Sub-Cell™ GT, Wide Mini-Sub™ Cell GT, and Mini-Sub Cell GT Agarose Gel Electrophoresis Systems

Instruction Manual

Catalog # 1704401 to 1704406
1704481 to 1704486

BIO-RAD

Bio-Rad Technical Support

Phone: 1-800-424-6723, option 2

Email: **Support@bio-rad.com** (U.S./Canada only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at **bio-rad.com**.

Notice

Safety and Regulatory Compliance

Important Safety Information

Please read these instructions before operating the Sub-Cell GT, Wide Mini-Sub Cell GT, and Mini-Sub Cell GT Agarose Gel Electrophoresis Systems. These instruments are intended for laboratory research use only. These devices are meant for use by specialized personnel who know the health risks associated with reagents used in electrophoresis.

Warranty

The Sub-Cell GT, Wide Mini-Sub Cell GT, and Mini-Sub Cell GT Agarose Gel Electrophoresis Systems are warranted against defects in materials and workmanship for one year. If any defect occurs in the instrument during this warranty period, Bio-Rad Laboratories, Inc. will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

- Defects caused by improper operation
- Repair or modification done by anyone other than Bio-Rad Laboratories, Inc. or the company's authorized agent
- Use of spare parts supplied by anyone other than Bio-Rad Laboratories, Inc.
- Damage caused by accident or misuse
- Damage caused by disaster
- Corrosion caused by improper solvents or samples

This warranty does not apply to:

- Platinum electrode wires

To ensure the best performance from the Sub-Cell GT, Wide Mini-Sub Cell GT, or Mini-Sub Cell GT Agarose Gel Electrophoresis System, Bio-Rad recommends that you become fully acquainted with these operating instructions by reading this manual carefully.

Bio-Rad also recommends that all Sub-Cell GT, Wide Mini-Sub Cell GT, and Mini-Sub Cell GT Agarose Gel Electrophoresis System components and accessories be inspected for damage, cleaned as recommended in this manual, and rinsed thoroughly with distilled water before use.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

General Precautions

- Read the user guide carefully
- Use the instrument only for the intended purpose in research laboratories
- Connect the instrument to a grounded power source and to a circuit breaker

Regulatory Notices

The Sub-Cell GT, Wide Mini-Sub Cell GT, and Mini-Sub Cell GT Agarose Gel Electrophoresis Systems are designed and certified to meet UL/CSA 61010-1, the internationally accepted electrical safety standard. Certified products are safe to use when operated in accordance with this user guide. Do not modify or alter these instruments in any way. Modification or alteration of these instruments will:

- Void the manufacturer's warranty
- Void the regulatory certifications
- Create a potential safety hazard

Bio-Rad Laboratories, Inc. is not responsible for any injury or damage caused by use of these instruments for purposes other than those for which they are intended or by modifications of the instruments not performed by Bio-Rad Laboratories, Inc. or an authorized agent.

Safety Alerts

Alert icons appear in cautionary and warning paragraphs in this guide to call attention to safety hazards.

Types of Safety Hazards

Most alert icons depict the relevant type of safety hazard.

Alert Icon	Explanation
	**General** — indicates a potential hazard requiring special attention. This icon appears when the hazard or condition is of a general nature
	**Electrical hazard** — indicates a potential hazard requiring special attention when you are working with electricity or electrical equipment
	**Extreme heat and flammable materials** — indicates a potential hazard requiring special attention when you are working with extreme heat and flammable materials

Levels of Potential Risk

Each alert icon appears in a paragraph in this guide that indicates the level of potential risk posed by the hazard or action described.

Cautions

A caution (example shown below) alerts you to take or avoid a specific action that could result in loss of data or damage to the instrument. A caution can also indicate that minor or moderate injury might occur if the precaution against a potential hazard is not taken.

Caution: Refer all servicing to qualified Bio-Rad personnel or their agents.

Instrument Safety Warnings

Before you operate the instrument, carefully read the explanation for each safety icon.

Safety Icon	Explanation
	**Caution:** Refer all servicing to qualified Bio-Rad personnel or their agents. If you experience technical difficulties with the instrument, contact Bio-Rad to schedule service. The instrument should not be modified or altered in any way. Alteration voids the manufacturer's warranty and might create a potential safety hazard for the user.
	**WARNING!** This instrument must be connected to an appropriate AC voltage outlet that is properly grounded.

Safety and Regulatory Compliance

Safety Compliance

The Sub-Cell GT, Wide Mini-Sub Cell GT, and Mini Sub-Cell GT Agarose Gel Electrophoresis Systems have been tested and found to be in compliance with all applicable requirements of the following safety standards:

- IEC 61010-1:2010 + A1:2016 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General requirements
- CAN/CSA-C22.2 No. 61010-1-12 + GI + GI2 (R2017) + A1 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General Requirements
- EN 61010-1:2010 + A1:2019 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General requirements
- UL 61010-1:2012 R7.19 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 1: General Requirements

Table of Contents

Section 1
General Information

1.1 Introduction

The Sub-Cell™ GT family of instruments (Sub-Cell GT, Wide Mini-Sub™ Cell GT, and Mini-Sub Cell GT) are comprehensive and flexible gel electrophoresis systems that effectively separate nucleic acids using submerged agarose gels. Submarine agarose gels are easy to cast and readily dissipate heat. These gels allow sample underlaying and prevent electrical field discontinuities caused by wicks or sample well dehydration. Agarose gels are ideal for the separation of DNA restriction digestions, PCR-amplified fragments, and genomic DNA and RNA prior to Southern or northern blotting. If operated correctly, agarose gel submarine electrophoresis can effectively separate nucleic acids from 20 base pairs to 20 kilobase pairs in length.

