nebo bezpečnost vnitrostátně uznanými zkušebními laboratoř.
- 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 vybavena. Nemají připojení výměník tepla s vodními setřepná nebo jakékoli chladicí kapaliny zdroje, kde tlak vody je neregulo.
- Nikdy zavést prostředek proti zamrznutí nebo jakákoli organická rozpouštědla do jakékoli části z tohoto nástroje. Rozpustidlům 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 godkendede 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.
- Sikkerhedlåget må være på plads før forbinding strømforsyningsblyet til en strømforsyning.
- Drejer alle strømforsyningskontroller af og afbryder kraftblyet før fjerning sikkerhedlåget.
- 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 unregulated.
- 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 specifications. Overheding vil forårsage uboelig skade til enheden!

Belangrijke Informatie – Dutch

- Indien deze uitrusting in een manier wordt gebruikt die niet door Hoefer, 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 Hoefer, 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/ethyleenglycol 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 specificeerde technische specificaties. Oververhittend zal onherstelbare schade aan de eenheid veroorzaken!

Tärkeää Tietoa – Finnish

- Jos tätä varusteita käytetään tavassa ei määritetty Hoefer, Inc. suojelu ehkäisty varusteille saattaa olla avuton.
- Tämä väline suunnitellaan sisälaboratoriokäytölle vain.
- Vain lisävarusteet ja osat hyväksyivät tai toimitti Hoefer, 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 tunnustettnut testaaminen laboratoriota.
- Turvallisuuskansi täytyy olla paikallaan ennen yhdistäminen käyttöjännitelyijyjä käyttöjännitteeseen.
- Kiertää kaikki käyttöjännitevalvonnat ja irrottaa valtalyijyt ennen poistaminen turvallisuuskantta.
- Kiertää vain vesi tai 50/50 vesi/ethyleneä 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. Orgaaniset 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 spécifié par Hoefer, 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 Hoefer, 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 mene 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'exchanger de chaleur si si équipé. Ne pas connecter l'exchanger 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 ungeregelt 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 nazionalmente riconosciuto testando il laboratorio.
- Il coperchio di sicurezza deve essere nel luogo prima di collegare i piombi di alimentatore a un alimentatore.
- Spegne 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'antigelo 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!

Viktig Informasjon – Norwegian

- Hvis dette utstyret blir brukt i en måte ikke spesifisert ved Hoefer, Inc. beskyttelsen som ha blitt git 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 forbinding 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 vanntapp eller noe kjølemiddelkilde hvor vannet trykket er unregulated.
- Introduserer Aldri antifreeze eller noe organisk løsemiddel inn i noe del av instrumentet. Organiske løsemiddler 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 controlos 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 Hoefer, 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 Hoefer, Inc. skyddet tillhandahöll vid utrustningen kan skadas.
- Detta instrument formges för inomhuslaboratorium användning bara.
- Bara medhjälpare och delar godkände eller levererade vid Hoefer, 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 irreparabla 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 

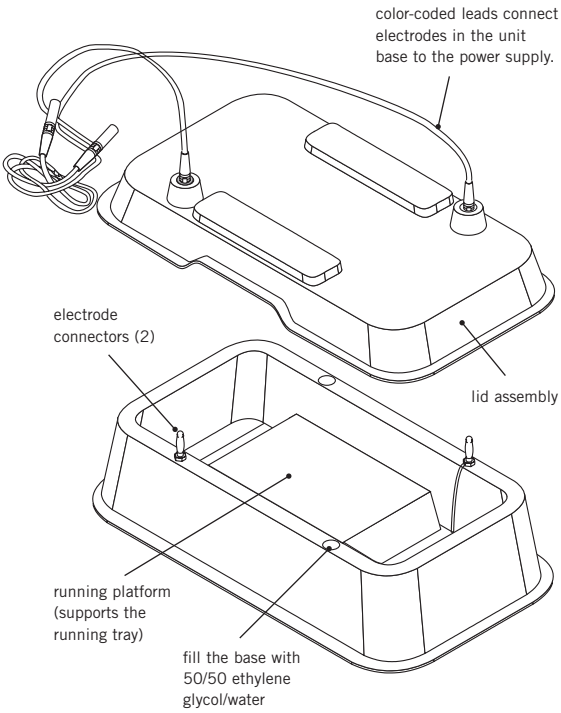
Denna symbol anger att elektriska och elektroniska utrustningar inte får avyttras som osorterat hushållsavfall och måste samlas in separat. Var god kontakta en auktoriserad tillverkarrepresentant för information angående avyttring av utrustningen.

