BIO-RAD

DNA SUB CELL™ ELECTROPHORESIS SYSTEM

Instruction Manual

Catalog Number 170-4300 to 170-4306

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DNA SUB-CELL™ ELECTROPHORESIS SYSTEM

SECTION 1· INTRODUCTION

The Bio-Rad Sub-Cell Electrophoresis System comprises a flexible cell which efficiently separates DNA restriction fragments and other nucleic acids, a power supply. and precut blotting media. Although the system is principally for simple electrophoresis of submersible gels (1-3), it may also be used with agarose gel bridges (4-5).

Submarine gels are easy to cast, and readily dissipate heat which could lead to band distortion. These gels prevent the electrical field discontinuities caused by wicks or sample well dehydration, and allow sample underlaying.

Agarose gel bridges are especially useful for casting the soft gels required for separating high molecular weight DNA, or for thicker preparative gels requiring high currents. Agarose legs are more conductive than paper wicks. and prevent field strength discontinuities caused by uneven wetting.

Agarose legs can be reused if the electrophoretic polarity is not altered.

The Model 250/2.5 variable voltage power supply is recommended for running the Submarine Cell. This power supply permits voltage regulation from 0 to 250 volts at up to 2.5 amperes, depending on resistive load.

SECTION 2: EQUIPMENT

2.1 DNA Sub-Cell Electrophoresis System

Description	Catalog Number
Basic 15 x 15 cm, complete, includes basic unit. lid with cables, gel forming gates, 1.5 mm 15 well comb and holder, and buffer recirculation connectors.	170-4300
With Gel Tray 15 x 15 cm, complete, includes basic unit, lid with cables. gel forming gates, 15 x 15 cm removable UVTP gel tray, 1.5 mm 15 well comb and holder, and buffer recirculation connectors.	170-4302
With Gel Tray, 15 x 10 cm, complete, as above.	170-4301
With Gel Tray, 15 x 20 cm, complete, as above.	170-4304

2.2 Agarose Bridge Cells

: x 15 cm, complete, includes basic unit, lid with cables, gel forming gates, 15 x 15 cm removable UVTP agarose gel bridge, 1.5 mm 15 well comb and holder, and buffer recirculation connectors.	170-4305
15 x 20 cm, complete, as above.	170-4306

2.3 Accessories

UV Transparent Gel Trays	
15 x 10 cm	170-4310
15 x 15 cm	170-4311
15 x 20 cm	170-4312
15 x 25 cm	170-4315
UVTP Agarose Gel Bridges	
15 x 15 cm	170-4313
15 x 20 cm	170-4314
Comb Holder and Combs	
Comb Holder	170-4320
20 well comb, 1.5 mm	170-4321
20 well comb, 0.75 mm	170-4322
15 well comb, 1.5 mm	170-4324
15 well comb, 0.75 mm	170-4323
10 well comb, 1.5 mm	170-4326
10 well comb, 0.75 mm	170-4325
1 well comb, 3 mm (contains 2 outside wells for standards)	170-4328

Gates

Gel Casting Gates	170-4329

Power Supply

Model 250/2.5 Constant Voltage Power Supply, 100V, 50/60 Hz	165-4752
Model 250/2.5 Constant Voltage Power Supply, 120V, 60 Hz (U.S. version)	165-4753
Model 250/2.5 Constant Voltage Power Supply, 220V, 50 Hz	165-4754
Model 250/2.5 Constant Voltage Power Supply, 240V, 50 Hz	165-4755

Blotting Cell

Trans-Blot™ Electrophoretic Transfer Cell	170-3905

2.4 Chemicals

Agarose. Standard Low -m_r, 100 g (for routine DNA Electrophoresis)	162-0100
Agarose, Standard Low -m_r, 500 g	162-0102
Ultra Pure DNA Grade, 100 g	162-0125
Agarose, Ultra Pure DNA Grade, 500 g	162-0126
Agarose, Low Gel Temperature, 25 g (for preparative recovery of DNA)	162-0017
Agarose, Low Gel Temperature, 250 g	162-0020
Tris 500 g	161-0716
Sucrose, RNAse free, 1 kg	161-0720
Urea, 250 g	161-0730
Urea, 1 kg	161-0731
Bromophenol Blue, 10 g	161-0404
Methyl Green, 10 g	161-0405
Bromocresol Green, 5 g	161-0415
Xylene Cyanole FF, 25 g	161-0423

