BIO-RAD

WIDE MINI-SUB™ DNA ELECTROPHORESIS CELL

Instruction Manual

Catalog Number 170-4343

Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547 • Phone (510) 741-1000

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SECTION 1: INTRODUCTION

The Wide Mini-Sub® cell is ideally suited for high capacity rapid screening applications. Rapid separations can be achieved because of the Wide Mini-Sub cell's short interelectrode distance (14 cm). The cell's width, 16 cm, is roughly twice that of the Bio-Rad Mini-Sub® cell, providing double sample capacity. Separations which require 4-12 hours in conventional equipment can be completed in 1-3 hours using the Wide Mini-Sub cell, and it offers a capacity of up to 30 samples.

The Wide Mini-Sub assembly is part of a comprehensive nucleic acids separation system which includes the versatile Sub-Cell® electrophoresis cell, the Mini-Sub cell, the economical Model 200/2.0 (200 V, 2.0 Amps) or Model 100/200 (100 V, 200 Amps) power supply, a gel casting system for submersible polyacrylamide gels, and the Trans-Blot® electrophoretic transfer cell with precut blotting media.

Because the width of the Wide Mini-Sub cell is the same as the Sub-Cell electrophoresis cell, the comb holder and all combs are interchangeable between these two units.

SECTION 2: EQUIPMENT

	Description	Catalog Number
2.1	**Wide Mini-Sub DNA Electrophoresis System**	
	Wide Mini-Sub Cell, Basic Unit, includes cell body; lid with cables; 15 x 10 cm gel tray; 30 Well Comb, 1.5 mm thick; Comb Holder; buffer recirculation connectors.	170-4343
2.2	**Accessories**	
	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
	30 Well Comb, 1.5 mm	170-4344
	2 Well Prep Comb, 3.0 mm (contains 2 outside wells for standards)	170-4345
	Ultraviolet Transparent (UVTP) Gel Tray, 15 x 10 cm	170-4310

*The Comb Holder, 15 x 10 cm gel tray, and all combs listed are interchangeable with the Sub-Cell DNA electrophoresis cell.

	Description	Catalog Number
2.3	**Polyacrylamide Gel Casting Equipment**	
	Polyacrylamide Gel Caster, 18 cm x 10 cm x 1.5 mm, with 1 mm thick x 2.5 mm wide multi-tooth comb.	170-4337
	Polyacrylamide Gel Caster Comb, 2.5 mm wide x 1 mm thick teeth.	170-4339
	Polyacrylamide Gel Caster Comb, 5.5 mm wide x 1 mm thick teeth.	170-4338
	Glass Plates, 7 x 10 cm	170-4101
	Glass Plates, 10 x 15 cm	170-4107
2.4	**Power Supply**	
	Model 200/2.0 Constant Voltage Power Supply, 100/120 V, 50/60 Hz	165-4761
	Model 200/2.0 Constant Voltage Power Supply, 220/240 V, 50/60 Hz	165-4762
	Model 100/200 Miniature Constant Voltage Power Supply, 100 V, 50/60 Hz	165-0556
	Model 100/200 Miniature Constant Voltage Power Supply, 120 V, 60 Hz	165-0557
	Model 100/200 Miniature Constant Voltage Power Supply, 220 V, 50 Hz	165-0558
	Model 100/200 Miniature Constant Voltage Power Supply, 240 V, 50 Hz	165-0559
2.5	**Blotting System**	
	Trans-Blot Electrophoretic Transfer Cell	170-3910
	Cooling Coil, plastic	170-3911
	Super-Cooling Coil	170-3912