Mini submarine electrophoresis unit function

The Hoefer® HE33 horizontal agarose unit is intended for rapid electrophoresis of small quantities of nucleic acids in agarose gels. A gel is cast in the gel caster, which holds one or two combs. (Eight different combs are available; a maximum of 32 samples can be run if two 16-well combs are used.) After the gel sets, the running tray is transferred to the platform of the horizontal unit. The base of the unit holds coolant that can be chilled before the run. This passive cooling capacity allows fast, high voltage runs.

Fig 1. Main components.

(See Figure 2 for an illustration of the casting kit.)



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 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 for repacking should it become necessary to return the unit.

Specifications

Max. voltage	500 V for 5 minutes or less
Max. wattage	15 W
Max. current	500 mA
Max. operating temp.	50 °C
Max. buffer volume	250 ml
Coolant required	≈600 ml 50/50 water/ethylene glycol
Gel size	7 × 10 cm
Environmental operating conditions	Indoor use: 4–40 °C Humidity up to 80% Altitude up to 2000 m Installation category II Pollution degree 2
Dimensions	width × depth × height 24 × 13 × 7 cm (9.5 × 5.2 × 2.8 in.)
Weight (base, lid, leads only)	0.4 kg (0.9 lbs)
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.

Operating instructions

Agarose gels are first prepared using the gel casting kit. Samples are then loaded into wells and electrophoretically separated. The fluorescent dye ethidium bromide can be added to the gel or electrophoresis buffer or both to track separation progress. After electrophoresis, the gel may be stained and photographed, blotted, or dried for autoradiography.

Important! Do not fill the base with commercial antifreeze, organic solvents, or pure water.

Note: It is not necessary to replace the coolant.

Fill the base with coolant

Even if no cooling is required, it is important to fill the base with the proper coolant solution before the first use because the solution provides a necessary heat sink.

❶

Prepare 600 ml of 50/50 ethylene glycol/water.

Optional: To help see wells more clearly while loading the sample, add a drop or two of soluble dye or food color to the coolant solution.

Locate the two inlet holes in the top edge of the base. Fill the base cavity as full as possible with coolant using a 50-ml syringe or a pump.

❷

Push a gray rubber plug into each hole, taking care that the plug is securely seated.

❸

Place the prepared base in an ice bucket or into a refrigerator or freezer set no lower than -20 °C for about an hour before use. (The base will always be ready if you store it in the refrigerator or freezer.)

Caution: Ethidium bromide is a known mutagen. Always wear gloves when handling.

Prepare solutions

❶

Prepare 250 ml of running buffer. (Refer to page 12 for recipes of commonly used electrophoretic running buffers.)

❷

Prepare the sample loading buffer. (Refer to page 14 for a recipe and a table of volume requirements for each comb size.)

❸

Prepare approximately 7 ml agarose solution per mm of gel thickness. (For example, a 3-mm gel requires 0.3 cm × 7 cm × 10 cm = 21 ml)

Dissolve agarose in running buffer, heat according to instructions accompanying the agarose, and allow the solution to cool to 50 °C before pouring into the casting tray.

Optional: Add 0.5 µg/ml ethidium bromide to the gel solution to observe separation during electrophoresis.

