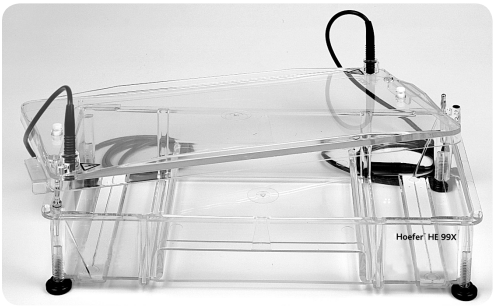
user manual

Hoefer HE99X

Submarine electrophoresis unit



um HE99X-IM/Rev. L0/07-12

Hoefer®

Contents

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 käyttöjänniteva lvonnat 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 

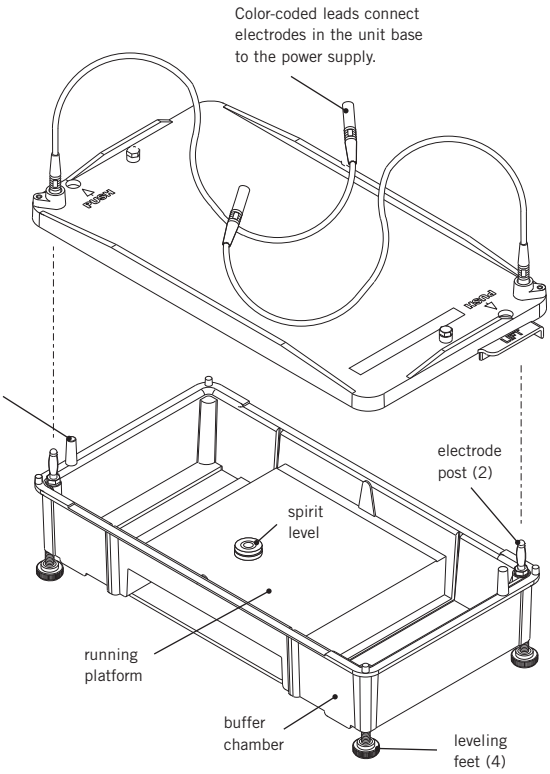
Denna symbol anger att elektriska och elektroniska utrustningar inte får avyttras som osorterat hushållsavfall och måste samlas in separat. Var god kontakta en auktoriserad tillverkarrepresentant för information angående avyttring av utrustningen.

Submarine Electrophoresis Unit
Function and description

The Hoefer® HE99X unit electrophoretically separates nucleic acid fragments in a submarine gel. The gel is first cast in a gel caster, which is available in three lengths. Once the gel sets, the running tray is transferred to the platform of the electrophoresis unit and the gel is submerged under running buffer.

Fig 1. Horizontal submarine unit main components.

Gel casting kits, combs and comb backs may be ordered separately; Fig 2 illustrates a casting kit, and the ordering section tabulates all comb sizes and accessories.



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 Hoefer, Inc. 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.

Specifications

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.

Max. voltage	200 V
Max. wattage	20 W
Max. amperage	100 mA
Max. operating temp.	45 °C
Max. buffer volume	1.2 liters
Gel size	15 cm wide × 10, 15, or 20 cm long
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 × l × d) (includes electrode posts)	18.2 × 36 × 14 cm (7.2 × 14.2 × 5.5 in.)
Weight (base, lid, and leads only)	0.82 kg (1.8 lb)
Product certifications	EN61010–1, UL61010A-1, CSA C22.2 1010.1, CE Certified

Before you start...

1. Wash all components with a dilute solution of laboratory detergent and rinse thoroughly.
2. Level the unit by placing the spirit level on the running platform and adjusting the leveling feet.

Volume for 3-mm thick gels

tray size (cm)	agarose (ml)
15 × 10	45
15 × 15	68
15 × 20	90

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

Operating instructions

Agarose gels are first cast in the gel casting kit, and samples are then loaded into the wells and electrophoretically separated. The fluorescent dye ethidium bromide can be added to the gel or electrophoresis buffer or both in order to track separation progress. At the completion of electrophoresis, the gel may be stained and photographed, blot transferred, or dried for autoradiography.

Casting the gel

Prepare the solutions

❶

Prepare about 1.3 liters of running buffer. Up to 100 ml of buffer is required for the gel and 1.2 liters for the buffer chamber. Refer to page 10 for recipes of three commonly used electrophoretic running buffers.

❷

Prepare the sample loading buffer. Refer to page 12 for a recipe and tabulated volume capacity for each comb size.

❸

Prepare agarose solution(s).

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 running tray.

Optional: Add 0.5 µg/ml ethidium bromide to the gel solution in order to facilitate observation of separation progress during electrophoresis.

Prepare the casting tray and pour the gel

❶

Install a foam pad at each end of the casting tray.