The Sub-Cell GT family is designed to provide reproducible results under rigorous use conditions for years. These rugged systems incorporate many features that make casting and running agarose gels simple and efficient. The gel caster provides tape-free gel casting in trays. Gels can also be cast in the Sub-Cell, Wide Mini-Sub Cell, and Mini-Sub Cell GT bases using specially designed casting gates. Replaceable electrode cassettes provide a simple way to replace electrode wires. A comprehensive assortment of base and tray sizes, including a variety of preparative, analytical, and multichannel pipet-compatible combs, makes these systems ideal for any agarose gel application.

Note: This manual contains instructions for the GT family of Sub-Cell electrophoresis systems only. Prior to the release of the GT instruments, Bio-Rad supplied similar agarose gel electrophoresis cells: the original Sub-Cell DNA Electrophoresis Cell, Wide Mini-Sub Cell, and Mini-Sub Cell Systems. This manual does not provide information on these earlier versions. Contact your local Bio-Rad representative for information concerning the original Sub-Cell Systems.

1.2 Safety

The Sub-Cell GT Electrophoresis Systems are designed for maximum user safety. The buffer chambers are made of 2.75 mm thick injection-molded acrylic to create a leak-free electrophoresis environment. The safety lids surround the buffer chamber to protect the user from exposure to electrical currents.

Before use, inspect the GT base for cracks or chips, which may allow the buffer to leak from the base and cause a potential electrical hazard. Additionally, inspect all electrical cables, banana jacks, and plugs for loose connections, cracks, breaks, or corrosion. Do not use any part that is cracked, charred, or corroded. These parts may also cause a potential electrical hazard. Contact your local Bio-Rad representative before using a part that may be considered hazardous.

During electrophoresis, inspect the base and workbench for any signs of buffer leakage. If leaking buffer is detected, disconnect the power to the cell immediately and contact your local Bio-Rad representative.

Power to Sub-Cell GT Systems is supplied by an external DC voltage power supply. This power supply must be ground-isolated in such a way that the DC voltage output floats with respect to ground. All of Bio-Rad's power supplies meet this important safety requirement. The recommended power supply for these units is the PowerPac Basic Power Supply. The PowerPac Basic Power Supply contains safety features such as no load, overload, rapid resistance change, and ground leak detection capabilities. The maximum specified operating parameters for the systems in the Sub-Cell GT family are given in Table 1.

Table 1. Operating parameters for Sub-Cell GT Systems.

	Sub-Cell GT	Wide Mini-Sub Cell GT	Mini-Sub Cell GT
Maximum voltage limit, VDC	200	150	150
Maximum power limit, W	40	45	10
Maximum buffer temperature, °C	40	40	40

1.3 Environment Requirements

The Sub-Cell GT, Wide Mini-Sub Cell GT, and Mini-Sub Cell GT System has each been designed to be safely operated under the environmental conditions listed in Table 2.

Table 2. Environment requirements.

Parameter	Specification
Environment	Indoor use only
Operating altitude	Up to 2,000 meters above sea level
Operating temperature	4–40°C 39–104°F*
Transport and storage temperature	–20° to 60°C –4 to 140°F**
Relative humidity	50–80% (noncondensing)
Mains supply voltage fluctuation	±0.5% (unless otherwise specified)
Overvoltage category	II
Pollution degree	2

* Operating the instrument outside of this temperature range may not meet performance specifications. A room temperature between 4–40°C (39–104°F) is considered safe.

** Store and transport the instrument in its shipping container to meet these temperature conditions.

Electric current to the cell from the external power supply enters the unit through the lid assembly, which provides a safety interlock. Current to the cell is broken when the lid is removed. Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid or when working with the cell.

Important: These Bio-Rad instruments are certified to meet UL/CSA 61010-1 safety standards. UL/CSA-certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the UL/CSA 61010-1 safety certification
- Create a potential safety hazard

There are no user-serviceable parts in these apparatuses. To ensure electrical safety, do not attempt to service these apparatuses.

1.3 System Components

Each system in the Sub-Cell GT family comes with the components listed in Table 3 (see Figure 1 for part illustrations). Check your instrument to be sure all items are present. Note any damage to the unit that may have occurred during shipping. Notify Bio-Rad Laboratories if any items are missing or damaged.

Table 3. Components of the Sub-Cell GT Systems.

	Sub-Cell GT	Wide Mini-Sub Cell GT	Mini-Sub Cell GT
Item	**Quantity**	**Quantity**	**Quantity**
GT base (buffer chamber)	1	1	1
Gel casting gates (optional)	2	2	2
Safety lid and cables	1	1	1
UVTP gel tray	1	1	1
Fixed-position comb	2 (15 well, 1.5 mm thick) (20 well, 1.5 mm thick)	2 (15 well, 1.5 mm thick) (20 well, 1.5 mm thick)	2 (8 well, 1.5 mm thick) (15 well, 1.5 mm thick)
Leveling bubble	1	1	1
Gel caster (optional)	1	1	1
Instruction manual	1	1	1

UVTP, ultraviolet-transparent plastic.