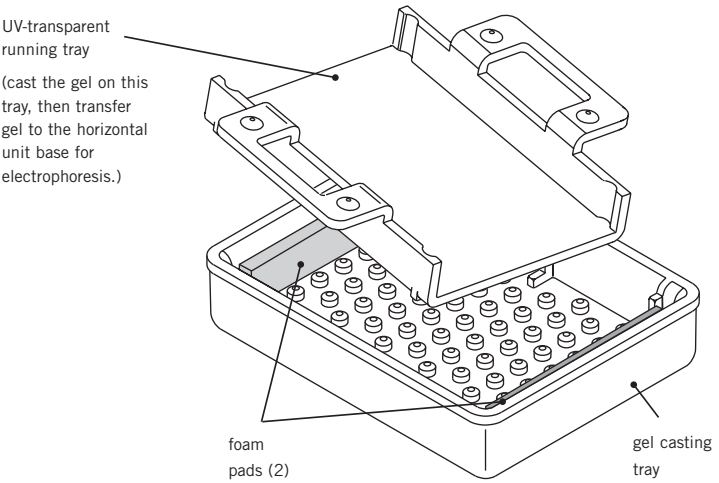


Fig 2. Gel casting kit.

Approach the foam pad with one end of the running tray and then gently press the tray edge against the pad, compressing it enough to allow the opposite end of the running tray to drop fully into the casting tray before sealing against the foam pad.

Cast the gel

❶

Install the running tray

Firmly grasp the casting tray with one hand. With the other hand, place one end of the running tray against the foam pad at the bottom edge, press the tray against the pad, and then lower it to rest on the bottom of the casting tray, seating the other end of the tray against the opposite foam pad.

❷

Prepare the comb(s)

Fit the two slots in the comb between the (loosened) thumb screw heads and the comb back. Tighten the screws until the comb is just supported. Seat the comb assembly on the rim of the casting tray and adjust the bottom of the comb so that it is about 1.0 mm from the running tray. Tighten the screws to secure the comb. To run twice as many samples, prepare two combs.

❸

Remove the comb assembly. Place the casting assembly on a leveling surface and level, using the spirit level on the running tray as a guide.

❹

Pour the agarose solution (cooled to 50 °C) into the casting tray. Orient the comb assembly so that the comb faces the nearest foam pad and seat it on the tray rim. Check that the comb is vertical to prevent well shape distortions. To run twice as many samples, place the second comb assembly in the center of the tray. Allow a minimum of 30 minutes for the gel to set.

❺

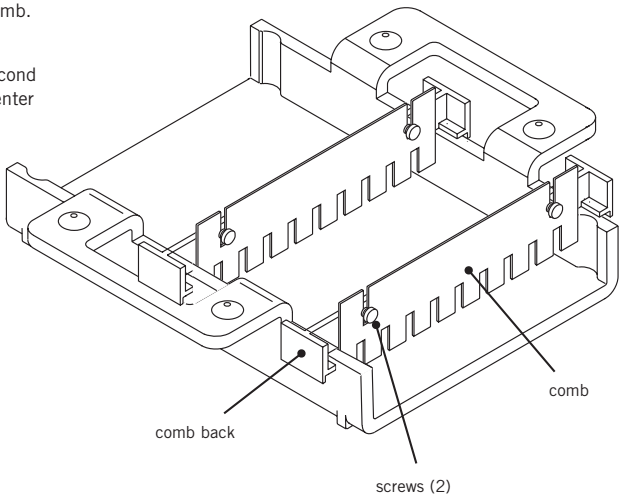
Once the gel is set, remove the comb carefully. Partially lift and slightly tilt the comb at one end and then slowly withdraw it from the gel. (Pulling the comb straight up creates a vacuum in the wells that may lift the gel out of the tray.)

❻

Remove the running tray and gel by grasping the handles of the tray and pressing against one of the foam pads. Once the tray clears the opposite pad, lift it out. Transfer the running tray and gel to the chilled base.

Fig. 3. A comb back, which fits onto the rim of the casting tray, positions the comb in the gel. Two screws hold the adjustable comb.

For twice as many wells, a second comb can be placed in the center of the gel.



