

Model 475 Gradient Delivery System

Instruction Manual

**Catalog Number
170-9042**

BIO-RAD

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Section 1 Introduction

The Model 475 Gradient Delivery System is a cam-operated manual gradient former that creates reproducible linear polyacrylamide gradient gels. The Model 475 gradient system delivers and mixes the high and low density solutions without using a peristaltic pump or magnetic stirrer. Since gradient formation is independent of the speed of the cam rotation, the gradient concentrations formed are very accurate and reproducible. All delivery volumes are accessible by using a sliding lever to scale the ratios of the cam profiles.

The Model 475 Gradient Delivery System has a capacity range of 7–50 ml, making it ideal for the construction of one small 7.5 x 10 cm gel or one large 16 x 16 cm gel. To obtain optimal gradients, tubing with an inside diameter $\leq 1/16$ " must be used. The Model 475 gradient system can be used with the DCode™ system (catalog numbers 170-9080–170-9085 and 170-9102–170-9104) to create either a perpendicular or parallel denaturing gradient gel. The perpendicular denaturing gradient gel is formed by a bottom-filling method. The parallel denaturing gradient gel is formed by a top-filling method.

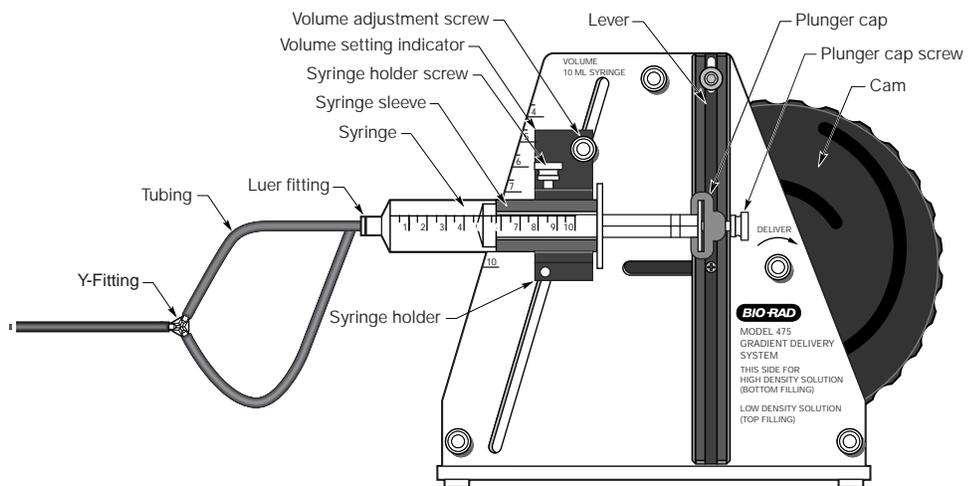


Fig. 1. Model 475 Gradient Delivery System.

1.1 Specifications

Overall size	22 cm x 21 cm
Weight	5 lb
Capacity	50 ml total (25 ml/syringe)

1.2 Description of Components

1. Syringe

There are two pairs each of 10 and 30 ml disposable syringes. The 10 ml syringes are used for gel volumes less than 10 ml. The 30 ml syringes are used for gel volumes greater than 10 ml. For proper casting of gels, use matching syringe sizes.

2. Plunger Caps/Plunger Cap Screw

There are two plunger caps; one for each syringe. The plunger caps fit both the 10 and 30 ml syringes (Figure 2).

3. Syringe Sleeves

One pair of syringe sleeves for each size syringe is provided (Figure 3). The syringe sleeve is a movable adaptor used to mount the syringe in the syringe holder. The sleeve should conform to the syringe. If the syringe is too tight or too loose, adjust the sleeve by pushing or pulling.

4. Lever Attachment Screw

The lever attachment screw is on the plunger cap. This screw fits into the groove of the lever and conducts the driving force of the cam in dispensing the gel solution.

5. Lever

The position of the lever is controlled by the rotation of the cam (Figure 1). The lever must be in the vertical or start position before use.

6. Syringe Holder/Syringe Holder Screw

The syringe holder is adjacent to the lever. It holds the syringe in place and is also used to control the delivery volume. The syringe is held in the syringe holder by tightening the syringe holder screw against the syringe sleeve.