Description	Catalog Number
Nitrocellulose Membrane (0.45 micron)	
Rolls, 33 cm x 3 m, pkg 1	162-0115
Sheets, 20 x 20 cm, pkg 5	162-0113
Sheets, 15 x 15 cm, pkg 10	162-0116
Sheets, 15 x 9.2 cm, pkg 10	162-0114
2-Aminophenylthioether (APT) Paper	
Sheets, 20 x 20 cm, pkg 1	162-0122
Sheets, 15 x 15 cm, pkg 2	162-0120
Sheets, 15 x 9.2 cm, pkg 2	162-0119
DEAE Ion Exchanger Paper	
Sheets, 20 x 20 cm, pkg 25	162-0109
Sheets, 15 x 9.2 cm, pkg 25	162-0110
Blot Absorbent Filter Paper	
Rolls, 33 cm x 3 m, pkg 1 (thin paper)	162-0118
Sheets, 18 x 34 cm, pkg 25 (thick paper)	165-0921
Blotting Media Selection Kit	
Sheets, 15 x 9.2 cm (includes Nitrocellulose Membrane, pkg 2, APT-Paper, pkg 1, and ABM-Paper, pkg 1)	162-0123
Zeta-Probe® Blotting Membranes	
10 x 15 cm, 15 sheets	162-0154
15 x 15 cm, 15 sheets	162-0155
15 x 20 cm, 15 sheets	162-0156
20 x 20 cm, 15 sheets	162-0157
20 x 25 cm, 8 sheets	162-0158

2.6 Electrophoresis Purity Reagents

Standard Low -m_r Agarose, 100 g (for routine DNA electrophoresis)	162-0100
Standard Low -m_r Agarose, 500 g	162-0102
Ultra Pure DNA Grade Agarose, 100 g	162-0125
Ultra Pure DNA Grade Agarose, 500 g	162-0126

Description	Catalog Number
Low Gel Temperature Agarose, 25 g (for preparative recovery of DNA)	162-0017
Low Gel Temperature Agarose, 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

SECTION 3: PREPARING AGAROSE SLAB GELS

3.1 Casting Agarose Gels on UVTP (Ultraviolet Transparent Plastic) Gel Tray

1. Seal the ends of the UVTP gel tray securely with 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 UVTP gel tray. Adjust the comb with the aid of the thumbscrews so that it remains 1 mm above the base of the UV tray.
4. Prepare the desired concentration of agarose in electrophoresis buffer (see Appendix A). To pour a 2 mm gel on the 15 x 10 cm UVTP gel tray, prepare 28 ml of agarose solution.
5. Cool the agarose to 60° C before pouring, to prevent warping the tray.
6. Pour the molten agarose into the gel tray.
7. Allow the gel to solidify at room temperature for up to 1 hour.
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 DNA cell. Leveling with the aid of a leveling table is recommended.
11. If recirculation is not required, secure the buffer recirculation ports with the provided plugs.
12. Submerge the gel under the minimum amount of buffer (no more than 4 mm above the surface of the gel).

3.2 Casting Agarose Gels on Glass Plates

1. Level the glass plate on a leveling table or workbench.
2. Prepare the desired concentration of agarose in electrophoresis buffer (see Appendix A). To pour a 2 mm gel on the 15 x 10 cm plate, prepare 28 ml of agarose solution.
3. To improve adhesion between the plate and the agarose, precoat the plate with a thin film of agarose (0.02%), and dry it before casting the gel. Precoat by wetting Kim Wipes™ tissues in molten agarose and rubbing across the plate.

Kim Wipes™ is a trademark of Kimberly-Clark.

4. Heat a pipet on a hotplate and pipet 28 ml of agarose solution into a pre-warmed test tube. Carefully pour the agarose from the test tube onto the center of the glass plate. The agarose will be 2 mm thick. Use the lip of the test tube to pull the hot agarose to the corners of the plate.

5. Immediately position the comb and holder over the glass plate. The comb should have been adjusted with the aid of the thumbscrews so that it remains 0.5 to 1 mm above the glass plate before pouring the gel.

6. Allow the gel to solidify at room temperature for up to one hour.

7. Carefully remove the comb and the holder from the solidified gel. Gels can be covered with Parafilm™ laboratory film and placed in a humid chamber to store for future use.

8. Place the gel on the stage of the DNA cell. Level the cell with the aid of a leveling table.

9. If recirculation is not required, secure the buffer recirculation ports with the plugs provided.

10. Submerge the gel in the minimum amount of buffer (no more than 4 mm above the surface of the gel).

SECTION 4: PREPARING POLYACRYLAMIDE GELS

This technique requires the optional Polyacrylamide Gel Caster (catalog number 170-4337).

