1 SuperSub Function and Description

The Hoefer HE 100 SuperSub™ Submarine Electrophoresis Unit electrophoretically separates nucleic acid fragments in agarose gel. The technique is simple and sensitive, and by varying the agarose concentration, fragments over a large size range can be separated. Fragments stained with ethidium bromide can be easily visualized under UV light.

All gels are 20 cm wide. Gel casters for three lengths—15, 20, and 25 cm—are available. Gel thickness can range from 3 to 7 mm. The gel is first cast in the running tray with or without a casting tray. After the gel sets, the running tray is transferred to the horizontal unit.

Two features contribute to the success of higher voltage runs without loss of resolution: 1) The built-in buffer circulation system, activated by a magnetic stirrer, maintains uniform buffer pH and ionic strength as well as uniform temperature at the gel surface, which can be critical in certain applications such as glyoxylated RNA separations. 2) A heat exchanger under the gel platform controls buffer temperature when attached to an external cooling system. Temperature control is especially important when using field inversion techniques to separate large DNA fragments.

Specifications

Max. voltage	500 V⎓
Max. wattage	40 W
Max. amperage	500 mA
Max. operating temperature	45 °C
Max. buffer volume	950–1175 ml, depending on gel size
Gel size	20 cm wide × 15, 20, or 25 cm long 3–7 cm thick
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 x l x d) (includes electrode posts)	25 x 32 x 8.5 cm (9.9×12.6×3.4 in.)
Product certifications	EN61010–1, UL3101–1, CSA C22.2 1010.1, CE

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

- used in laboratory locations,
- used as delivered from Amersham Biosciences except for alterations described in the User Manual, and
- connected to other CE labeled instruments or products recommended or approved by Amersham Biosciences.

Important information

- **The safety lid must be in place before connecting the power leads to a power supply.**
- **Turn all power supply controls off and disconnect the power leads before removing the safety lid.**

- **Circulate only water or 50/50 water/ethylene glycol through the heat exchanger.** Never introduce anti-freeze or any organic solvent into any part of the instrument. **Organic solvents will cause irreparable damage to the unit!**
- **Do not connect the heat exchanger to a water tap or any coolant source where the water pressure is unregulated.**
- Cool the agarose to 50 °C before pouring into the casting kit to prevent plastic parts from warping.
- **Do not operate with buffer temperature above 45°C.** All plastic parts are rated for 45°C continuous duty. Circulate coolant through the heat exchanger during electrophoresis to minimize heating. **Overheating will cause irreparable damage to the unit!**

- If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
- Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining, and servicing this product.

Informations importantes

- **Le couvercle de sécurité doit être en place avant de brancher les prises au générateur.**
- **Eteindre le générateur et débrancher les prises avant d'enlever le couvercle de sécurité.**

- **Faire circuler seulement de l'eau ou 50/50 d'eau et d'éthylène glycol dans l'échangeur vertical à cirulation d'eau.** Ne jamais utiliser d'anti-gel ou tout autre solvant organique avec cet instrument. **Les solvants organiques causeraient des dommages irréparables à l'appareil.**
- **Ne pas connecter l'échangeur vertical à circulation d'eau à un robinet ou quelque source de refroidissement dont la pression n'est pas régulière.**
- Refroidir l'agarose entre 50 °C avant de la verser dans l'unité de moulage afin d'éviter que les piéces en plastique ne se déforment.
- **Ne pas utiliser avec un tampon à une température au dessus de 45 °C.** Toutes les piéces en plastique sont prévues pour résister à une température constante de 45 °C. **Faire circuler l'eau dans l'échangeur vertical durant l'électrophorèse pour minimiser l'échauffement afin d'éviter des dommages irréparables à l'instrument.**

- Si l'instrument n'est pas utilisé en conformité avec les recommandations du fabriquant, les protections de sécurité qui équipent cet appareil peuvent être rendues inéfficaces.
- Seulement les accessoires et piéces detachées approuvés ou fournis par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

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 Amersham Biosciences sales office. Inspect all components for damage that may have occurred while the unit was in transit. If any part appears damaged, contact the carrier immediately. Be sure to keep all packing material for damage claims or to use should it become necessary to return the unit.