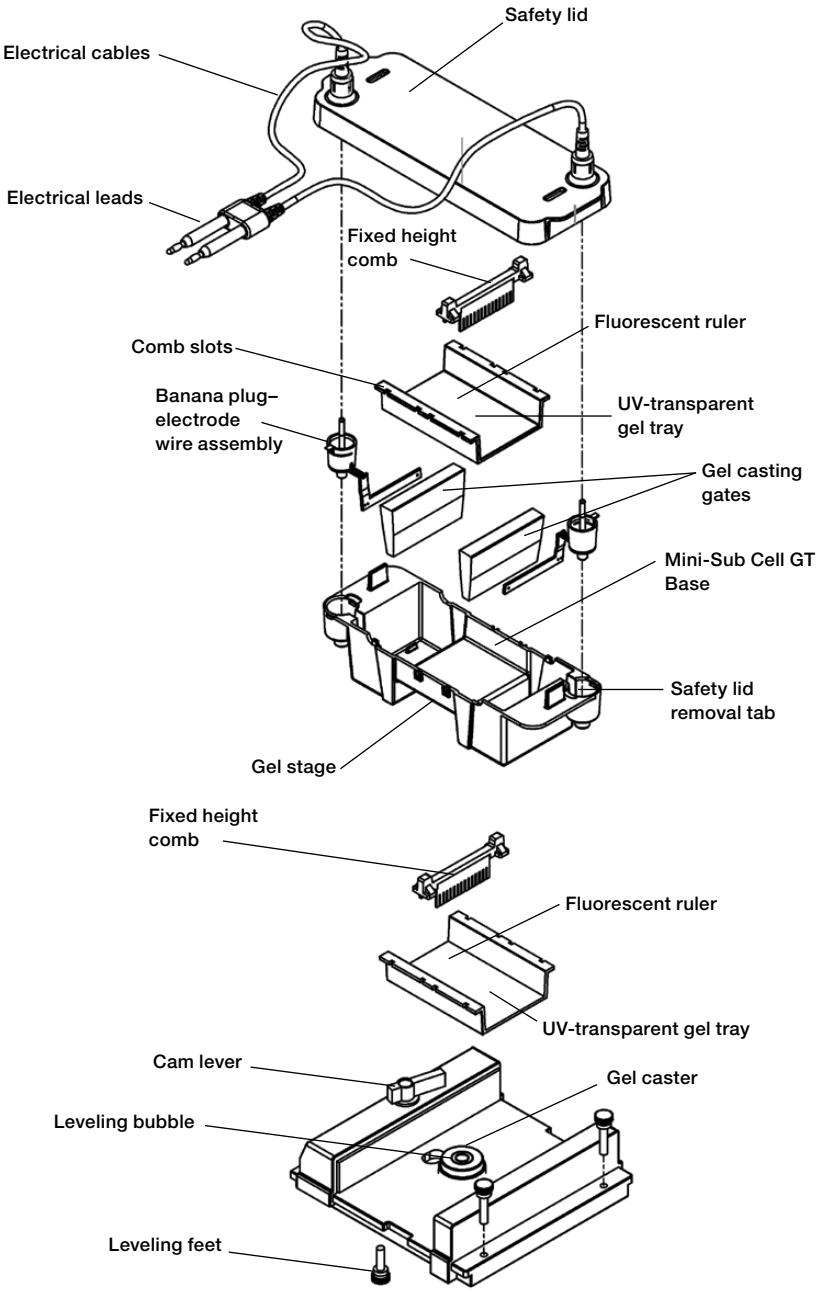

Fig. 1. System components. The illustration shows the parts in the Mini-Sub Cell GT System; the parts are similar but not identical in the Sub-Cell GT and Wide Mini-Sub Cell GT Systems.

1.4 Specifications

Table 4 shows the specifications of the instruments in the Sub-Cell GT family of instruments.

Table 4. Specifications.

	Sub-Cell GT System	Wide Mini-Sub Cell GT System	Mini-Sub Cell GT System
GT base footprint (L x W x H), cm	40.5 x 18 x 9.4	25.5 x 17.8 x 6.8	25.5 x 9.2 x 5.6
GT base buffer volume,* ml	1,500–2,000	650–900	265–320
GT base gel size, cm	15 x 15	15 x 7	7 x 7
Gel tray sizes, cm	15 x 10	15 x 7	7 x 7
	15 x 15	15 x 10	7 x 10
	15 x 20		
	15 x 25		
Construction			
GT base	Molded clear plastic		
Gel casting gates	Aluminum		
Safety lid	Molded clear plastic		
Electrode wire guard	Molded polycarbonate		
Banana plugs	Gold-plated brass, 4.4 cm length		
Electrodes	Platinum, 0.25 mm diameter		
Electrical cables	Dual, 20 AWG, tinned copper wire cable		
	Flame-retardant polyurethane insulation jacket		
Electrical leads	Nickel silver		
Gel tray	Ultraviolet transparent acrylic plastic		
Combs	Molded plastic and machined acrylic		
Gel casting device	Polycarbonate and 0.64 cm silicone foam		

* GT base buffer volumes will vary depending on the size and thickness of the gel used.

Section 2
Operating Instructions

Note: See Section 3, Gel and Electrophoresis Reagent Preparation, for information on the preparation of RNA gels. See Section 7, References, to find more information on DNA and RNA electrophoresis.

2.1 DNA Gel Preparation

DNA agarose gels can be used to separate and visualize DNA of various sizes. Before casting an agarose gel, consult Table 5 to determine the appropriate percent agarose gel to use based on the size of DNA to be separated.

Procedure

1. Determine the amount of agarose (grams) required to make the desired agarose gel concentration and volume. Use Tables 5 and 6 as guides for agarose concentration and gel volume requirements.

   Example: For a 1% agarose gel, add 1 g of agarose to 100 ml of 1x electrophoresis buffer.