The electrophoresis run

Refer to the notes, buffers, and volumes section for additional information and guidelines.

1

Chill the base before use, especially when higher voltage settings will be used or when the separation will require more than 30 minutes.

Note: To monitor separation progress, either add 0.5 µg/ml (final conc.) of ethidium bromide to the running buffer now, or add 50 µg/ml (final conc.) ethidium bromide to the sample buffer. To visualize progress, turn off the power supply, remove the lid assembly, and hold a portable UV lamp near the gel.

Adding ethidium bromide to the running or sample buffer slows migration slightly. Detection by this method is not as sensitive as staining and viewing on a transilluminator. (See DNA detection, page 15.)

Caution: Ethidium bromide is a known mutagen. Always wear gloves when handling.

Wear UV safety goggles and protect skin when using a UV lamp.

2

Fill both buffer chambers with running buffer until the buffer is ~1 mm deep over the gel. (This requires about 220 ml.)

3

Load the samples.

Add sample to 5X sample loading buffer and mix (1/5 of the final volume is loading buffer, see page 14). Use a micro-pipette to load each sample, taking care to avoid puncturing the well bottom or entrapping any bubbles.

Note: If no dye was added to the coolant, place the base on a dark background to see the wells more easily.

4

Place the lid so that the cathode (–) black lead is at the end nearest the sample well. (Nucleic acid samples migrate toward the anode (+) red lead. Connect the color-coded leads (red to red, and black to black) to an approved power supply, such as the PS300B. Set the voltage level and timer (if available) according to the degree of resolution sought.

Note: At the maximum setting the unit begins overheating as soon as the chilled base reaches ambient temperature. If overheating is not controlled, the gel will melt and/or the base of the unit will warp!

Quick, high-voltage runs

Certain applications, such as screening samples or checking sample purity, can be accomplished quickly under high voltage conditions. Chill the base (-20 °C) and limit the run to 5 minutes or less at 500 V.

Slower, lower voltage runs

Note: To calculate the voltage gradient, divide the voltage setting by the distance between the electrodes (12.7 cm).

A voltage gradient of 12 V/cm (150 V) separates 0.1 to 23 kb fragments of a *Hin*d III digest of λ DNA in 30 to 40 minutes (using 1% agarose gel and 0.5X TBE running buffer). Alternatively, using the same solutions, this sample could be run at 24 V/cm (300 V) with acceptable band resolution in 20 to 30 minutes. Chill the base before use.

Table 1: Voltage settings and recommended run settings†

voltage (V)	gradient (V/cm)	time (min)
500	40	5*
400	31	10*
300	24	20*
200	16	30 to 40
150	12	30 to 60

*For rapid runs of 20 minutes or less, use 0.5X TBE and chill the base to -20 °C before use.

†Voltage and times are for 1% Agarose NA, 0.5X TBE and a chilled base.

After the separation

❶

Important! Turn off the power supply, disconnect the leads, and remove the lid.

❷

If no ethidium bromide was added to the gel or sample before the run, stain the gel in a solution of 0.5 to 1.0 µg/ml ethidium bromide in water or buffer.

❸

Clean the unit as described below.

Care and maintenance

Important! Never autoclave any component of the electrophoresis unit or casting kit.

Cleaning

After each use, clean the unit with a mild detergent and water, rinse thoroughly with distilled water, and allow to air dry. Never use abrasive cleansers. Do not expose the unit to solutions or vapors of aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 30%), or concentrated acids (over 25%).

To reduce DNase and RNase contamination, soak the buffer chamber or casting kit for 10 minutes in a 3% hydrogen peroxide (H_2O_2) solution, then rinse thoroughly with DEPC-treated, autoclaved, deionized water. (Sambrook, *et al.* 1:7:40)

Replacing foam pads

Remove worn foam pads. Peel off the adhesive cover on a new foam pad. Align the pad so that it will rest on the bottom of the tray along a short (7 cm) side, adhesive side toward the inside wall, and then press it in place. Repeat with second pad on the wall opposite the first pad.