7. Volume Adjustment Screw

The volume adjustment screw is on both sides of the syringe holder (Figure 1). It adjusts the holder to the desired delivery volume.

8. Volume Setting Indicator

The volume setting indicator is at the top corner of the syringe holder nearest the volume setting numbers (Figure 1).

9. Tygon® Tubing

One length of Tygon tubing is provided. The Tygon tubing should be cut into two 15.5 cm lengths and one 9 cm length. The longer pieces are used to transport the gel solution from the syringes into the Y-fitting. The short piece will transport the gel solution from the Y-fitting to the gel sandwich.

10. Y-fitting

The Y-fitting mixes the high and low density gel solutions (Figure 4).

11. Luer fitting/Coupling

There are two luer fittings that fit both the 10 and 30 ml syringes. The fittings twist onto the syringe and connect to the Tygon tubing on the other end. A luer coupling is used for one end of the 9 cm tubing to connect it to the gel sandwich stopcock.

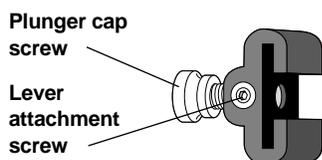


Fig. 2. Plunger cap.

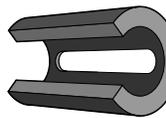


Fig. 3. Syringe sleeve.

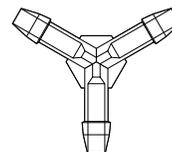


Fig. 4. Y-Fitting.

Section 2 Casting a Linear Gradient Gel

The Model 475 Gradient Delivery System uses a pair of disposable syringe of two sizes, 10 or 30 ml. One-half the total volume needed to cast the gel is placed into each syringe. See Section 4.1 for the correct gel volumes needed for the different gel sizes.

Note: We advise you to familiarize yourself thoroughly with the sequence of operations before casting. **Use caution: wear gloves and eye protection at all times.**

2.1 Casting Perpendicular Gradient Gels

1. Refer to the DCode system instruction manual for assembling the gel sandwich for a perpendicular gradient gel.
2. One length of Tygon tubing is provided. Cut the tubing into two 15.5 cm lengths and one 9 cm length. The longer pieces of Tygon tubing will be used to conduct the gel solution from the syringes into the Y-fitting. The short piece of Tygon tubing will conduct the gel solution from the Y-fitting to the gel sandwich. Connect one end of the 9 cm Tygon tubing to the Y-fitting and connect a luer coupling to the other end of the 9 cm tubing. Connect luer fittings to one end of each of the two long pieces of tubing. Connect the luer fittings to 10 ml or 30 ml syringes. Do not connect the long Tygon tubing to the Y-fitting at this time.
3. Label one of the syringes LO (for the low density solution) and one HI (for the high density solution). Attach a plunger cap to each syringe plunger "head." Position the plunger "head" in the middle of the plunger cap and tighten enough to hold the plunger cap in place. Slide each syringe into a syringe sleeve. Move the sleeve to the middle of the syringe, keeping the volume gradations visible. Make sure that the lever attachment screw is in the same plane as the flat or back side of the sleeve. This is very important for proper attachment of the syringe to the lever.

Note: Insure that the tubing is free of any gel material by pushing water through the tubing with the syringe. The tubing should be free of material before casting. Remove any remaining water from the tubing.

4. Rotate the cam wheel counterclockwise to the vertical or start position. To set the desired delivery volume, loosen the volume adjustment screw. Set the volume setting indicator on the syringe holder to the desired volume setting. Tighten the volume adjustment screw. For 7.5 x 10 cm gels (1 mm thick), set the volume setting indicator to 4.5. For 16 x 16 cm gels (1 mm thick), set the volume setting indicator to 14.5. Refer to Section 4.1.
5. From the stock solutions, pipet the desired amounts of the high and low density gel solutions into two disposable test tubes. (See Section 4.1.)

Optional: To visually check the formation of the gradient, add 100 µl of DCode dye solution per 5 ml high density solution.

Note: The gel solution volume should be greater than the amount set on the volume adjustment lever. For example, if the setting indicator is set at 4.5, the syringe should contain 5 ml of the gel solution. This extra solution is needed to push the correct amount into the gel sandwich.