Buffer chamber. Both platinum electrodes are located at the outer lower edges of each buffer chamber (so that bubbles generated during electrophoresis do not build up near the gel). Below the running platform are housed both an internal buffer circulation system that can be driven by a magnetic stirrer, and a heat exchanger that can be attached to an external circulator bath.

Safety lid. The safety lid houses the electrode connectors and leads. It fits on the buffer chamber in only one orientation. The fully-shielded 4 mm safety plugs connect to a compatible DC power supply.

Running trays. All running trays are 20 cm wide. Three tray lengths are available: 15, 20, and 25 cm. The two shorter trays each can hold one or two combs, and the 25-cm long tray can hold up to four combs. The tray has an “anchor notch” at each end to help keep the gel in place during transport or when higher circulation rates are required. Gels can be cast either by setting the running tray into a casting tray or by taping the ends of the tray. All trays are UV-transparent for convenient visualizing of the stained results.

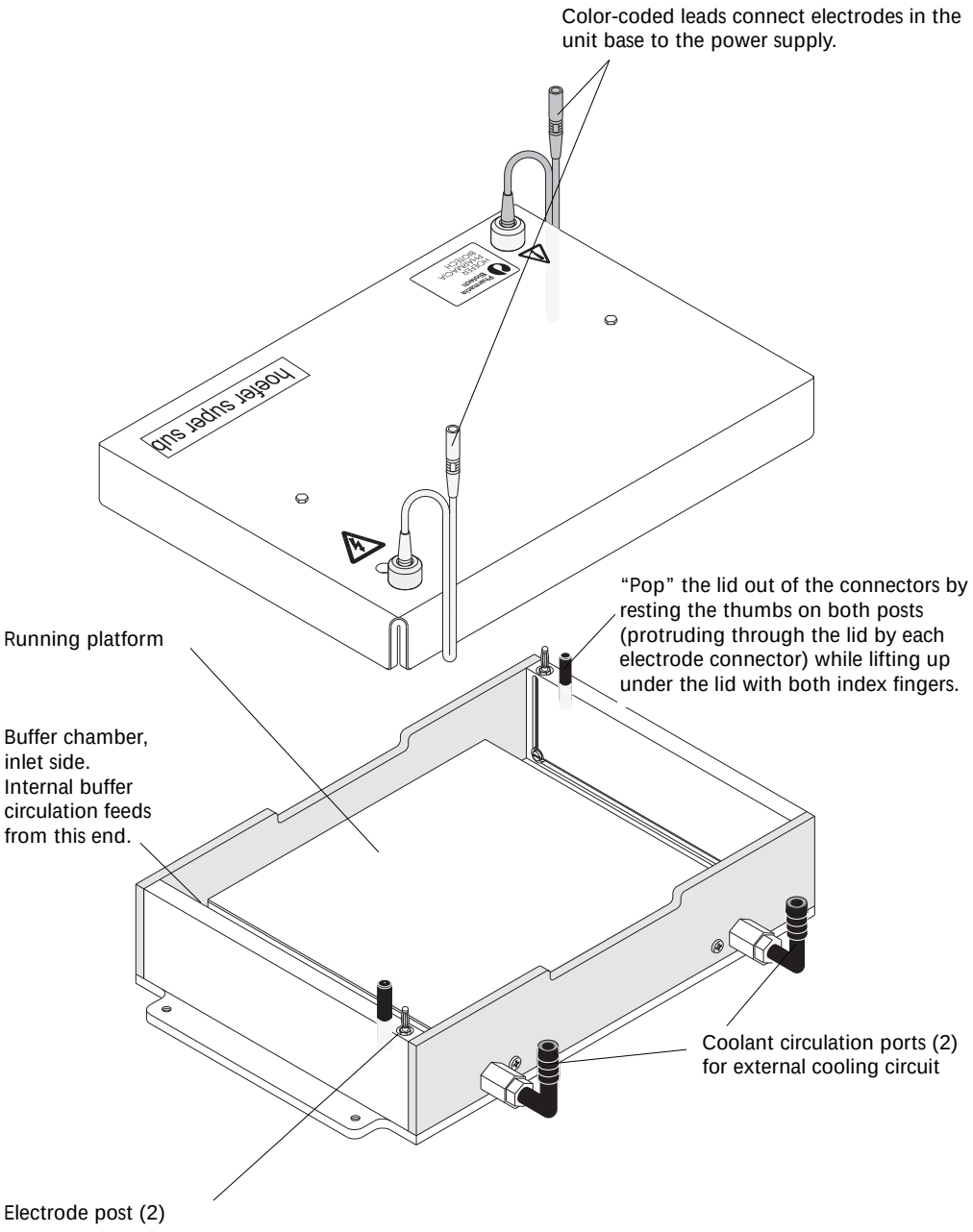
Casting tray. Using the appropriate length casting tray eliminates the taping step. Agarose should be cooled to 50 °C before pouring to prevent warping of the tray.