Nitrocellulose Membrane, 33 cm x 3 m roll	162-0115
Nitrocellulose Membrane, 20 x 20 cm, pkg 5	162-0113
Nitrocellulose Membrane, 15 x 15 cm, pkg 10	162-0116
Nitrocellulose Membrane, 15 x 9.2 cm pkg 10	162-0114
ABM-Paper, 20 x 15 cm, pkg 2	162-0104
ABM-Paper, 20 x 20 cm, pkg 1	162-0103
ABM-Paper, 15 x 15 cm, pkg 2	162-0105
ABM-Paper, 15 x 9.2 cm. pkg 2	162-0106
APT-Paper, 20 x 20 cm, pkg 1	162-0122
APT-Paper, 15 x 15 cm. pkg 2	162-0120
APT-Paper, 15 x 9.2 cm, pkg 2	162-0119
DEAE-Paper, 20 x 20 cm, pkg 25	162-0109
DEAE-Paper, 15 x 9.2 cm, pkg 25	162-0110
Blot Absorbant Filter Paper, 33 cm x 1 m roll	162-0118
Filter Paper (thick) 18 x 34 cm, pkg 25	165-0921
Blotting Media Selection Kit, 15 x 9.2 cm, includes:	162-0123
Nitrocellulose Membrane, 2 sheets	
APT-Paper, 1 sheet	
ABM-Paper, 1 sheet	

SECTION 3: PREPARING AGAROSE GEL SLABS

There are two ways to cast submarine gels. Fixed size (15 x 15 cm) gels may be cast on the basic cell, using the gel casting gates on the base of the unit. Variable size (15 x 10, 15 x 15, 15 x 20 cm and 15 x 25 cm) gels are cast on removable UV transparent plastic (UVTP) trays with the aid of plastic sealing tape or Scotch-tape. The UVTP trays allow multiple gels to be cast. They are also convenient for storing gels, or for transporting low percentage gels for staining, blotting, or photographing.

DNA Agarose Bridges use agarose wicks which are cast by sealing the bridge legs with plastic sealing tape and pouring the gel into the legs. These wicks may be formed from a higher percentage gel than the running gel if a soft running gel is required. After the wicks are formed, the running gel, or agarose bridge, is poured.

3 1 Fixed Submarine Mode

In the fixed submarine mode, fixed size gels are cast on the unit wihtout the removable gel trays.

1. Level the cell.

2. Slide the gel casting gates into the slots at opposite ends of the gel running stage.

3. Prepare the desired concentration of agarose in electrophoresis buffer. (See Appendix A for method).

4. Cool the agarose to at least 60°C before pouring.

WARNING

Hot agarose may cause the plastic in the cell to warp.

5. Place the comb holder next to the outside walls of the cell. The holder can be secured to the walls by lightly tightening the thumb screw. Avoid overtightening the screw as the holder may move or bow when the agarose is poured into the cell.

6. Adjust the comb to 1 to 2 mm above the base of the cell with the aid of the thumb screws. Pour the molten agarose between the gates.

7. Allow 1 to 2 hours for the gel to solidify. The gel should solidify at room temperature.

8. Remove the comb and holder carefully from the solidified gel. Loosen the screw securing the holder to the outer wall before removing the holder.

9. Submerge the gel beneath 2 to 6 mm of buffer. Use greater depth overlay with increasing voltages to avoid pH and heat effects.

10. If buffer recirculation is not required, seal the buffer recirculation ports in the cell with the plugs provided.

IMPORTANT

Buffer recirculation is required when greater than 1.2 V/cm (~ V) is applied to a 4 mm gel overnight or for lengthy runs. Recirculation outlets are provided. Failure to recirculate higher voltage runs may result in distorted bands and lanes as a result of gross pH and ionic changes which normally occur in Tris-Acetate running buffers. See Troubleshooting.

3.2 Removable Gel Tray Submarine Mode

1. Seal the ends of the UVTP gel tray securely with strips of Bio-Rad plastic sealing tape or Scotch-tape. Press the tape firmly to the edges of the gel tray to form a fluid-tight seal.

2. Level the gel tray on a leveling table or workbench.

3. Position the comb on the walls of the UV Gel Tray. Adjust the combs with the aid of the thumb screws so that it remains 1 to 2 mm above the base of the UV tray.

4. Prepare the desired concentration of agarose in electrophoresis buffer. (See Appendix A for method).

5. Cool the agarose to at least 60°C before pouring.

WARNING

Hot agarose will cause the gel tray to warp, which may result in wells of uneven depth.