The steps below are time-sensitive (about 7–10 minutes). Insure that steps 1 through 5 are done before proceeding further. Be thoroughly familiar with the following steps before casting the gel.

6. Add a final concentration of 0.09% (v/v) each of ammonium persulfate and TEMED solutions. The 0.09% (v/v) concentrations allow about 5–7 minutes to finish casting the gel before polymerization. Cap and mix by inverting several times. With the syringe connected to the tubing, withdraw all of the high density solution into the HI syringe. Do the same for the low density solution into the LO syringe.

Note: Acrylamide is a very hazardous substance. Use caution: wear gloves and eye protection at all times. Avoid skin contact.

7. Carefully remove air bubbles from the LO syringe by turning it upside down (plunger cap towards the bench) and gently tapping. Push the gel solution to the end of the tubing. Do not push it out of the tubing, as loss of gel solution will disturb the volume required to cast the desired gel.
8. Place the LO syringe into the gradient delivery system syringe holder (LO density side) by holding the syringe by the plunger and inserting the lever attachment screw into the lever groove. Do not handle the syringe. It will dispense the gel solution out of the syringe. Casting a perpendicular gel is referred to as a **bottom filling method**, so place the LO syringe on the correct side of the gradient system.
9. Carefully remove the air bubbles from the HI syringe by turning it upside down (plunger cap toward the bench) and gently tapping. Push the gel solution to the end of the tubing. Do not push it out of the tubing, as loss of gel solution will disturb the volume required to cast the desired gel.
10. Place the HI syringe into the gradient delivery system syringe holder (HI density side) by holding the syringe by the plunger and inserting the lever attachment screw into the lever groove. Do not handle the syringe. It will dispense the gel solution out of the syringe.
11. Slide the tubing from the low density syringe to one end of the Y-fitting. Do the same for the high density syringe.
12. Connect the 9 cm tubing with the luer coupling on the sandwich assembly stopcock. Insure that the stopcock is open and that the vent port is unplugged for the half of the sandwich being cast.

Note: For a 16 x 16 cm single gel, both stopcocks are open during casting. After casting, both stopcocks are closed.

13. Rotate the cam wheel slowly and steadily to deliver the gel solution. It is important to cast the gel solution at a steady pace to avoid disturbances between gel solutions within the sandwich.

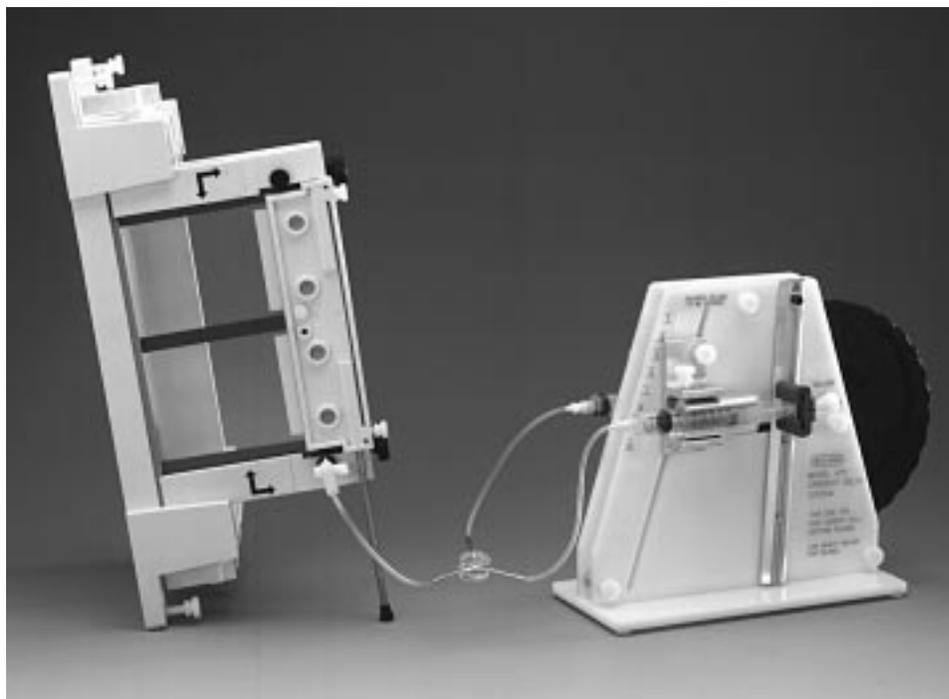


Fig. 5. Casting a perpendicular gradient gel using the Model 475 Gradient Delivery System.