Combs. Available combs range in thickness from 1 to 6 mm, and have 6, 12, 15, 20, 30 or 36 wells. Preparative combs with 2 or 3 reference wells are also available. The 20-well combs are designed for loading wells with a multi-channel pipetter.

Well Locating Decal. Place on the running platform under the running tray to see wells more easily while loading samples.

Figure 1.
Horizontal submarine unit main components

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



2 Operating Instructions

Agarose gels are first cast in the gel casting tray. The running tray is then transferred to the platform of the unit, samples are loaded into wells created by a comb, and the sample is electrophoretically separated. The fluorescent dye ethidium bromide can be added to the gel or electrophoresis buffer or both in order to track separation progress. After electrophoresis, the gel may be stained and photographed, blotted, or dried for autoradiography.

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 levelling feet.

Casting the gel

Prepare the solutions

1 **Prepare about 1.5 liters of running buffer.** Approximately 300 ml of buffer is required for the gel and 1.2 liters for the buffer chamber. Refer to p. 13 for recipes of three commonly used electrophoretic running buffers.

Optional: Prechill the buffer either before pouring into the SuperSub, or after, by connecting the heat exchanger to an external circulator bath.

2 **Prepare the sample loading buffer.** Refer to page 14 for a recipe and tabulated volume capacity for each comb size.

3 **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.

Caution!

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

Table 1.

Volume of agarose required for different gel sizes.

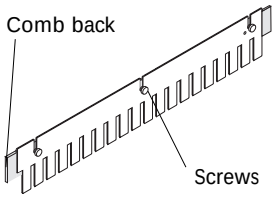
	Approximate volume of agarose (ml) for various gel thicknesses (mm)				
Tray size (cm)	3	4	5	6	7
20 x 15	90	120	150	180	210
20 x 20	120	160	200	240	380
20 x 25	150	200	250	300	350

Casting the gel

Prepare the combs

1 Align the three slots in the comb with the loosened thumb screws on the comb back. Tighten the screws until the comb is just supported.

2 Place the comb assembly into a set of slots on the casting tray and adjust the comb so that the bottom is ≈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 combs. Prepare up to four combs for the 25-cm tray.



Pour the gel

1 Press the running tray into the casting tray. The running tray should lay flush against the bottom of the casting tray.

Alternative casting assembly: Tape both ends of the running tray, making sure the tape is high enough to contain the agarose.

2 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 will allow ≈1 mm of space between the comb bottom and the running tray. Remove the level and the comb assembly.

3 Pour the agarose solution **(cooled to 50 °C)** into the casting assembly. Orient the comb assembly so that the comb side faces the gel. Fit the comb assembly into the slots. (The 20 cm and 15 cm trays can hold two combs; one at the cathode end, indicated by the black dot, and one in the center. The 25 cm tray can hold 4 combs.)

5 Allow a minimum of one hour for the gel to set, then 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.)

Lift the running tray out of the casting tray, or, remove the tape from the ends of the running tray if no casting tray was used.

6 Remove any agarose adhering to the underside of the running tray.

Optional: Store the cast gel in running buffer for up to two days at 4 °C.