Table 5. Gel concentration required for DNA separation.*

Gel Concentration (%)	DNA Size
0.50	1–30 kb
0.75	800 bp–10 kb
1.00	500 bp–10 kb
1.25	400 bp–7 kb
1.50	200 bp–3 kb
2.00**	100 bp–2.5 kb
3.00**	40 bp–2 kb
4.00***	10–400 bp

* See References for more information.
** Sieving agarose such as Bio-Rad's Certified PCR Agarose.
*** Sieving agarose such as Bio-Rad's Certified Low Range Ultra Agarose.

Table 6. Gel volume requirements.

Gel Size (thickness)	0.25 cm	0.5 cm	0.75 cm	1.0 cm
Base				
7 x 7 cm	10 ml	20 ml	30 ml	40 ml
15 x 7 cm	20 ml	40 ml	60 ml	80 ml
15 x 15 cm	50 ml	100 ml	150 ml	200 ml
Tray				
7 x 7 cm	10 ml	20 ml	30 ml	40 ml
7 x 10 cm	15 ml	30 ml	45 ml	60 ml
15 x 7 cm	20 ml	40 ml	60 ml	80 ml
15 x 10 cm	30 ml	60 ml	90 ml	120 ml
15 x 15 cm	50 ml	100 ml	150 ml	200 ml
15 x 20 cm	70 ml	140 ml	210 ml	280 ml
15 x 25 cm	90 ml	180 ml	270 ml	360 ml

2. Add the agarose to a suitable container (for example, 250 ml Erlenmeyer flask, Wheaton bottle, etc.). Add the appropriate amount of 1x electrophoresis buffer (see Section 3, Gel and Electrophoresis Reagent Preparation, for electrophoresis buffer preparation) and swirl to suspend the agarose powder in the buffer. If using an Erlenmeyer flask, invert a 25 ml Erlenmeyer flask into the open end of the 250 ml Erlenmeyer flask containing the agarose. The small flask acts as a reflux chamber, allowing long or vigorous boiling without much evaporation.

Note: A mark can be put on the lower flask at the same level as the liquid. If evaporation occurs, water can be added to bring the liquid back to the original starting level.

3. The agarose can be melted by boiling on a magnetic hot plate (Step 4a) or in a microwave oven (Step 4b).

   Caution: Always wear protective gloves, safety glasses, and a lab coat while preparing and casting agarose gels. The vessels containing hot agarose can cause severe burns if allowed to contact skin. Additionally, molten agarose can boil over when swirled.

Magnetic Hot Plate Method

4a. Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Bubbles or foam should disrupt before rising to the neck of the flask.

Microwave Oven Method

4b. Place the gel solution in the microwave. Using a low to medium setting, set the timer for a minimum of 5 minutes, stopping the microwave oven every 30 seconds and swirling the flask gently to suspend the undissolved agarose. This technique is the fastest and safest way to dissolve agarose.

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

2.2 Casting Agarose Gel Slabs

There are several ways to cast agarose submarine gels using the Sub-Cell GT Systems. Gels may be cast with an ultraviolet transparent plastic (UVTP) tray directly on the gel stage of the Sub-Cell GT bases using the gel casting gates. Gels may also be cast on the removable UVTP trays with the aid of the gel caster or with standard laboratory tape.

Casting Gels on the Base Stage with the UVTP Tray

1. Level the cell using the leveling bubble provided.
2. Place the UVTP tray on the gel stage.

   Note: The Mini-Sub Cell GT System requires the 7 x 7 cm UVTP tray for casting in the GT base. The Wide Mini-Sub Cell GT System requires the 15 x 7 cm UVTP tray, and the Sub-Cell GT System requires the 15 x 15 cm UVTP tray for casting in the GT base.

3. Slide the gel casting gates into the slots at opposite ends of the GT gel stage. Ensure that the gates are evenly seated in the slots and the gates uniformly contact all edges of the UVTP tray. The weight of the gates provides a tight seal to prevent any leakage problems during gel casting.

   Note: If leakage occurs while pouring the gel on the casting tray atop the stage, chill the casting gates in the freezer for 2–3 minutes. Place the casting gates into the slots when ready to pour the gel. The chilled casting gates will prevent the gel solution from leaking out of the tray and into the chambers.

4. Place the comb(s) into the appropriate slot(s) of the trays so that the sample wells are near the cathode (black). DNA samples will migrate toward the anode (red) during electrophoresis.

5. Prepare the desired concentration and amount of agarose in 1x electrophoresis buffer (see Section 2.1, DNA Gel Preparation). When the agarose solution has cooled to 50–60°C, pour the molten agarose between the gates.

   

   Warning! Hot agarose (>60°C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.

6. Allow 20–40 minutes for the gel to solidify at room temperature.

7. Carefully remove the comb from the solidified gel. Remove the gel casting gates.

8. Submerge the gel beneath 2–6 mm of 1x electrophoresis buffer (see Section 3, Gel and Electrophoresis Reagent Preparation). Use greater depth overlay (more buffer) with increasing voltages to prevent pH and heat effects.

Removable Tray (UVTP) Gel Casting Using a Sub-Cell GT Gel Caster or Mini-Sub Cell GT Gel Caster

1. Level the Sub-Cell GT Gel Caster or Mini-Sub Cell GT Gel Caster using the leveling feet in the gel caster and the leveling bubble provided.

2. Disengage and slide the movable wall to the open end of the Sub-Cell GT Gel Caster or Mini-Sub Cell GT Gel Caster by turning and lifting the cam peg upward.