14. Plug the vent port and close the stopcock on the gel sandwich when the cam wheel has reached the stop position. Carefully level the gel sandwich by adjusting the gasket tilt rod. Be sure to loosen the tilt rod screw and not the sandwich clamp screw.

Note: For a properly cast perpendicular gradient gel it is extremely important to level the sandwich assembly after casting.

15. Immediately remove the tubing from the sandwich assembly stopcock. Place the tubing into a beaker of water and reverse the cam on the gradient delivery system. This will rinse the tubing and Y-fitting. Remove both syringes from the syringe holder on the gradient delivery system. Detach the syringe tubing from the Y-fitting. Run or push water through the syringe, tubing, and Y-fitting several times to get rid of any residual gel solution. It is extremely important that this is done quickly after casting to avoid any gel polymerization.
16. Let the gel polymerize for about 60 minutes. To cast the other half of the 7.5 x 10 cm gel, remove the gasket tilt rod and place it on the other side of the comb gasket. Repeat steps 4 through 15.

Note: If casting a single 7.5 x 10 cm gel, let the gel solution polymerize for 60 minutes. Carefully remove the comb gasket; leave the comb in place and pipette (on an opening near the spacer) a 10 ml gel solution plus initiators in the uncast half of the sandwich to create a dam.

17. After polymerization, remove the comb by pulling it straight up slowly and gently.

2.2 Casting Parallel Gradient Gels

1. Assemble the gel sandwich for a parallel gradient gel as in the DCode system instruction manual. Position the gel assembly upright (see Figure 6).
2. One length of Tygon tubing is provided. Cut the tubing into two 15.5 cm lengths and one 9 cm length. The longer pieces of Tygon tubing will be used to conduct the gel solution from the syringes into the Y-fitting. The short piece of Tygon tubing will conduct the gel solution from the Y-fitting to the gel sandwich. Connect one end of the 9 cm Tygon tubing to the Y-fitting and connect a luer coupling to the other end of the 9 cm tubing. Connect luer fittings to one end of each of the two long pieces of tubing. Connect the luer fittings to 30 ml syringes. Do not connect the long Tygon tubing to the Y-fitting at this time.
3. Label one of the syringes LO (for the low density solution) and one HI (for the high density solution). Attach a plunger cap onto each syringe plunger "head." Position the plunger "head" in the middle of the plunger cap and tighten enough to hold the plunger in place. Position the cap in the middle for proper alignment with the lever on the gradient delivery system. Slide each syringe into a syringe sleeve. Move the sleeve to the middle of the syringe, keeping the volume gradations visible. Make sure that the lever attachment screw is in the same plane as the flat or back side of the sleeve. This is very important for proper attachment of the syringe to the lever.

Note: Insure that the tubing is free of any gel material by pushing water through the tubing with the syringe. The tubing should be free of material before casting, remove any remaining water from the tubing.

4. Rotate the cam wheel counterclockwise to the vertical or start position. To set the desired delivery volume, loosen the volume adjustment screw (refer to Section 4.1 for volume settings). Place the volume setting indicator located on the syringe holder to the desired volume setting. Tighten the volume adjustment screw.
5. From the stock solutions, pipet the desired amounts of the high and low density gel solutions into two disposable test tubes (refer to the Section 4.1).

Optional: To visually check the formation of the gradient, add 100 μ l of DCode dye solution per 5 ml high density solution.

The steps below are time-sensitive (about 7–10 minutes). Insure that steps 1 through 5 are done before proceeding further. Be thoroughly familiar with the following steps before casting the gel.

6. Add the final concentration of 0.09% (v/v) each of ammonium persulfate and TEMED solutions. The 0.09% (v/v) concentrations allow about 5–7 minutes to finish casting the gel before polymerization. Cap and mix by inverting several times. With the syringe connected to the tubing, withdraw all of the high density solution into the HI syringe. Do the same for the low density solution into the LO syringe.