1. Place the polyacrylamide gel casting stand on a leveling table.

2. Prepare the desired monomer solution as follows:

NOTE

Prepare 30 ml of solution for each 10 x 15 cm plate.

STOCK SOLUTION	VOLUME	FINAL CONCENTRATION
5x acrylamide/bis	6 ml	1x
5x TBE buffer	6 ml	1x
0.75% ammonium persulfate	3.0 ml	0.075%
Distilled deionized water	15 ml	--
Degas for 10-15 minutes under 28 mm Hg pressure.		
Add TEMED "neat" or 100%	30 µl	0.10%

Parafilm™ is a trademark of American Can Company.

3. Choose the appropriate comb, and screw it between the two casting stand pieces. Lightly grease the surfaces of the casting stand which will come into contact with the comb. Avoid getting grease on the comb teeth or the casting surface of the stand. Tighten screws fingertight.

4. Place the plate on the spacer rails of the casting stand. The plate should be thoroughly cleaned.

5. Pipet the monomer solution between the plate and the casting tray.

CAUTION

Always wear gloves during this procedure, as acrylamide is a neurotoxin.

a) Be sure to clear the bubble from the end of the pipet before expelling the monomer.
b) Hold the pipet at a 45 degree angle.
c) Begin flowing the solution at the side of the plate closest to the comb.
d) Slowly pipet the solution under the plate so as to avoid trapping air bubbles along the comb teeth or other areas of the sandwich. Inspect the poured gel carefully to assure that no bubbles are present, especially around the comb teeth.
e) If a bubble is trapped, remove it by sliding the plate slowly sideways until the bubble is exposed and withdraw it with the pipet. The plate can also be raised upward at a 45 degree angle to remove bubbles. The end of a Pasteur pipet can be used to catch the bubbles. Monomer solution will remain on the surface between the edge of the plate and casting stand due to surface tension.

6. Allow the gel to polymerize for thirty minutes to one hour.

7. To remove the gel from the tray after polymerization, insert the flat end of a spatula slightly under the gel near the middle of the plate. Do not place the spatula between the spacer and glass plate or between the glass plate and gel as this may dislodge the gel from the glass plate. When air appears under the gel, grasp the edge of the plate and remove it from the tray. Briefly rinse the gel with running buffer to wash away any unpolymerized monomer on the surface of the gel.

8. The gel may be used immediately, or it may be stored at 4° C for several days. To store the gel, lay Parafilm laboratory film over it and place it in a humid chamber. If gel is used immediately allow at least 2 hours from pouring to complete polymerization. After removing the gel from the stand, put the gel in running buffer until electrophoresis is begun. Pre-electro-phoresis for 1 hour at 100 V may improve resolution of low molecular weight DNA.

9. Place the plate onto the DNA cell. Leveling with the aid of a leveling table is recommended.

10. If recirculation is not required, secure the buffer recirculation ports with the provided plugs.

11. Submerge the gel under just enough buffer to barely cover sample wells.

SECTION 5: ELECTROPHORESIS

5.1 Buffer Selection

A. Agarose Gels

Tris-EDTA-Acetate (TEA) buffer is most often used for native gels. (See Reference 2 for a general discussion of DNA electrophoresis). TEA buffer can be modified with respect to pH, acetate concentration, or EDTA concentration. The most frequently used form is 40 mM Tris, pH 7.6, 20 mM sodium acetate, and 2 mM EDTA.

B. Polyacrylamide Gels

The buffer of choice for native polyacrylamide gels is 89 mM Tris, 89 mM sodium borate, pH 8.3 (TBE). Reference 2 discusses general DNA electrophoresis.

5.2 Sample Application

A. Sample Preparation

Samples are made dense for underlayering into sample wells, using 50% glycerol or 2% Ficoll® detergent. A convenient stock sample buffer consists of 50% glycerol, 0.05% bromophenol blue, and 0.05% xylene cyanole in 2 X TEA or TBE buffer.