Prepare the unit

1 **Optional cooling:** Connect the heat exchanger to a thermostatic circulator such as the MultiTemp® III. Slide hose clamps (4 total) onto each end of two lengths of 8 or 9 mm i.d. (approx. 3/8") vinyl or silicone tubing. Attach one end of each length of tubing to a heat exchanger port. Attach the free ends of each length of tubing to the circulator ports, one to the inlet and the other to the outlet. Secure the connections with the hose clamps.

Note

If the cooling option is used frequently, it is convenient to attach Quick-fit fittings to the tubing. The valves in these fittings prevent coolant spillage.

Important!

- Use only water or 50/50 water/ethylene glycol as a coolant. Never use a commercial antifreeze or any alcohol-based mixture or irreparable damage to the heat exchanger will result.
- Do not connect the heat exchanger to a water tap or any other source where the water pressure is unregulated.

A starting point temperature setting for the circulator is 20 °C for electrophoresis at or below 200 V. Adjust as necessary for variables such as ambient temperature, changes in power output, and circulator efficiency. If accurate temperature control is critical, measure the temperature and adjust as necessary.

Optional: After establishing buffer circulation (step 2 below), run the circulator to prechill the buffer for about 30 minutes before starting electrophoresis.

2 **Establish buffer circulation**. Locate the inlet buffer chamber at the right side of the unit (if the coolant ports are in the back.) The inlet is a groove along the chamber floor. Slowly add buffer to this chamber until the spiral conduit is filled, allowing air bubbles to escape. If necessary, tip the unit so that the inlet chamber is lower than the outlet chamber.

Note: A large number of bubbles in the pumping chamber will interfere with circulation efficiency, but a few remaining should not.

Place a magnetic stirrer on a leveling plate or other level surface, then place the SuperSub on the magnetic stirrer. Place the spirit level on the running platform and level the unit.

Turn on the magnetic stirrer and move the unit as necessary until the stir bar spins freely. Adjust the stirring speed to achieve the required circulation rate—the maximum rate for prechilling, and the lowest rate during electrophoresis. Circulation at the maximum rate is not recommended for electrophoresis because high flow rates may result in uneven cooling and turbulence that could wash sample out of the wells.

Separating the sample

Refer to the Notes, Buffers, and Volume section for additional information and guidelines.

1 Turn off the magnetic stirrer to stop buffer circulation.

Optional: To more easily see wells for sample loading, place the Well Locating Decal on the running platform, printed side down (to prevent the lines from becoming defaced): First fold the tabs down at the four corners, and then place, making sure that the side marked "+" is next to the positive electrode. Note: Gels cast in the 25 cm tray may not coincide perfectly with the shaded areas on the Decal.

Alternatively, if your wells are always in the same location, apply a length of red tape across the running platform to mark that area.

2 Transfer the running tray with the gel to the running platform, orienting it 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.

3 Add buffer until the gel is submerged under ≈1 mm of liquid. This is the optimal depth; too much buffer will cause excessive heat to be generated, and too little buffer will cause excessive turbulence around the gel.

Caution

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

4 **Optional:** Although this technique is not recommended, migration progress can be monitored by either adding 0.5 µg/ml (final conc.) of ethidium bromide to the running buffer now, or, adding 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 staining and viewing on a transilluminator. See the DNA detection section for more details.

5 **Load the samples.** Add the sample to 5X sample loading buffer (1/5 of the final volume is loading buffer, see p. 12). Mix and load each sample into a well with a micro-pipet, taking care to avoid puncturing the well bottom or entrapping bubbles in a well.

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

7 Connect the color-coded leads (red to red, and black to black) to an approved power supply such as the EPS 600 or the Hoefer EPS 2A200, and set the voltage and timer (if available.)

Starting point guidelines for DNA separations (*Hin*d III digest of lambda phage, 2–27 kb, 0.5X TBE buffer) are 200 V constant voltage, a maximum current setting of 200 mA, and the circulator set to 20 °C. This separation typically requires 3–4 hours.