6. Pour the molten agarose into the gel tray.

7. Allow the gel to solidify at room temperature for 1 to 2 hours.

8. Carefully remove the comb and holder from the solidified gel.

9. Remove the tape from the edges of the gel tray.

10. Place the tray onto the leveled DNA cell.

11. Submerge the gel under 2 to 6 mm buffer. Use greater depth overlay with increasing voltages to avoid pH and heat effects.

12. If buffer recirculation is not required, seal the buffer recirculation ports in the cell with the plugs provided.

IMPORTANT

Buffer recirculation is required when greater than 1.2 V/cm (~36 V) is applied to a 4 mm gel overnight or for lengthy runs. Recirculation outlets are provided. Failure to recirculate higher voltage runs may result in distorted bands and lanes as a result of gross pH and ionic changes which normally occur in Tris-Acetate running buffers. See Troubleshooting.

3.3 Agarose Bridge Mode

1. Seal the open slots in the legs of the UVTP agarose gel bridge securely with strips of Bio-Rad plastic sealing tape or Scotch-tape.

2. Level the gel bridge on a leveling table or workbench.

3. Position the comb holder at the outside of the gel bridge. Secure the holder with the thumb screw.

4. Adjust the comb with the aid of the thumb screws to 1 to 2 mm above the base of the bridge.

5. Prepare the desired concentration of agarose in electrophoresis buffer. (See Appendix A for method).

6. Cool the agarose to at least 60°C before pouring.

CAUTION

Hot agarose will warp the gel bridge.

7. Pour agarose into both legs of the bridge, to the top of the running surface. A higher agarose concnetration may be used in the leg wicks to support low percentage running gels. Agarose legs may be reused if the electrophoretic polarity is never reversed.

8. Pour the separating gel when agarose has cooled to 60°C. Hot gel will damage the bridge and cause the comb holder to bow, resulting in wells of uneven depth.

9. Allow the gel to solidify at room temperature for 1 to 2 hours.

10. Carefully remove the holder and comb from the gel. Be sure to loosen the thumb screw on the holder before removal.

11. Remove the tape from the slots on the gel bridge.

12. Place the bridge on the stage of the DNA cell.

13. Add buffer to a level above the slots in the gel legs.

14. Buffer circulation at about 10 ml/min. is recommended to prevent pH and ionic changes.

SECTION 4: ELECTROPHORESIS

4.1 Buffer

Tris-EDTA-Acetate (TEA) buffer is most often used for native gels. (See References 4 through 7 for running denaturing gels and Reference 6 for general DNA electrophoresis.) TEA buffer can be modified with respect to pH, acetate concentration, or EDTA concentration. The most often used form is 40 mM Tris pH 7.6, 20 mM sodium acetate, and 2 mM EDTA.

4.2 Sample Application

A. Submarine Mode

Samples are made dense for underlaying into sample wells using 10 to 50% glycerol, 5 to 10% sucrose, or 1 to 2% Ficoll. A convenient stock sample buffer consists of 50% glycerol, 0.05% bromophenol blue, and 0.05% xylene cyanole in 1x TEA buffer.

B. Agarose Bridge Mode

Flood the sample wells with buffer. Remove air bubbles. Load samples as in the submarine mode.

C. Choice of Well Comb and Gel Thickness

For larger sample volumes, the gel thickness may be increased or wider sample combs may be used. The 0.75 mm thick combs are recommended for high resolution requirements.

4.3 Running Samples

A. Submarine Mode

Power requirements vary depending on the gel thickness, the gel length, and the buffer used. Using the Sub-Cell System, it is recommended that gels be run at 36V (1.2 V/cm) overnight. Such runs do not require buffer recirculation. Greater field strengths or lengthy runs require buffer recirculation. Short runs at higher power may be performed, however, buffer recirculation requirements must be independently determined. Typical running conditions for a 15 x 15 cm long, 3 mm thick gel are 30 to 36 constant volts overnight without recirculation or 60V for 6 hours with recirculation. Thicker gels require proportionately greater currents and may affect recirculation requirements.

1. Place the lid on the DNA cell carefully Do not disturb the samples.

2. If buffer recirculation is desired, run the samples into the gel for 30 minutes to 2 hours before starting circulation.

B. Agarose Bridge Mode

Power requirements vary depending on the gel thickness, the gel length and the buffer used. Generally electrophoresis is carried out at room temperature for up to 24 hours at 0.5 to 2 V/cm. Buffer recirculation is recommended.