   Note: If casting more than one gel with the Sub-Cell GT Gel Caster, add the removable gel casting wall to the gel caster. The removable wall will allow casting using two 15 x 10 cm trays, four 7 x 10 cm trays, or one 15 x 10 cm and one 15 x 15 cm tray.

3. Place the open edge of the UVTP tray against the fixed wall of the Sub-Cell GT Gel Caster or Mini-Sub Cell GT Gel Caster.
4. Slide the movable wall against the edge of the UVTP tray (Figure 2).

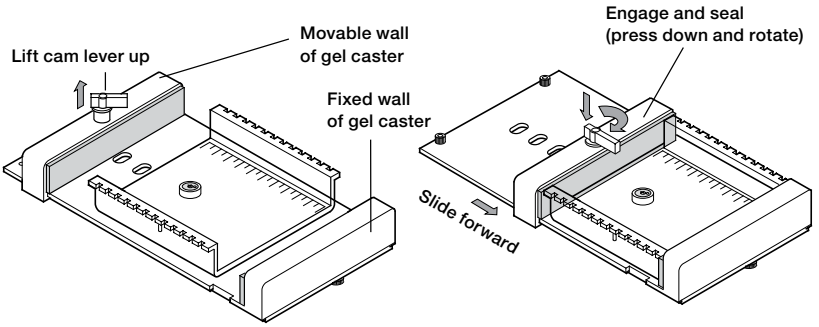

Fig. 2. Sealing the UVTP tray for gel casting.

5. To seal the open tray ends, engage the cam peg by turning and pressing downward simultaneously.
6. When the cam peg has dropped into the appropriate slot, turn the peg in either direction until resistance is felt. This action seals the edges of the tray for casting.
7. Place the comb(s) into the appropriate slot(s) of the tray.
8. Prepare the desired concentration and amount of agarose in 1x electrophoresis buffer (see Section 2.1, DNA Gel Preparation). When the agarose solution has cooled to 50–60°C pour the molten agarose between the gates.

   Warning! Hot agarose (>60°C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.
9. Allow 20–40 min for the gel to solidify at room temperature.
10. Carefully remove the comb from the solidified gel.
11. Disengage the cam peg by turning and lifting upward. Slide the movable wall away from the tray. Remove the tray from the Sub-Cell GT Gel Caster or Mini-Sub Cell GT Gel Caster.

    Note: While the gel is solidifying, a light seal is formed between the gasket and the gel (especially for low-percentage agarose gels [<0.8%]). Before moving the wall away from the tray, carefully lift the tray on one side to release the seal or use a spatula to break the seal between the agarose and gasket.

12. Place the tray onto the leveled Sub-Cell base so that the sample wells are near the cathode (black). DNA samples will migrate toward the anode (red) during electrophoresis.
13. Submerge the gel beneath 2–6 mm of 1x electrophoresis buffer (see Section 3, Gel and Electrophoresis Reagent Preparation). Use greater depth overlay (more buffer) with increasing voltages to avoid pH and heat effects.

Removable Tray (UVTP) Gel Casting Using Tape

1. Seal the ends of the UVTP gel tray securely with strips of standard laboratory 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 using the leveling bubble provided with the instrument.
3. Prepare the desired concentration and amount of agarose in 1x electrophoresis buffer (see Section 2.1, DNA Gel Preparation). When the agarose solution has cooled to 50–60°C, pour the molten agarose into the gel tray.

Warning! Hot agarose (>60°C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.

4. Allow 20–40 minutes for the gel to solidify at room temperature.
5. Carefully remove the comb from the solidified gel.
6. Remove the tape from the edges of the gel tray.
7. Place the tray onto the leveled Sub-Cell base so that the sample wells are near the cathode (black). DNA samples will migrate toward the anode (red) during electrophoresis.
8. Submerge the gel beneath 2–6 mm of 1x electrophoresis buffer (see Section 3, Gel and Electrophoresis Reagent Preparation). Use greater depth overlay (more buffer) with increasing voltages to avoid pH and heat effects.

2.3 Electrophoresis

After the agarose gel has solidified, sample loading and electrophoresis can begin. Agarose gels can be run in many different types of electrophoresis buffers. Nucleic acid agarose gel electrophoresis is usually conducted with either Tris-acetate-EDTA (TAE) buffer or Tris-borate-EDTA (TBE) buffer. While TAE buffers provide faster electrophoretic migration of linear DNA and better resolution of supercoiled DNA, TBE buffers have a stronger buffering capacity for longer or higher-voltage electrophoresis runs. Bio-Rad offers premixed 50x TAE and 10x TBE buffers, as well as individual buffer reagents for use with the Sub-Cell GT Systems.

1. Prepare samples for gel loading. The maximum sample loading volumes for Bio-Rad's combs are listed in the tables in Section 6.2, Sub-Cell GT System Accessories, under Well Volume Capacity. Loading volume is dependent upon the type of comb used (well thickness and length) and thickness of the gel.
2. When loading volume is determined, add standard nucleic acid sample loading dye to a final 1x concentration to make samples dense for underlaying into sample wells (see Section 3, Gel and Electrophoresis Reagent Preparation, for sample loading dye preparation).
3. Load the samples into the wells using standard pipets. Multichannel pipets can be used only for loading samples with Bio-Rad Multichannel Pipet Compatible Fixed Height Combs (see Section 6.2, Sub-Cell GT System Accessories).