Replacing the electrode

It is recommended that electrodes be replaced only by Hoefer technicians. Call your local representative for advice.

Troubleshooting

problem	solution
Deformed sample well	Allow the gel to set for a minimum of 30 minutes and make sure it is at room temperature before removing the comb.
	When removing the comb, hold it at a slight angle and lift very slowly to prevent the gel from breaking.
	Take care to not damage the well with the pipet while loading the sample; aim for the center of the well and do not puncture the bottom with the pipet tip.
Samples not running along a straight path	If a comb or running tray is warped, replace.
	Reduce the voltage.
	Choose a buffer with the appropriate ionic strength and buffering capacity. (The buffering capacity of TBE, for example, is higher than that of TAE.) If the buffer is depleted, stop the run, remove the lid, and pipette the buffer from each chamber into the opposite chamber to replenish the buffer.
	If the gel is uneven, level the casting tray before pouring the gel.
Double-banded pattern	The comb must be vertical to prevent well shape distortion.
	Decrease the buffer level to 1 mm above the top of the gel, to reduce the vertical temperature gradient.
Poor band resolution	Add Ficoll™, glycerol, or sucrose to the sample loading buffer to ensure that the sample sinks to the bottom of the well. (Ficoll is preferred.)
	Make sure the sample is completely dissolved.
	Reduce the voltage.
	Reduce the sample concentration.
	Reduce the sample volume.
	At least 1 mm of gel below the bottom of the comb is required to prevent samples from leaking out of the well bottom.
	Reduce the salt concentration of the sample.
	Check enzyme activity; the sample may require longer digestion or a different restriction buffer.
	Prepare fresh sample if you suspect nuclease contamination.
	Choose agarose with a low endosmosis value.
Foam pads peel off	Install the running tray as as described on page 5; do not press straight down into place.

Notes, buffers, and volumes

Agarose gel electrophoresis notes

Agarose gel electrophoresis can be used to separate DNA fragments as small as 0.1 kb or less. Polyacrylamide gels are usually used for fragments smaller than 1 kb.

DNA mobility

Suggested agarose concentration for separating fragments of various sizes is given in Table 2 below. Other factors affecting separation results include the running buffer, voltage setting, temperature, conformation, and the presence of ethidium bromide. Special agaroses are available that can extend resolution ranges.

A common standard is a *Hin*d III digest of lambda phage, which gives eight fragments ranging in size from 0.1 to 23 kb pairs. For good resolution, run 45 minutes on a 10-cm long, 1% agarose gel in 0.5X TBE gel at 150 V.

Table 2:
Agarose concentrations for separating DNA fragments of various sizes

agarose (%)	effective range of resolution of linear DNA fragments* (kb)
0.5	1.0–30
0.7	0.8–12
1.0	0.5–10
1.2	0.4–7
1.5	0.2–3

*Current Protocols in Molecular Biology, p 2.5.2 (1993)

RNA mobility

RNA can also be separated on the basis of size. To avoid anomalies due to secondary structure, RNA is denatured either before or during electrophoresis. For example, RNA fragments previously denatured with glyoxal and dimethylsulfoxide can be separated on neutral agarose gels, or RNA can be fractionated on agarose gels containing methylmercuric hydroxide or formaldehyde.

RNA samples usually require longer runs or buffers that are easily depleted, and so require circulation. The Hoefer SUB20C and SUB25C horizontal units are recommended for this application rather than the HE33.

Important! Do not adjust the pH of these buffers once they are prepared according to the recipe!

Running buffers for DNA in agarose gels

Recipes for the two most commonly used running buffers for DNA electrophoresis are listed below. The ionic strength of these buffers is appropriate for the application.