1. Place the lid on the DNA cell carefully. Do not disturb the sample.

2. Apply power for 15 to 30 minutes to allow samples to enter the gel.

3. Turn off the power and remove the lid.

4. Place a piece of Saran Wrap over the exposed surface and sample wells to prevent evaporation. Molten agarose can be dripped into sample wells as an alternative to Saran Wrap. Make sure the sample wells are free of air bubbles.

5. Replace the lid, and turn on the power to finish the run.

SECTION 5: STAINING AND POST-ELECTROPHORESIS GEL ANALYSIS

Gels can be removed from the DNA cell, gel tray, or agarose bridge for staining. The gel can also be transported on the gel tray and placed into staining solution. Gels are generally stained for about 30 minutes in 0.5 ug/ml solution of Ethidium Bromide.

DANGER

> Ethidium bromide is a suspected carcinogen and should be handled with extreme care. Always wear gloves. Dispose of used solutions and gels appropriately.

Gels can be briefly destained (10 minutes) in H_2O, but Ethidium Bromide will be removed from the DNA with extended destaining. This will cause lower sensitivity of detection.

DNA-Ethidium Bromide complexes may be illuminated with UV light of 254, 300, or 366 nm. Sensitivity decreases with illumination at higher wavelength; however, nicking of DNA may be troublesome below 300 nm. The following chart indicates the percentage of transmittance of UV light through UV transparent plastic.

Wavelength (nm)	% Transmittance
254	0
300	45
360	92

Gels are generally photographed with a yellow, orange, or red cut-off filter. Red gives the cleanest background.

Blotting may be performed by the method of Southern (6) or electrophoretically as described by Bittner (8). Preparative recovery of DNA or RNA may be performed with the aid of low melting temperature agarose (9), by blotting to DEAE paper (10,11).

SYMPTOMS	CAUSE	SOLUTIONS
Skewed bands	1. pH or ionic strength abnormalities.	1. Recirculate buffer. Check pH of anode and cathode for gross variation. Reduce voltage (V/cm) if recirculation not desired.
Slanted lanes (bands)	2. Gel not aged long enough.	2. Age at least 2 hours.
Bands washed out	3. Sample wells dehydrated in agarose bridge mode.	3. Keep wells filled. Use Saran Wrap over wells.
"Double Exposure" appearance of stained bands	4. Agarose legs collapsed.	4. Cast new legs. Avoid pH changes and electrophorese in single polarity.
Curved line or distortion in agarose gel	5. Bubbles in sample wells.	5. Remove bubbles prior to electrophoresis.
Differential relative mobilities	6. Sample spilled out of wells.	6. Samples should be proper density. Apply carefully.
	7. Unit not leveled.	7. Level unit. Place on steady work bench.
Curved bands, smiles	1. Sample overload.	1. Reduce load.
Ragged bands	2. Sample density incorrect.	2. See sample application instructions.
	3. Sample well deformed.	3. Carefully remove comb, especially from soft gels. Be sure gel is aged. Cooling soft gels aids in comb removal.
	4. Excessive power or heating.	4. Reduce voltage.

SYMPTOMS	CAUSE	SOLUTIONS
Band smearing and streaking	1. Agarose has improper endosmosis ($m-_r$).	1. Consult Bio-Rad about agarose.
	2. Salt concentration in sample too high.	2. Reduce salt concentration to $\leq$ 0.1 M.
	3. Excessive power and heating.	3. See running conditions instructions.
	4. Sample spilled out of well.	4. Take care in applying sample. Increase gel thickness for large sample volumes.
	5. Incomplete digest, nuclease contamination, bad enzyme.	5. Heat sample. Check enzyme activity. Digest sample further.
	6. Sample wells cast through the gel. Sample leaks along bottom of running surface.	6. Comb should be placed 1 to 2 mm above the base of the running surface.
	7. Sample overload.	7. Dilute sample.
Bands sharp but too few bands	1. Too high gel percentage.	1. Lower gel percentage.
	2. Incomplete digest.	2. Check enzyme activity, digest further.
High MW bands sharp Low MW bands smeared	1. Gel percentage too low.	1. Increase gel percentage. 2. Switch to polyacrylmaide.
Gels crack	1. Too high voltage gradient especially with low melting temperature agarose or low gel strength gels.	1. Reduce voltage. Run gel at lower temperatures.
	2. pH or ionic strength abnormalities.	2. Check pH of starting buffer and anode and cathode after electrophoresis for gross variations. Recirculate buffer.