   Note: Sample wells are often difficult to see. Well visibility can be enhanced by placing black paper or tape under the base or trays in the area of comb placement and well formation.
4. Place the safety lid on the Sub-Cell base carefully. Do not disturb the samples. The Sub-Cell GT System safety lids attach to the base in only one orientation. To attach the safety lid correctly, match the red and black banana jacks on the safety lid with the red and black banana plugs of the base.
5. Power requirements vary depending on gel thickness, length, agarose, concentration, and on the type of electrophoresis buffer used. Refer to Tables 7 and 8 for relative sample migration rates for the different Sub-Cell GT Systems and for DNA size migration with sample loading dyes.

   Note: Buffer recirculation is not required for most standard DNA and RNA agarose gel electrophoresis. If buffer recirculation is required, simply turn off the power supply, remove the safety lid, and mix the running buffer as desired. After the buffer has been mixed, reconnect the safety lid and continue with electrophoresis.

Table 7. Relative sample migration rates.*

Bromophenol Blue Cell Type	Voltage	Migration Rate
Sub-Cell GT System, 15 x 15 cm gel	75 V	3.0 cm/hr
Wide Mini-Sub Cell GT System, 15 x 10 cm gel	75 V	4.5 cm/hr
Mini-Sub Cell GT System, 7 x 10 cm gel	75 V	4.5 cm/hr

* These sample migration rates were determined based on a 0.5 cm thick 1.0% agarose gel using Bio-Rad's Certified Molecular Biology Agarose in 1x TAE electrophoresis buffer (diluted from Bio-Rad's premixed 50x TAE Buffer). Migration rates will vary depending on the voltage, current, and type of agarose or buffer used.

Table 8. DNA size migration with sample loading dyes.

Agarose Concentration, %	Xylene Cyanole	Bromophenol Blue
0.5–1.5	4–5 kb	400–500 bp
2.0–3.0*	750 bp	100 bp
>3.0**	125 bp	25 bp

* Sieving agarose such as Certified PCR Agarose.
** Sieving agarose such as Certified Low Range Ultra Agarose.

2.4 Nucleic Acid Staining and Visualization

Gels can be removed from the Sub-Cell GT base or gel tray for nucleic acid staining. The gel can also remain on the UVTP gel tray for staining.

Ethidium Bromide (EtBr) Staining Procedure

1. Place the gel into the appropriate volume of 0.5 µg/ml EtBr stain for 15–30 minutes. Use enough staining solution to cover the entire gel.

   Caution: EtBr is a suspected carcinogen and should be handled with extreme care. Always wear gloves, safety glasses, and a laboratory coat. Dispose of used EtBr solutions and gels appropriately (see the ethidium bromide material safety data sheet [MSDS] for proper disposal methods).

2. Destain the gel for 10–30 minutes in dH_2O with the same volume used for staining.

   Note: EtBr can be removed from the DNA with extended destaining. This will cause lower sensitivity of detection. However, insufficient destaining will create higher background fluorescence.

3. Rinse the gel briefly with dH_2O to remove any residual staining solution.

4. Place the gel on a UV transilluminator for nucleic acid visualization and analysis. DNA-EtBr complexes may be illuminated with UV light of 254, 302, or 366 nm. Sensitivity decreases with illumination at higher wavelengths. However, nicking of DNA will increase below 302 nm. Table 9 gives the percentage of transmittance of UV light through 1/4" (0.64 cm) UVTP.

Note: Nucleic acids in the gel can be visualized through the UVTP trays. If a UVTP tray is not used, place household plastic wrap between the UV transilluminator and the gel to avoid contaminating the transilluminator with nucleic acids or EtBr.

Table 9. Percent UV transmittance through 1/4" (0.64 cm) UV-transparent plastic.

Approximate Wavelength, nm	% Transmittance
254	0
302	80
366	90

5. Image the gel using the GelDoc™ Go Gel Imaging System, ChemiDoc™ Go Imaging System, or ChemiDoc MP Imaging System for nucleic acid gel analysis. On the imager touch screen, select your nucleic acid application. Image Lab Touch Software will automatically select the appropriate filter and illumination source based on the selected application.

2.5 Note on Blotting

Nucleic acids within the gel can be transferred to membranes using the techniques of Southern and northern blotting. It is beyond the scope of this instruction manual to include blotting procedures. Consult the References for blotting techniques. Bio-Rad offers a full line of nitrocellulose and positively charged nylon membranes, as well as vacuum and electrophoretic blotting apparatuses for Southern and northern blotting.

Section 3
Gel and Electrophoresis Reagent Preparation

RNA Agarose Formaldehyde Gels

For 100 ml of a 1% agarose formaldehyde gel, prepare as follows:

- 62 ml of 1.6% melted agarose
- 20 ml 5x MOPS electrophoresis buffer (1x final concentration)
- 18 ml 12.3 M (37.5%) formaldehyde (2.2 M final concentration)

Caution: Formaldehyde solutions and formaldehyde vapors are toxic. When handling solutions or gels that contain formaldehyde, use a chemical hood. Always wear gloves, safety glasses, and a laboratory coat when using formaldehyde. See the formaldehyde MSDS for safety information.

Nucleic Acid Electrophoresis Buffers (see References)

DNA agarose gel electrophoresis is usually performed using either Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE). While TAE buffers provide faster electrophoretic migration of linear DNA and better resolution of supercoiled DNA, TBE buffers have a stronger buffering capacity for longer or higher-voltage electrophoresis runs. Bio-Rad offers premixed 50x TAE and 10x TBE buffers for use with the Sub-Cell GT Systems. RNA formaldehyde gels require a MOPS [3-(N-morpholino)-propanesulfonic acid] electrophoresis buffer.