B. Varying Sample Volumes

For large sample volumes run in agarose gels, the gel thickness can be varied. Wider sample combs can be used for agarose gels, or for polyacrylamide gels cast in the polyacrylamide gel casting stand. Calculate the volume of the sample well before applying the sample.

5.3 Running the Gel

Agarose

Electrophoretic power requirements vary depending on the gel thickness and the buffer used. In the Wide Mini-Sub cell, 1.5 to 3 mm gels should be run at 60 V (4 V/cm) for 1 to 2 hours. Such runs do not require buffer recirculation. If the field strength for a 1 to 2 hour run is greater than 60 V, buffer recirculation is required to prevent band or lane distortions caused by pH and ionic effects (Refer to Section 7, Troubleshooting). Shorter runs at higher power may be performed, but buffer recirculation requirements must be determined independently. For example, DNA digests run for 15 minutes at 180 V have been efficiently resolved with minimal lane distortion and without buffer recirculation.

Ficoll® is a registered trademark of Pharmacia Fine Chemicals.

1. Carefully place the lid on the cell. Do not disturb the samples.

2. If buffer recirculation is required, run the samples into the gel for approximately 10 minutes. The exact amount of time is best indicated by the xylene cyanole dye entering the gel. Then begin buffer recirculation. If use of a recirculating pump is not desired, hand recirculation may be performed after the sample has entered the gel. Turn off the power and rock the cell back and forth 5 to 10 times every 15 to 20 minutes. This will effectively mix the buffer. Be careful not to dislodge the gel from its support, since this may cause artifacts.

Polyacrylamide

Polyacrylamide gels in the Wide Mini-Sub cell should be run at no more than 60 V (4 V/cm) to avoid band slanting or electrodecantation. That is, the top of the band migrates slightly in front of the band bottom due to the slightly greater resistance of the gel than the running buffer. Greater voltages require minimal buffer overlay and potentially require buffer recirculation as discussed for agarose gels.

Short pre-electrophoresis of the gel or equilibration in running buffer prior to electrophoresis in order to remove gel polymerization reactants is recommended in order to improve resolution of low molecular weight DNA.

Gels can be pre-electrophoresed for up to 1 hour at 100 V. Typically a HaeIII digest of Ø x 174 DNA will be well resolved in 2 hours at 60 V.

SECTION 6: STAINING AND POST-ELECTROPHORESIS GEL ANALYSIS

6.1 Ethidium Bromide Staining

Polyacrylamide gels or agarose gels can be stained rapidly with ethidium bromide. Stain the gels for approximately 10 minutes in a 0.5 µg/ml solution of ethidium bromide. If necessary, destain the gel briefly in distilled water. Polyacrylamide gels may be easily removed from glass plates by wedging a razor or spatula edge under the gel and plate and directing a stream of water between the gel and the plate until the gel comes loose.

CAUTION

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

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, like that of the UVTP Gel Tray.

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.

6.2 Silver Staining

Polyacrylamide gels may be silver stained for highly sensitive detection of single or double stranded DNA or RNA. Refer to Bio-Rad Silver Stain Instructions, Bulletin 1089, (Reference 3) for protocols. Silver staining of most agarose gels is impractical at present due to excessive background staining and problems inherent in staining gels thicker than 1.5 mm. Gels can be removed from glass plates as discussed under Ethidium Bromide Staining.

6.3 Blotting

Analytical blotting may be performed by the method of Southern (2) or electrophoretically as described by Bittner (4) and others (5). Preparative blotting to DEAE paper can also be performed (6,7).

6.4 Preparative Recovery

Besides recovery of DNA and RNA from DEAE paper after blotting (6-8), recovery can be performed with the aid of low melting temperature agarose (9).