Use a comb as a placement guide so that the pad adheres ≈1 mm from the bottom of the tray: Lay the comb into the bottom of the tray, oriented so that it fits completely across the tray along the side that is 16 cm wide. Peel off the adhesive backing on the foam pad, align the pad on the comb, adhesive side toward the inside wall of the tray, and slide the comb against the wall. Press the foam pad in place and repeat with second pad on the wall opposite the first pad.

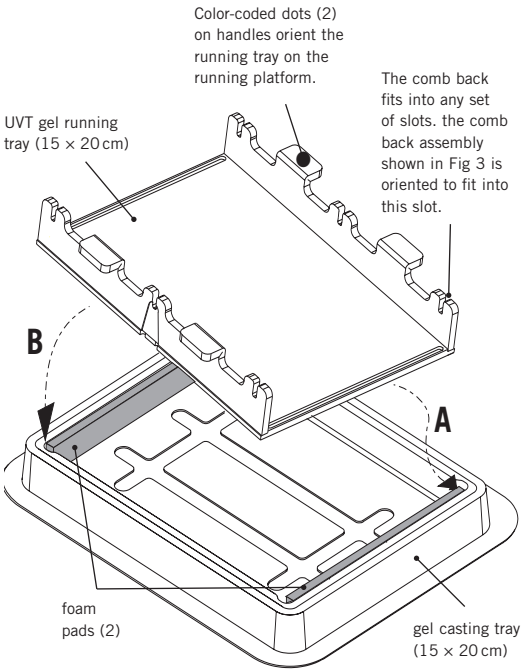


Fig 2. Gel casting kit.

Running tray installation: Approach the foam pad with one end of the running tray (Arrow A) 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 (Arrow B) before sealing against the foam pad.

Note: Grooves in the running tray create ridges at both ends of the gel to prevent it from slipping or floating. If these ridges are not desired, either tape over the grooves before casting, or trim off ridges with a spatula after the run.

❷

Seat the running tray between the foam pads in the casting tray by placing one end of the tray against the foam pad, slightly compressing it, then seating the other end of the tray against the opposite foam pad. (See arrows A and B in Fig 2.) The running tray should lay flush against the bottom of the casting tray.

❸

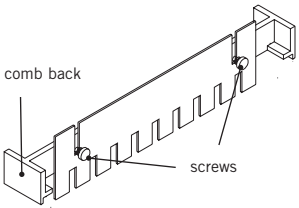
Place the casting tray assembly on a leveling surface and level, using the spirit level on the running tray as a guide. Check that the comb assembly leaves ≈1 mm of space between the comb bottom and the running tray. Remove the level and the comb assembly.

Prepare the combs

❶

Align the two slots in the comb with the loosened thumb screws of the comb back. Tighten the screws until the comb is just supported.

Fig 3. Assembled comb.



❷

Place the comb assembly into a set of slots on the running tray seated in the casting tray. Adjust the comb so that the bottom of the teeth are ≈1.0 mm from the running tray. Tighten the screws to secure the comb.

To run twice as many samples on the 15 and 20 cm trays, prepare two comb assemblies and place one near the cathode end, indicated by the black dot, and one at the center.

Final casting steps

❶

Pour the agarose solution (cooled to 50 °C) onto the running tray seated in the casting tray. Orient the comb assembly so that it is at the end of the tray opposite the direction of migration (typically at the cathode [–] end, which is marked by a black dot on the handle). Fit the comb assembly into the slots.

❷

Allow a minimum of 30 min for the gel to set, then remove the comb carefully: partially lift and slightly tilt the comb at one end and 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.)

❸

Lift the running tray out of the casting tray and transfer it with the gel to the horizontal unit. Orient the running platform so that the sample will "run to red". That is, place the sample wells at the cathode (–) end, which is indicated by a black dot. A notch on either side of the running tray centers the tray on the running platform.

Preparing for electrophoresis

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

Note: Refer to the Buffers, volumes, and notes section for additional information and guidelines on page 10.

See page 12 for a sample loading buffer recipe and well volumes for various comb sizes in gels of different thicknesses.

Important! If running two sets of samples in one gel, monitor the run closely and stop electrophoresis when the marker dye approaches the wells in the center.

❶

Optional: 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. **Note:** Adding ethidium bromide to the running or sample buffer slows migration slightly. Detection by this method is not as sensitive as by staining after the electrophoresis run. See the DNA detection section, for more details (page 14).

❷

Fill the chamber with buffer until the gel is submerged ≈1 mm.

❸

Load the samples. Add the sample to 1/5 volume of the sample loading buffer. Mix each sample and load into a well with a micro-pipet, taking care to avoid puncturing the well bottom or entrapping bubbles.

❹

Place the lid on the unit so that the cathode (black lead) is at the end nearest the samples. (Nucleic acid samples migrate toward the anode.)