1. 10X Tris-borate-EDTA (TBE) stock buffer[†]

(0.89 M Tris, 0.89 M boric acid, 20 mM EDTA, pH ~8.2, 1000 ml)

Tris base (FW 121.1)	0.89 M	108.0 g
Boric acid (FW 61.8)	0.89 M	55.0 g
EDTA solution (0.5 M, pH 8.0, soln. 3)	0.02 M	40.0 ml
Deionized H_2O		to 1000.0 ml

Stir. Do not adjust pH.

Before use dilute either to: 0.5X, to yield 45 mM Tris base, 45 mM boric acid, and 1 mM EDTA. This dilution is often used because current remains low, resulting in less heat.

—or—

1X, to yield 89 mM Tris base, 89 mM boric acid, and 2 mM EDTA.

2. 10X Tris-acetate-EDTA (TAE) stock buffer[†]

(0.4 M Tris, 0.2 M acetic acid, 10 mM EDTA, pH ~8.4, 1000 ml)

Tris base (FW 121.1)	0.40 M	48.4 g
Acetic acid (99.5%)	0.20 M	11.4 ml
EDTA solution (0.5 M, pH 8.0, soln. 3)	0.01 M	20.0 ml
Deionized H_2O		to 1000.0 ml

Stir. Do not adjust pH. Dilute to 1X before use to yield 40 mM Tris base, 20 mM acetic acid, and 1 mM EDTA.

3. EDTA solution (ethylenediamine tetraacetic acid)[†]

(0.5 M, pH 8.0, 100 ml)

$Na_2EDTA{\cdot}2H_2O$, (FW 372.2)	0.5 M	18.6 g
Deionized H_2O		to 70.0 ml
NaOH (10 M) to pH 8.0		~5.0 ml
Deionized H_2O		to 100.0 ml

[†]Modified from Sambrook, J., Molecular Cloning: A Laboratory Manual, p. B.23 (1989). See also Current Protocols in Molecular Biology, p. A.2.1 (1993).

Sample loading buffer

Loading buffer

(5X, 25% Ficoll 400, 0.25% Bromophenol blue†,10 ml)

Deionized H_2O	to 7.0 ml
Ficoll 400	2.5 g
Bromophenol blue (FW 691.9)	25.0 mg
Deionized H_2O	to 10.0 ml

Add to sample in proportion so that 1/5 of the final volume is loading buffer. (Loading buffer increases solution density.)

Note 1: Sucrose or glycerol may be used instead of Ficoll 400.

Note 2: Xylene cyanol (0.25%), which migrates more slowly than bromophenol blue, can be added as an additional marker if desired. The agarose concentration determines the position of the dye bands relative to a polynucleotide.

†Tracking dyes may be omitted to eliminate obscuring and dragging effects caused by comigration with smaller nucleic acids.

Table 3: Comb specifications and well volumes

comb code no.	no. of wells	thickness (mm)	well width (mm)	sample vol. per 1 mm depth (µl)
HE31A-P-1.0	1 prep/2 ref	1.0	44/6	44/6*
HE31A-P-1.5	1 prep/2 ref	1.5	44/6	66/9*
HE31A-8-1.0	8	1.0	6.5	6.5
HE31A-8-1.5	8	1.5	6.5	9.7
HE31A-12-1.0	12	1.0	3.9	3.9
HE31A-12-1.5	12	1.5	3.9	5.8
HE31A-16-1.0	16	1.0	2.6	2.6
HE31A-16-1.5	16	1.5	2.6	3.9

*The preparative combs form two reference wells (for MW standards), one on each side of the preparative well. The first number is sample volume/mm in the preparative well; the second is volume/mm in the reference well.

Caution! Ethidium bromide is a known mutagen. Always wear gloves when handling.

Caution! Wear UV safety goggles and protect skin when using any UV light source.

Note: Ethidium bromide slows DNA migration by about 15%.

Note: Minimize the staining time to prevent small nucleic acid fragments from diffusing out of the gel.

DNA detection

DNA can be detected either by the fluorescence of bound ethidium bromide or by autoradiography of radiolabeled DNA.