SECTION 7: REFERENCES

7.1 Submarine Gels

1. Favaloro, J., Treisman, R. and Kaman, R., Methods in Enzymol., 65, 718-749, Academic Press, New York (1979).

2. Serwer, P., Biochem., 19, 3001 (1980).

3. Herrick, G., Anal. Biochem., 108, 346-347 (1980).

7.2 Agarose Bridge Gels

4. McDonnell, M.W., Simon, M.N. and Studier, F.W., J. Mol. Biol., 110, 119 (1977).

5. Kaplan, D.A., Russo, R. and Wilcox, G., Anal. Biochem., 78, 235-243 (1977).

7.3 General DNA Electrophoresis

Native Gels

6. Southern, E., Methods in Enzymol., 68, 152-176, Academic Press, New York (1979).

Denaturing Gels

7. Maniatis, T., Jeffry, A. and van de Sande, H., Biochem., 14, 3787 (1975).

7.4 DNA Blotting

8. Bittner, M., Kupferer, P. and Morris, C.F., Anal. Biochem., 102, 459 (1980).

7.5 Preparative Recovery

9. Bio-Rad Product Information #2061 - DNA, RNA Separation and Recovery with Low Gel Temperature Agarose.

10. Winberg, G. and Hammarskjold, M. L., Nucleic Acid Res., 8, 253 (1980).

11. Kutateladze, T. V., Axelrod, V. D., Gorbulev, V. G., Belzhelarskaya, S. N. and Vartikyan, R. M., Anal. Biochem., 100, 129 (1979).

12. Polsky, F. I., Edgell, M. H., Seidman, J. G. and Leder, P., Anal. Biochem., 87, 397 (1978).

APPENDIX A

How to prepare a solution of agarose in electrophoresis buffer:

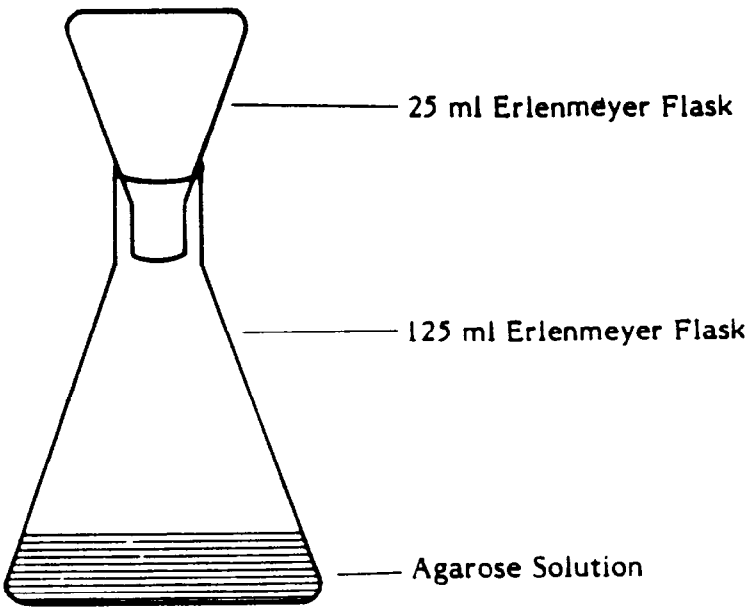
1. Weigh out the appropriate amount of agarose powder to make the agarose solution. Pour the powder into a clean 125 ml Erlenmeyer flask.

2. Pour the appropriate amount of electrophoresis buffer into the 125 ml flask, swirling to suspend the agarose powder in the liquid. Add a stir bar.

3. Place a 25 ml flask as shown on top of the 125 ml flask.

4. Heat to a boil while stirring on a hot plate. Bubbles or foam should break up before rising to the neck of the flask. If you wish to prepare larger volumes, use a 250 ml flask. The small flask acts as a reflux chamber, thus allowing long or vigorous boiling without much evaporation. This system prevents the formation of a film of agarose on the surface of the solution while boiling - a common problem.

5. Boil the solution until all of the small transparent particles are dissolved. With the small flask in place, set aside to cool to 60°C before pouring.



6. A microwave oven works very well as an alternative to a hot plate. Leave out the stir bar while heating. Stop the microwave oven every 30 seconds and swirl the flasks gently to maintain a suspension of the undissolved agarose. This is the fatest way to dissolve agarose.