❺

Connect the color-coded leads (red to red, and black to black) to an approved power supply. Set the voltage and timer (if available). Agarose gels are typically run at constant voltage under a voltage gradient in the range of 2–5 V/cm. The distance between the electrodes is ≈26 cm, so a setting of 130 V results in a gradient of 5 V/cm.

After electrophoresis

❶

Important! Always turn off the power supply and disconnect the leads before removing the lid.

❷

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

❸

Clean the unit as described in the next section.

Care and maintenance

Cleaning

- Never autoclave or heat any component above 45 °C.
- 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%).

The unit is resistant to all common electrophoresis buffers, but we recommend a thorough washing with a mild detergent after each use. Rinse with distilled water and allow to air dry.

To remove DNase and RNase contamination, fill the unit with 3% hydrogen peroxide (H_2O_2), soak for 10 minutes, then rinse thoroughly with DEPC-treated, autoclaved, deionized water. (Sambrook and Russell, *et al.* 1:7.82)

Troubleshooting

problem	solution
Sample well deformed	Allow the gel to set for a minimum of 1 hour and make sure it is at room temperature before removing the comb.
	Remove the comb at a slight angle and 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 the comb is warped, replace.
	If the running tray is warped, replace. (Cool agarose to 50 °C to prevent the tray from warping.)
	Circulate buffer if it becomes depleted by stopping the run and pipetting the buffer from one chamber to the other.
Double-banded pattern	Make sure the comb remains vertical after the gel is cast so that the well shape is not distorted.
	Decrease the buffer level to 1 mm above the top of the gel in order to reduce the temperature gradient in the gel.
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 the recommended agent.)
	Make sure the sample is completely dissolved.
	Reduce the sample concentration.
	Reduce the sample volume.
	Reduce voltage to 5 V/cm.
	Be sure the well floor is at least 1 mm thick to prevent samples from leaking through the 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	Do not press the running tray into place. Install as described on page 4.

Notes, buffers, and volumes

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 three most commonly used running buffers for DNA electrophoresis are listed below. The buffering capacity of both TBE and TPE is usually sufficient so that buffer circulation is unnecessary. Circulation may be required during runs longer than 3 hours or when using the TAE buffer.

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

(0.89 M Tris, 0.89 M boric acid, 20 mM EDTA, pH ≈8.3, 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, solution 4)	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-phosphate-EDTA (TPE) stock buffer[a]

(0.89 M Tris, 0.89 M phosphoric acid, 20 mM EDTA, pH ≈8.1, 1000 ml)

Tris base (FW 121.1)	0.89 M	108.0 g
Phosphoric acid (85%)	0.23 M	15.5 ml
EDTA solution (0.5 M, pH 8.0, solution 4)	0.02 M	40.0 ml
Deionized H_2O		to 1000.0 ml

Stir. Do not adjust pH. Dilute to 1X, to yield 89 mM Tris base, 23 mM phosphoric acid, and 2 mM EDTA.

3. 10X Tris-acetate-EDTA (TAE) stock buffer[a]

(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, solution 4)	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.

4. EDTA solution (ethylenediamine tetraacetic acid)[b]

(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

[a]Sambrook J. and Russell, D.W., (2001) Molecular Cloning: A Laboratory Manual, A1.17

[b]Current Protocols in Molecular Biology (1993) A.2.1

Loading buffer and sample volumes

Loading buffer

(5X, 25% Ficoll 400, 0.25% Bromphenol 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 1 volume loading buffer to 4 volumes of sample. (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 or dragging effects caused by comigration with smaller nucleic acids.

Comb specifications and well volumes

code number	no. of wells	well width (mm)	well thickness (mm)	sample vol. per 1 mm depth (µl)
HE91A-P-1.5	1/2	113/10	1.5	171/14.5*
HE91A-P-3.0	1/2	113/10	3.0	342/29.0*
HE91A-10-1.5	10	9.7	1.5	14.5
HE91A-10-3.0	10	9.7	3.0	30.0
HE91A-15-1.0	15	7.1	1.0	7.1
HE91A-15-1.5	15	7.1	1.5	10.6
HE91A-15-3.0	15	7.1	3.0	21.3
HE91A-20-1.0	20	4.7	1.0	4.7
HE91A-20-1.5	20	4.7	1.5	7.1
HE91A-20-3.0	20	4.7	3.0	14.2
HE91A-30-1.0	30	3.0	1.0	3.0

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

Agarose gel electrophoresis notes

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

DNA mobility

The suggested agarose concentration for separating fragments of various sizes is listed below. Other factors affecting separation results include the selected running buffer, the voltage setting, the temperature, and the presence of ethidium bromide.