Ethidium bromide (0.5 µg/ml) can be added to running buffer to monitor sample progress because the dye's fluorescence under a UV lamp reveals band location. (To check progress, turn off the power supply and remove the lid of the agarose unit. Hold a portable UV-lamp near the running tray. Replace the lid and turn on the power again to resume electrophoresis.)

Alternatively, after electrophoresis, stain the gel in an ethidium bromide solution (0.5 µg/ml H_2O) for 15 to 60 minutes and then view or photograph the sample on a UV transilluminator.

To photograph the gel, either place the running tray on the transilluminator surface or slide the gel onto the surface for maximum exposure. (The running tray is 95% transparent to 302-nm light and 40% transparent to 254 nm light.) View the sample under 366-nm UV light or reduced intensity 302-nm UV light to reduce photonicking.

To reduce the background fluorescence of unbound ethidium bromide, the gel can be destained by soaking it for 5 minutes in 0.01 m $MgCl_2$, or for 1 hour in 0.001 M $MgSO_4$. Destaining makes it easier to detect small quantities (less than 10 ng) of DNA. (Sambrook, section 6.15).

Bibliography

Ausubel, et al., (eds). *Current Protocols in Molecular Biology*. Greene Publishing and Wiley-Interscience. New York (1993).

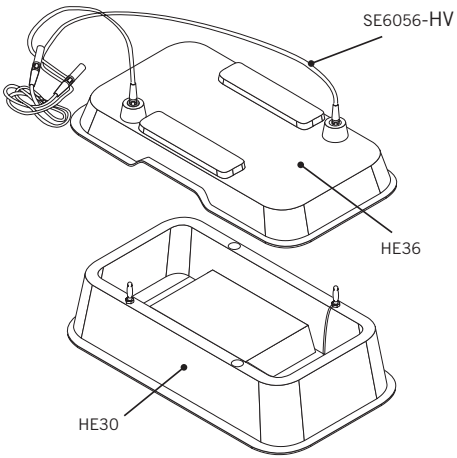
Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press (1989).

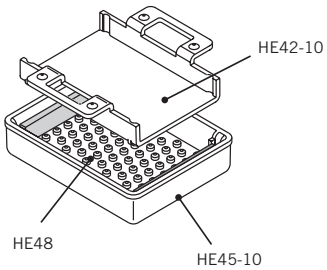
Ordering information

All quantities are 1 except where indicated.

Basic unit and kit	code no.
Mini Submarine Electrophoresis Unit, basic. Includes gel running tray, gel casting tray and bubble level. (Order comb and comb back separately.)	HE33B
Mini Submarine Electrophoresis Unit, kit. Same as above plus one 8-well, 1.5 mm thick comb, comb back, and screws.	HE33-8-1.5

Replacement parts	
Buffer chamber assembly	HE30
Lid with high voltage leads	HE36
Fill plug, bottom	HE38
Fill plug, top	HE38TP
Gel running tray, UVT, 7 × 10 cm	HE42-10
Casting tray, 7 × 10 cm	HE45-10
Gel casting kit with gel casting tray and running tray, 7 × 10 cm	HE47-10
Foam gaskets (pk/2)	HE48
High voltage leads	SE6056-HV
Electrode replacement kit	HE39
Bubble level	SER11





Combs

Specify thickness and number of wells, as tabulated below. (Order a comb back separately.)

thickness (mm)	no. of wells	code no.
1.0	Preparative	HE31A-P-1.0
1.0	8	HE31A-8-1.0
1.0	12	HE31A-12-1.0
1.0	16	HE31A-16-1.0
1.5	Preparative	HE31A-P-1.5
1.5	8	HE31A-8-1.5
1.5	12	HE31A-12-1.5
1.5	16	HE31A-16-1.5

Comb back with 2 screws	HE31-BK
Replacement screws for comb backs (pk/12)	HE31-S

Companion products

Hoefer PS300B power supply 300 V, 500 mA, 90 W	PS300B
MacroVue UV-20 Transilluminator 115 V~	UV20-115V
230 V~	UV20-230V

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