SYMPTOMS	CAUSE	SOLUTIONS
Skewed bands Slanted lanes (bands)	1. pH or ionic strength abnormalities are the main cause of distortions.	1. Recirculate buffer. Check pH at anode and cathode for gross variation. Reduce voltage (V/cm) if recirculation not desired.
Bands washed out	2. Gel not aged long enough.	2. Age at least 2 hours.
"Double exposure" appearance of stained bands	3. Sample wells dehydrated in agarose bridge mode.	3. Keep wells filled. Use Saran Wrap™ plastic wrap over wells.
Curved line or distortion in agarose gel	4. Agarose legs collapsed.	4. Cast new legs. Avoid pH changes and electrophorese in single polarity.
Differential relative mobilities	5. Bubbles in sample wells.	5. Remove bubbles prior to electrophoresis.
	6. Sample spilled out of wells.	6. Samples should be proper density. Apply carefully.
	7. Unit not leveled.	7. Level unit. Place on steady workbench.
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.
In polyacrylamide gels, bands look "doubled" or slanted, often forward near top of gel	1. Excessive power and conductance above gel.	1. Reduce volume of buffer over gel and reduce power.
	2. Excessive polymerization reactants in gels.	2. Pre-electrophorese gel for 30 minutes at 100 V.

Saran Wrap is a trademark of the Dow Chemical Company.

SYMPTOMS	CAUSE	SOLUTIONS
Band smearing and streaking	1. Agarose has improper endosmosis ($-m_r$).	1. Use Ultra Pure DNA Grade Agarose.
	2. Salt concentration in sample too high.	2. Reduce salt concentration to ≤ 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 the 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 or reduce sample load.
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. 2. Ionic contaminants in gel.	1. Increase gel percentage. 2. Switch to polyacrylamide 3. Pre-electrophorese gel for 30 minutes to 1 hour.

SYMPTOMS	CAUSE	SOLUTIONS
Gels crack	1. Too high voltage gradient especially with low melting temperature agarose or low 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 8: REFERENCES

1. Kopchick, J.J., Cullen, B.R. and Stacey, D.W., Anal. Biochem., 115, 419 (1981).

2. Southern, E., "Methods in Enzymol.," Academic Press, N.Y., 68, 152 (1979).

3. The Bio-Rad Silver Stain - Bulletin 1089.

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

5. Bio-Rad Trans-Blot Cell Operation Instructions - Bulletin 1082.

6. Winberg, G. and Hammarskjold, M.L., Nucleic Acids Res., 8, 253 (1980).

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

8. Dretzen, G., Bellard, M., Sassone-Corsi, P. and Chambon, P., Anal. Biochem., 112, 295 (1981).

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

APPENDIX A

How to prepare a solution of agarose in electrophoresis buffer:

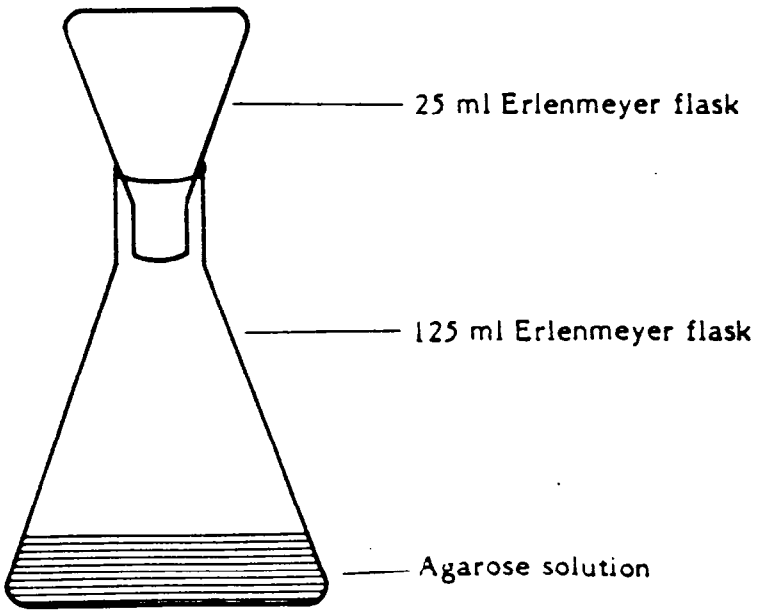
1. Weigh out the appropriate amount of agarose powder to make the agarose solution. Pour the powder into a clean 125 ml Erlenmeyer flask.

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 boiling 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 fastest way to dissolve agarose.

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