1x Tris-Acetate-EDTA (TAE): 40 mM Tris (pH 7.6), 20 mM acetic acid, and 1 mM EDTA

For 50x stock (1 L), dissolve the following in 600 ml distilled water:

- 242 g Tris base (FW = 121)
- 57.1 ml glacial acetic acid
- 100 ml 0.5 M EDTA, pH 8.0

Fill to a final volume of 1 L with distilled water.

1x Tris-Borate-EDTA (TBE): 89 mM Tris (pH 7.6), 89 mM boric acid, 2 mM EDTA

For 10x stock (1 L), dissolve the following in 600 ml distilled water:

- 108 g Tris base (FW = 121)
- 55 g boric acid (FW = 61.8)
- 40 ml 0.5 M EDTA, pH 8.0

Fill to a final volume of 1 L with distilled water.

1x MOPS Buffer (RNA gels): 0.02 M MOPS (pH 7.0), 8 mM sodium acetate, 1 mM EDTA

5x stock (1 L), dissolve in 600 ml distilled water treated with diethyl pyrocarbonate (DEPC):

- 20.6 g MOPS
- 13.3 ml 3 M sodium acetate (DEPC-treated), pH 7.4
- 10 ml 0.5 M EDTA (DEPC-treated), pH 8.0

Fill to a final volume of 1 L with DEPC-treated distilled water.

Caution: DEPC is a suspected carcinogen. Always wear gloves, safety glasses, and a laboratory coat. Use caution when handling DEPC-containing solutions. Consult the diethyl pyrocarbonate MSDS for more information.

DNA and RNA Sample Loading Dye (see References)

A convenient 10x sample buffer stock consists of 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanole FF in 1x TAE buffer. Only 1–10 ml of the 10x loading dye should be prepared.

RNA Sample Preparation (see References)

Prior to loading RNA onto an agarose formaldehyde gel, prepare each RNA sample as follows:

- 6 µl RNA in DEPC-treated water
- 10 µl 5x MOPS buffer (final concentration 1.67x)
- 9 µl 12.3 M formaldehyde (final concentration 3.7 M)
- 25 µl formamide (final concentration 50% v/v)

Caution: Formamide is a teratogen. Always wear gloves, safety glasses, and a laboratory coat. Use caution when handling formamide. Consult the formamide MSDS for more information.

Ethidium Bromide Solution

Add 10 mg of EtBr to 1 ml distilled water. Bio-Rad offers Ethidium Bromide Solution (10 mg/ml).

Section 4
Care and Maintenance

4.1 Cleaning Sub-Cell GT System Components

1. All Sub-Cell GT parts should be washed with a mild soap or detergent solution in warm water.

   Note: Be careful not to snag or break the electrode wire in the GT base while cleaning.

2. Thoroughly rinse all parts with warm water (distilled, if possible) and air dry.

4.2 Compatible Cleaning Agents

Chemically compatible cleaners must be used to ensure long life of parts. These include:

- **Aqueous solutions of soaps or mild detergents**:
  - Bio-Rad Cleaning Concentrate (catalog #1610722)
  - Dishwashing liquid
- **Organic solvents**:
  - Hexane
  - Aliphatic hydrocarbons

Do not leave plastic parts to soak in detergents more than 30 minutes. A short detergent rinse typically is all that is required.

Caution: Do not use the following chemicals to clean Sub-Cell GT parts. Exposure to these chemicals may cause the plastic parts to crack, craze, etch, or warp.

- **Chlorinated hydrocarbons**
  - Carbon tetrachloride
  - Chloroform
- **Aromatic hydrocarbons**
  - Benzene
  - Phenol
  - Toluene
  - Methyl ethyl ketone
  - Acetone
- **Alcohols**
  - Methanol
  - Ethanol
  - Isopropyl alcohol

Do not use abrasive or highly alkaline cleaners on Sub-Cell GT parts.

Do not expose Sub-Cell GT parts to temperatures >60°C. Do not sterilize Sub-Cell GT parts with an autoclave or dry heat.

4.3 Maintenance Schedule

See Table 10 for instructions on maintaining your Sub-Cell GT, Wide Mini-Sub Cell GT, or Mini-Sub Cell GT System

Table 10. System maintenance.

Item	Look for	Frequency	Action
All parts	Dried salts, agarose, grease, and dirt	Each use	Clean parts as described in Section 4.1
Electrical cables	Breaks or fraying	Each use	Replace cables
Trays	Chips or cracks	Each use	Replace tray
Electrode wires	Breaks	Each use	See Section 4.4
Cable connections (banana jacks and plugs)	Looseness	Weekly	Replace banana jacks or banana plug holders
GT base	Crazing, cracks	Monthly	Replace GT base

4.4 Electrode Replacement

The Sub-Cell GT family of systems allows easy replacement of broken electrode wires by removing the banana plug–electrode wire assembly and ordering a new electrode assembly from Bio-Rad (Figure 3). Order the new assembly using the part description and catalog numbers listed in Section 6, Ordering Information.

1. Remove the broken wire assembly by placing one finger on the banana plug and using another finger to press the QuickSnap feature on the outside of the upper part of the electrode wire assembly, then lifting upward. Discard the broken assembly.
2. Insert the new assembly into the electrode assembly chamber of the GT base. Make sure the tab of the QuickSnap feature is properly seated in the slot of the electrode assembly chamber.