Note: Acrylamide is a very hazardous substance. Use caution: wear gloves and eye protection at all times. Avoid skin contact.

7. Carefully remove air bubbles from the LO syringe by turning the syringe upside down (plunger cap towards the bench) and gently tapping the syringe. Push the gel solution to the end of the tubing. Do not push it out of the tubing, as loss of solution will disturb the volume required to cast the desired gel.

Note: The gel solution volume should be greater than the amount set on the volume adjustment lever. For example, if the indicator setting is set at 14.5, the syringe should contain 15 ml of solution. This extra solution is needed to deliver the correct amount for casting.

8. Place the LO syringe into the gradient delivery system syringe holder (LO density side) by holding the syringe by the plunger and inserting the lever attachment screw into the lever groove. Do not handle the syringe. It will dispense the gel solution out of the syringe. Casting a parallel gel is referred to as a **top filling method**, so place the LO syringe on the correct side of the gradient system.
9. Carefully remove the air bubbles from the HI syringe by turning the syringe upside down (plunger cap towards the bench) and gently tapping the syringe. Push the solution to the end of the tubing. Do not push it out of the tubing, as loss of solution will disturb the volume required to cast the desired gel.
10. Place the HI syringe into the gradient delivery system syringe holder (HI density side) by holding the syringe by the plunger and inserting the lever attachment screw into the lever. Do not handle the syringe. It will dispense the gel solution out of the syringe.
11. Slide the tubing from the low density syringe over one end on the Y-fitting. Do the same for the high density syringe.
12. Attach a 19 gauge needle to the coupling. Hold the beveled side of the needle at the top-center of the gel sandwich and cast (Figure 6). For convenience, the needle can be taped in place.

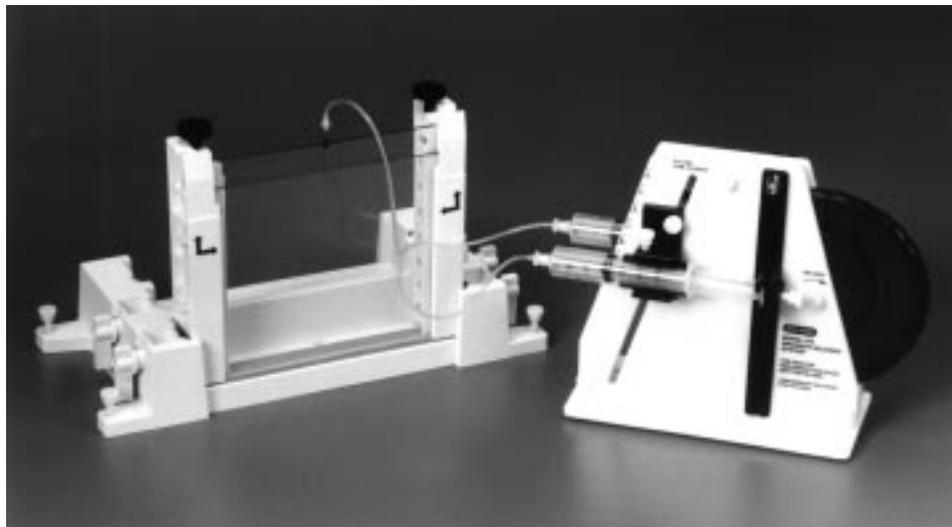


Fig. 6. Casting a parallel gradient gel.

13. Rotate the cam wheel slowly and steadily to deliver the gel solution. It is important to cast the gel solution at a steady pace to avoid any disturbances between the gel solutions within the gel sandwich.
14. Carefully insert the comb to the desired well depth and straighten. Let the gel polymerize for about 60 minutes.
15. Place the tubing and needle into a beaker of water and reverse the cam on the gradient delivery system. This will rinse the tubing and Y-fitting. Remove both syringes from the syringe holder on the gradient delivery system. Detach the syringe tubing from the Y-fitting. Run or push water out through the syringe, tubing, and Y-fitting several times to get rid of any residual gel solution. It is very important that this is done quickly after casting to avoid premature gel polymerization.
16. After polymerization, remove the comb by pulling it straight up slowly and gently.