Agarose concentrations for separating DNA fragments of various sizes

agarose (%)	effective range of resolution of linear DNA fragments (kb)[†]
0.5	1 to 30
0.7	0.8 to 12
1.0	0.5 to 10
1.2	0.4 to 7
1.5	0.2 to 3

[†]Current Protocols in Molecular Biology, p 2.5.2 (1993).

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. The bands are well resolved when run 2 hours on a 20 cm long 1% agarose gel in 0.5X TBE buffer at 150 V.

Note: For an example of RNA electrophoresis, refer to *Molecular Cloning: A Laboratory Manual* by J. Sambrook and D.W. Russell.

RNA mobility

RNA can also be separated on the basis of size. To avoid irregularities 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.

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 ≈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 radio-labeled DNA.

Ethidium bromide (0.5 µg/ml) can be added to running buffer to monitor sample progress because the dye's fluorescence reveals DNA under UV light. (To check band location, 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. If you

place the gel on the transilluminator, ensure that it lies flat by cutting off the ridges formed by the grooves in the running tray. (Do not damage the transilluminator surface; trim both ends of the gel with a spatula while it is still in the tray, lift away the ridges, and then slide the gel onto the transilluminator.) For viewing, 302 nm light is recommended for both acceptable sensitivity and reduced 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 and Russell, A9.4.).

Transfer

Before transfer, trim off the ridges at both ends of the gel to ensure even gel contact with the membrane.

Bibliography

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

Sambrook, J., and Russell, D.W., *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press. (2001).

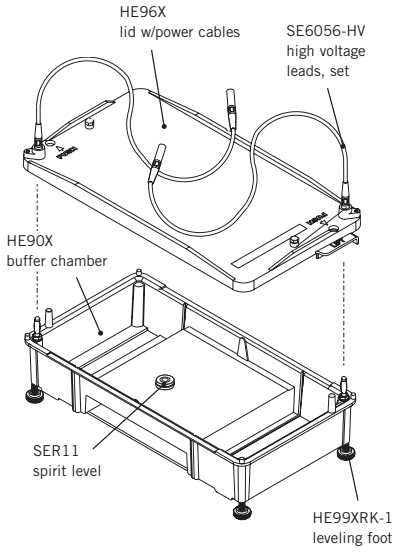
Ordering information

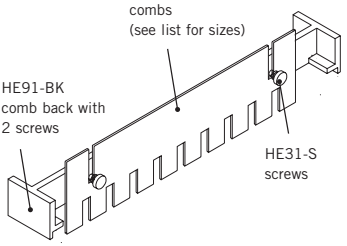
product	quantity	code number
HE99X Horizontal Agarose Submarine Unit, complete. Includes basic unit, 15 × 20 cm gel casting kit, one 1.5 mm-thick 15-well comb and comb back.	1	HE99X-15-1.5
HE99X Horizontal Agarose Submarine Unit, basic. Includes spirit level. (Order gel casting kit, comb and comb back separately.)	1	HE99X
Accessories and replacement parts		
Buffer chamber assembly only	1	HE90X
Comb back for HE99X comb with 2 screws	1	HE91-BK
Lid with power cables	1	HE96X
High voltage leads, set	1	SE6056-HV
Mylar sealing tape (1 roll, 66 mm)	1	SE1510
Foam sealing gaskets	4	HE98X
Leveling feet	4	HE99XRK-1
Companion products		
MacroVue™ UV-20 Transilluminator 115 V~	1	UV20-115V
MacroVue™ UV-20 Transilluminator 230 V~	1	UV20-230V
HE99X gel casting kits		
Each size includes 1 gel casting tray, 1 UVT running tray, and 4 foam sealing gaskets		
15 × 10 cm	1	HE97X-10
15 × 15 cm	1	HE97X-15
15 × 20 cm	1	HE97X-20
HE99X gel casting trays		
15 × 10 cm	1	HE95X-10
15 × 15 cm	1	HE95X-15
15 × 20 cm	1	HE95X-20
HE99X gel running trays		
15 × 10 cm	1	HE92X-10
15 × 15 cm	1	HE92X-15
15 × 20 cm	1	HE92X-20

Combs

no. of wells	comb thickness (mm)	well width (mm)	code number
1/2*	1.5	113/10	HE91A-P-1.5
1/2*	3.0	113/10	HE91A-P-3.0
10	1.5	9.7	HE91A-10-1.5
10	3.0	9.7	HE91A-10-3.0
15	1.0	7.1	HE91A-15-1.0
15	1.5	7.1	HE91A-15-1.5
15	3.0	7.1	HE91A-15-3.0
20	1.0	4.7	HE91A-20-1.0
20	1.5	4.7	HE91A-20-1.5
20	3.0	4.7	HE91A-20-3.0
30	1.0	3.0	HE91A-30-1.0

*Preparative combs form two marker wells, one on each side of the preparative well. The first value in each column refers to the prep well, the second to the reference well.





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