Important

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

8 **Important:** Electrophoresis should be under way several minutes before starting buffer circulation to prevent samples from being washed out of the wells. Once circulation is established, set the stirrer to the lowest speed (about 100 ml/min). If the stirrer is too powerful, try placing two glass plates on the stirrer to decrease the strength of the magnetic field.

After the separation

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

2 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. If the gel is still in the running tray, staining will take longer.

3 Visualize the gel on a transilluminator. Transfer the gel using the UV-transparent running tray. For maximum visualization, place the gel directly on the transilluminator (see step 4).

To reduce the background fluorescence of unbound ethidium bromide, the gel can be destained by soaking it for 10 min in distilled water or TBE buffer. Destaining makes it easier to detect quantities of DNA less than 50 ng.

4 Remove the gel from the running tray by gently pulling up on the outer edges of the gel. If the gel is fragile, run a spatula or the Hoefer Wonder Wedge between the gel and tray to free the gel "anchor".

5 Clean the unit as described below.

3 Care and Maintenance

- 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%).
- Adhesive from the sealing tape may be removed from the running tray by gently wiping the surface with kerosene.
- To remove DNase and RNase contamination, fill the unit with 3% hydrogen peroxide (H_2O_2), soak for 10 minutes and circulate the solution (see "Prepare the unit, step 2). Rinse thoroughly with DEPC-treated, autoclaved, deionized water. (Sambrook, *et al.* 1:7:40)

The unit is resistant to all common electrophoresis buffers, but we recommend a thorough washing after each use:

1 Rinse the SuperSub and then fill with deionized water.

2 Place the unit on a magnetic stirrer and allow it to circulate to rinse all internal surfaces. (See "Prepare the unit, step 2.)

3 Stop circulation and empty the unit. To empty the circulation chamber, turn the unit upside-down, outlet chamber facing down. Allow the liquid to drain.

Note: If a thorough cleaning is required, disassemble the base by removing the two fittings and six screws on the base and sides.

4 Troubleshooting

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 comb is warped, replace.
- ✓ If running tray is warped, replace. (Cool agarose to 50°C to prevent the tray from warping.)
- ✓ Circulate the buffer at about 100 ml/min. This is the slowest speed on most magnetic stirrers.

Double-banded pattern

- ✓ Make sure the comb is vertical during casting 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 through the gel.
- ✓ Avoid a temperature gradient in the gel; the cooling temperature should be set no lower than 20 °C at 200 V.

Poor band resolution

- ✓ Add Ficoll, glycerol, or sucrose to the sample loading buffer to ensure that the sample layers on the bottom of the well. (Ficoll is the recommended agent.)
- ✓ Make sure the sample is completely dissolved.
- ✓ Reduce the voltage.
- ✓ Reduce the sample concentration.
- ✓ Reduce the sample volume.
- ✓ Be sure there is at least 1 mm of gel below the bottom of the comb to prevent samples from leaking out the bottom of the well.
- ✓ Reduce salt concentration of the sample.
- ✓ Check enzyme activity; may require longer digestion or a different restriction buffer.
- ✓ Prepare fresh sample if you suspect nuclease contamination.
- ✓ Choose agarose with a low endosmosis value.

5 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 1 below. Other factors affecting separation results include the running buffer, the voltage setting, the temperature, and the presence of ethidium bromide. Special agaroses are available that can extend resolution ranges.

Table 2.

Agarose concentrations for separating DNA fragments of various sizes*

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

*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 pairs. The bands are well resolved when run 3 hours on a 20 cm long 1% agarose gel in 0.5X TBE gel at 200 V.

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.

Note

RNA samples usually require longer runs or buffers that are easily depleted, so it is necessary to circulate the buffer.

Request Hoefer Technical Bulletin #137, Low Formaldehyde Denaturing RNA Gel Electrophoresis - Protocol for Northern Blotting with the TE 80 TransVac™ Vacuum Transfer Unit, for an example of RNA electrophoresis.

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; do not adjust the pH of these buffers once they are prepared according to the recipe! 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 TAE buffer.