Section 3 Gradient Former Care and Maintenance

After use, remove the tubing from the gel sandwich stopcock. Remove both syringes from the syringe holder on the gradient delivery system. Detach the syringe tubing from the Y-fitting. Run or push water out through the tubing and Y-fitting. It is extremely important that this is done quickly after casting to avoid any gel polymerization. If gel polymerizes in the tubing, luer fittings, stopcocks, or Y-fitting, use a disposable inoculating needle to gently poke the polymerized gel. Rinse out the polymerized gel with water using a syringe. When cleaning the Y-fitting, use extreme care so as not to distort the Y-fitting channels. Do not use organic solvents, strong acid solutions, or ethanol in any of the gradient delivery system components.

Section 4 Gel Preparation

4.1 Gel Volumes

Linear Denaturing Gradient Gels

For casting linear gradient perpendicular and parallel gels, use half the volume of low density denaturing solution and half the volume of high density denaturing solution. For example, if the total gel volume is 22 ml, use 11 ml low density solution and 11 ml high density denaturing solution. The table below shows the required gradient former settings per gel sandwich size desired.

Spacer Size	Gel Size	Volume	Volume per Syringe	Volume Adjustment Setting
0.75 mm	7.5 x 10 cm	10 ml	5.0 ml	3.5
	16 x 10 cm	16 ml	8.0 ml	6.5
	16 x 16 cm	22 ml	11.0 ml	9.5
1.00 mm	7.5 x 10 cm	12 ml	6.0 ml	4.5
	16 x 10 cm	22 ml	11.0 ml	9.5
	16 x 16 cm	32 ml	16.0 ml	14.5
1.50 mm	7.5 x 10 cm	16 ml	8.0 ml	6.5
	16 x 10 cm	30 ml	15.0 ml	13.5
	16 x 16 cm	48 ml	24.0 ml	22.5

4.2 Stock Solution Preparation

40% Acrylamide/Bis (37.5:1)

Acrylamide 38.93 g
Bis-acrylamide 1.07 g

Add dH₂O to 100 ml. Filter through a Whatman No. 1 and store at 4 °C.

50x TAE Buffer (1 L)

Tris Base 242.0 g
Acetic acid, glacial 57.1 ml
0.5 M EDTA, pH 8.0 100.0 ml
dH₂O 600.0 ml

Mix and add dH₂O to 1 liter. Autoclave for 20–30 minutes. Store at room temperature.

Denaturing Solution (100 ml)

	7.5% Gel	10% Gel	12.0% Gel
40% Acrylamide/Bis (37.5:1)	18.8 ml	25.0 ml	30.0 ml
50x TAE buffer	2.0 ml	2.0 ml	2.0 ml
dH ₂ O	79.2 ml	73.0 ml	68.0 ml

Degas for about 10–15 minutes. Store at 4 °C in a brown bottle for about 1 month.

100% Denaturing Solution (100 ml)

	7.5% Gel	10% Gel	12.0% Gel
40% Acrylamide/Bis (37.5:1)	18.8 ml	25.0 ml	30.0 ml
50x TAE buffer	2.0 ml	2.0 ml	2.0 ml
Formamide (deionized)	40.0 ml	40.0 ml	40.0 ml
Urea	42.0 g	42.0 g	42.0 g
dH ₂ O	to 100 ml	to 100 ml	to 100 ml

Degas for about 10–15 minutes. Store at 4 °C in a brown bottle for about 1 month.

For other denaturing solutions, use the volumes in the 100% Denaturing Solution with the exception of the formamide and urea. For these reagents use the volumes indicated below.

Denaturing Solution per 100 ml solution

	10%	20%	30%	40%	50%	60%	70%	80%	90%
Formamide (ml)	4	8	12	16	20	24	28	32	36
Urea (g)	4.2	8.4	12.6	16.8	21	25.2	29.4	33.6	37.8

10% Ammonium Persulfate (1 ml)

Ammonium persulfate 0.1 g
dH₂O 1.0 ml

Mix and store at -20 °C for about a week.