Important

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

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

(0.90 M Tris, 0.90 M boric acid, 20 mM EDTA, pH ≈8.2, 1000 ml)

Tris base (FW 121.1)	0.90 M	109.0 g
Boric acid (FW 61.8)	0.90 M	55.6 g
EDTA solution (0.5 M, pH 8.0, soln. 3)	0.20 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 90 mM Tris base, 90 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 (85%)	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)

$Na_2EDTA\text{-}2H_2O$, (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

Important

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

†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% Bromphenol blue†, 10 ml)

Deionized H_2O	to 7.0 ml
Ficoll® 400 (Amersham Biosciences)	2.5 g
Bromphenol 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.

Sample volume required for various combs

wells			sample vol. per	
no.	**thickness (mm)**	**width (mm)**	**1 mm depth (µl)**	**Code. no.**
12	1.0	13.7	13.7	80-6044-47
12	1.5	13.7	20.5	80-6044-66
12	3.0	13.7	41.1	80-6044-85
20	1.0	7.3	7.3	80-6045-04
20	1.5	7.3	10.8	80-6045-23
20	3.0	7.3	21.8	80-6045-42
30	1.0	4.5	4.5	80-6045-61
30	3.0	4.5	13.5	80-6045-80
36	1.0	3.0	3.0	80-6045-99
36	1.5	3.0	4.5	80-6046-18
36	3.0	3.0	8.9	80-6046-37
1/2[a]	1.0	175.6/5.1	175.6/5.1	80-6046-75
1/2[a]	1.5	175.6/5.1	175.6/5.1	80-6046-94
1/2[a]	3.0	175.6/5.1	526/15.2	80-6047-13

[a]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.

DNA detection

DNA can be detected either by the fluorescence of bound ethidium bromide or by autoradiography of radio-labeled DNA.

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%.

Ethidium bromide (0.5 µg/ml) is often added to running buffer to monitor sample progress because the dye's fluorescence reveals DNA under a UV lamp. (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. **Note:** Minimize the staining time to prevent small nucleic acid fragments from diffusing out of the gel.

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, cut off the ridges formed by the grooves in the running tray so that the gel lies flat. (Do not damage the transilluminator surface; trim both ends of the gel with a spatula while 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 photoknicking.

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, p. 6.15)

Transfer

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

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6 Customer Service Information

Technical Service and Repair

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

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

Ordering Information

	Qty.	Code No.
HE 100 SuperSub Submarine Electrophoresis Unit, basic. Includes: spirit level. (Order gel casting kit, comb and comb back separately.)	1	80-6043-90
HE 100 SuperSub Submarine Electrophoresis Unit, complete. Includes basic unit, 1.0-mm thick 20-well comb and comb back, 20x25 cm running plate, and 20x25 cm casting tray.	1	80-6043-71

Accessories and replacement parts

Buffer chamber assembly only	1	80-6044-09
Comb back for HE 100 combs, with 3 screws	1	80-6046-56
Lid with power cables	1	80-6048-65
High voltage leads, set	1	80-6177-09
Mylar sealing tape (1 roll)	1	80-6124-65
Quick-fit coupler body, female, to fit 3/8" i.d. tubing	2	80-6115-15
Quick-fit coupler body, male, to fit 3/8" i.d. tubing	2	80-6115-53
Tubing for coolant, silicone, 8 mm i.d./12 mm o.d.	4 m	80-1106-56

HE 100 Combs

wells no.	thickness (mm)	width (mm)	
12	1.0	13.7	80-6044-47
12	1.5	13.7	80-6044-66
12	3.0	13.7	80-6044-85
20	1.0	7.3	80-6045-04
20	1.5	7.3	80-6045-23
20	3.0	7.3	80-6045-42
30	1.0	4.5	80-6045-61
30	3.0	4.5	80-6045-80
36	1.0	3.0	80-6045-99
36	1.5	3.0	80-6046-18
36	3.0	3.0	80-6046-37
1/2[a]	1.0	176/5	80-6046-75
1/2[a]	1.5	176/5	80-6046-94
1/2[a]	3.0	176/5	80-6047-13

[a] 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.