DCode Dye Solution (10 ml)

Bromophenol blue 0.05 g
Xylene cyanol 0.05 g
1x TAE buffer 10.0 ml

Store at room temperature.

Section 5 Troubleshooting

Problem	Cause	Solution
Cam		
Not rotating	Tubing components have polymerized gel	Insure that all tubing components are free of any gel material
Bad gradient	Syringes are installed in the wrong orientation	Insure syringes are oriented in the right way, <i>i.e.</i> bottom or top filling
Syringe		
Not moving	Lever attachment screw not within the lever groove	Insure that lever attachment screw is within the lever groove
	Polymerized gel material in tubing and components	Insure that tubings and components are free of any gel material before casting
No/poor gradient	HI and LO syringes are placed in the wrong position	Check gradient delivery system for proper positioning of HI and LO syringes depending on type of gradient to be cast
	Used wrong size syringes	Use the right size syringes for casting
	Lever not in start/vertical position	Insure lever is in start/vertical position before casting
	Acrylamide denaturing stock solution is old	Use new acrylamide stock solution
Gel cast is not the full amount	Displaced gel solution during set-up	Insure that the total volume of gel solution is in the syringes before use
Y-fitting		
Gel solution is not entering gel sandwich	Polymerized gel solution in Y-fitting	Insure that Y-fitting is free of any polymerized gel material
Gel		
Not polymerized	Did not add the proper volumes of 10% ammonium persulfate and TEMED	Use the proper concentration of 10% ammonium persulfate and TEMED
Gel polymerized before casting	Used too high a concentration of 10% ammonium persulfate and TEMED	Use the proper concentration of 10% ammonium persulfate and TEMED
	Syringe components not prepared before use	Insure that all gradient delivery system components are ready prior to casting

Problem	Cause	Solution
Glass plate cracked	Excessive force at comb gasket	Apply only one turn to thumb gasket screw after it touches glass
Gel solution leaks during casting	Not sufficient pressure on comb gasket	Make sure pressure clamp screws are turned two turns
	Improper assembly of gel sandwich	Using the alignment card, check that the spacers and glass plate bottom are flush prior to pouring gel
	Poor contact between comb gasket and spacers	Reassemble glass plates as in Section 4.1. Visually confirm contact at spacers and comb gasket
	Casting stand gasket positioned incorrectly	Position gray gasket so that it covers the entire bottom section of the glass plate
	Wrong comb gasket	Make sure correct comb gasket is used
	Misaligned comb gasket	Insure that comb gasket notches are against spacer notches
	Misaligned plates	Pressure clamp may force plates to shift if sandwich clamps are not tight. Insure that sandwich clamps are tightened
	Misalignment of spacers and glass plates	Check alignment at bottom of glass plates, using alignment slot on casting stand
	Damaged or dirty spacers and/or combs	Replace spacers or combs
	Different thickness of spacers and comb	Use spacers and comb of same thickness
	Dirty inlet fitting or missing O-ring	Replace fitting
	Loose stopcock	Tighten stopcock/inlet port screw connection
No air vent plug	Plug vent after casting gel	
Chipped glass plates	Insure glass plates are free of flaws. Use new set of glass plates	
Damaged or non-Bio-Rad glass plates	Replace with Bio-Rad glass plates only	

Section 6 Equipment and Accessories

6.1 Model 475 Gradient Former

Catalog Number	Product Description
170-9042	Model 475 Gradient Former , includes one set each of 10 and 30 ml syringes, Y-fitting, tubing, luer fittings and coupling
170-9196	Y-Fittings for Gradient Delivery System , 5
170-9228	DCode Gradient Delivery System Tubing and Fitting Kit , includes 3' tygon tubing, 5 Y-fittings, 4 vent port plugs, 4 female luer fittings, 4 male luer fittings, 2 stopcocks, two 15 ml syringes, two 30 ml syringes

6.2 Electrophoresis Reagents

170-9038	DCode Control Reagent Kit , includes mutant and wild-type DNA, two oligonucleotide primers, loading buffer, and instructions
170-9032	DCode Electrophoresis Reagent Kit , includes 40% acrylamide/bis (37.5:1), 50x TAE buffer, urea, formamide (deionized), ammonium persulfate, TEMED, ethidium bromide, and DCode dye solution



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