Casting and running trays

HE 100 Gel Casting Kit		
20×15 cm	1	80-6048-84
20×20 cm	1	80-6049-03
20×25 cm	1	80-6049-22
Includes: casting and running trays		
HE 100 Casting Tray		
20×15 cm	1	80-6048-08
20×20 cm	1	80-6048-27
20×25 cm	1	80-6048-46
HE 100 UVT Running Tray		
20×15 cm	1	80-6047-51
20×20 cm	1	80-6047-70
20×25 cm	1	80-6047-89

Reagents

Ethidium bromide	10 ml	17-1328-01
Agarose, NA	100 g	17-0554-02
Agarose, prep	50 g	80-1130-07
Ficoll 400	100 g	17-0400-01
Bromophenol Blue	10 g	17-1329-01
Tris	500 g	17-1321-01
EDTA, disodium salt	100 g	17-1324-01
Boric acid	500 g	17-1322-01

Manuals and Technical Bulletin

User manual	1	80-6297-36
Service manual	1	80-6312-12
Amersham Biosciences Technical Bulletin #137	1	80-6012-36

Companion products

EPS 200 power supply	1	19-0200-00
EPS 600 power supply	1	19-0600-00
EPS 2A200 power supply		
115 V~	1	80-6274-18
230 V~	1	80-6274-37
MultiTemp III Thermostatic Circulator		
115 V~	1	18-1102-77
230 V~	1	18-1102-78
ImageMaster VDS		
DU 115 V~, 60 Hz	1	80-6246-82
DE 230 V~, 50 Hz	1	80-6247-01
DJ 100 V~, 50/60 Hz	1	80-6247-20
MacroVue UV-20 Transilluminator		
115 V~	1	80-6245-11
230 V~	1	80-6245-30
MacroVue UV-25 Transilluminator		
115 V~	1	80-6224-78
230 V~	1	80-6224-97
Photoman Polaroid Direct	1	80-6077-34

Printed in the USA

Hoefer® HE 100 SuperSub™

Submarine Electrophoresis Unit

User Manual

1. SuperSub Function and Description . . . 1
   - Specifications . . . 2
   - Important Information . . . 3
   - Unpacking and Inventory . . . 4
2. Operating Instructions . . . 6
3. Care and Maintenance . . . 10
4. Troubleshooting . . . 11
5. Notes, Buffers, and Volumes . . . 12
   - Bibliography and References . . . 16
6. Customer Service Information . . . 17

80-6297-36 22845/Rev E/5-97

Important user information

English

Please read this entire manual to fully understand the safe and effective use of this product.

The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument.

The lightning symbol within an equilateral triangle is intended to alert the user to the risk of exposure to high voltages.

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

Amersham Biosciences Inc.
Marketing Department
654 Minnesota Street
San Francisco, CA 94107 USA

Amersham Biosciences reserves the right to make changes in the specifications without prior notice.

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Renseignements importants d'utilization

Français

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

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

Le symbole de l'éclair dans un triangle équilatéral a pour objet d'attirer l'attention de l'utilisateur sur un danger d'exposition à la haute tension.

Tous vos commentaires sur ce manuel seront les bienvenus et veuillez les adresser à:

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654 Minnesota Street
San Francisco, CA 94107 USA

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Información importante para el usuario

Español

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

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

El símbolo del rayo en un triángulo equilátero alerta al usuario sobre el riesgo de exposición a altas tensiones.

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Wichtige Benutzerinformationen

Deutsch

Für ein vollständiges Verständnis und eine sichere Handhabung dieses Produktes ist es notwendig, daß der Benutzer dieses Handbuch vollständig durchliest.

Ein Ausrufezeichen in einem gleichseitigen Dreieck soll den Benutzer auf die Anwesenheit wichtiger Betriebs- und Wartungsanweisungen in der dem Gerät beiliegenden Dokumentation hinweisen.

Ein Blitzsymbol in einem gleichseitigen Dreieck soll den Benutzer auf die Gefahr anliegender Hochspannungen hinweisen.

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

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San Francisco, CA 94107 USA

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Informazioni importanti per l'operatore

Italiano

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

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

Il simbolo del fulmine all'interno di un triangolo equilatero indica all'utente la presenza di un rischio di esposizione ad alte tensioni.

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