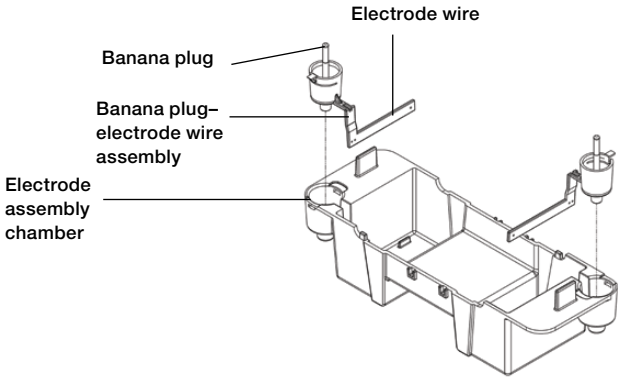

Fig. 3. Removal of banana plug–electrode wire assembly.

4.5 RNase Decontamination

Sub-Cell GT parts can be cleaned with a mild detergent and treated for 10 minutes with 3% hydrogen peroxide (H_2O_2), and then rinsed with 0.1% DEPC-treated distilled water to eliminate RNases prior to using any of the Sub-Cell GT Systems for RNA gels. Consult the References for other suggestions regarding the use of DEPC in RNase decontamination.

Caution: DEPC is a suspected carcinogen. Always wear gloves, safety glasses, and a laboratory coat. Use caution when handling DEPC-containing solutions. Consult the diethyl pyrocarbonate MSDS for more information.

Do not attempt to eliminate RNase contamination from Sub-Cell GT parts using extreme dry heat.

Note: Several commercial products are available for eliminating RNase contamination. RNase*ZAP* (Thermo Fisher Scientific Inc.) is a safe, simple, and effective method that if used properly does not craze or fog the Sub-Cell GT parts. See manufacturer's instructions for proper use.

Section 5
Troubleshooting

Table 11. Troubleshooting.

Symptom	Cause	Solution
Slanted lanes (bands)	▪ Gel not fully solidified	▪ Let gel solidify for at least 30–45 min
	▪ Comb warped or at an angle	▪ Check alignment of comb
Curved line or distortion of lanes (bands)	▪ Bubbles in sample wells	▪ Remove bubbles prior to electrophoresis
Differential relative mobilities	▪ Sample spilled out of wells	▪ Samples should have proper density
	▪ Unit not leveled	▪ Level unit
		▪ Place on steady work bench
Curved bands, smiles	▪ Sample overload	▪ Reduce load
Ragged bands	▪ Sample density incorrect	▪ See sample application instructions
	▪ Sample well deformed	▪ Carefully remove comb, especially from soft gels. Be sure gel has solidified. Cooling soft gels aids in comb removal
	▪ Excessive power or heating	▪ Reduce voltage. See electrophoresis instructions
Band smearing and streaking	▪ Agarose has improper endosmosis	▪ Consult Bio-Rad about agarose
	▪ Salt concentration in sample too high	▪ Reduce salt concentration to ≤0.1 M
	▪ Excessive power and heating	▪ Reduce voltage. See electrophoresis instructions
	▪ Sample spilled out of well	▪ Apply sample carefully. Increase gel thickness for large sample volumes. Adjust comb height
	▪ Incomplete digestion, nuclease contamination, bad enzyme	▪ Heat sample. Check enzyme activity. Digest sample further
	▪ Sample wells cast through the gel. Sample leaks along bottom of running surface	▪ Comb should be placed 1 to 2 mm above the base of the running surface
	▪ Sample overload	▪ Dilute sample
Bands sharp but too few bands seen	▪ Gel agarose percentage too high	▪ Lower agarose percentage
		▪ Check enzyme activity, digest further
	▪ Incomplete digestion	
High MW bands sharp; low MW bands smeared	▪ Gel agarose percentage too low	▪ Increase agarose percentage
		▪ Switch to polyacrylamide
Gels crack	▪ Too high voltage gradient, especially with low melting temperature agarose or low strength gels	▪ Reduce voltage
		▪ Run gel at lower temperature

Section 6
Ordering Information

6.1 Sub-Cell GT Agarose Gel Electrophoresis Systems

Catalog #	Product Description
1704401	**Sub-Cell GT System**, with 15 x 10 cm tray
1704402	**Sub-Cell GT System**, with 15 x 15 cm tray, casting gates
1704403	**Sub-Cell GT System**, with 15 x 20 cm tray
1704404	**Sub-Cell GT System**, with 15 x 25 cm tray
1704481	**Sub-Cell GT System**, with 15 x 10 cm tray, gel caster
1704482	**Sub-Cell GT System**, with 15 x 15 cm tray, casting gates, gel caster
1704483	**Sub-Cell GT System**, with 15 x 20 cm tray, gel caster
1704484	**Sub-Cell GT System**, with 15 x 25 cm tray, gel caster
1704405	**Wide Mini-Sub Cell GT System**, with 15 x 7 cm tray, casting gates
1704485	**Wide Mini-Sub Cell GT System**, with 15 x 7 cm tray, casting gates, mini-gel caster
1704468	**Wide Mini-Sub Cell GT System**, with 15 x 10 cm tray
1704469	**Wide Mini-Sub Cell GT System**, with 15 x 10 cm tray, gel caster
1704489	**Wide Mini ReadySub-Cell GT System**
1704406	**Mini-Sub Cell GT System**, with 7 x 7 cm tray, casting gates
1704466	**Mini-Sub Cell GT System**, with 7 x 10 cm tray
1704467	**Mini-Sub Cell GT System**, with 7 x 10 cm tray, mini-gel caster
1704487	**Mini ReadySub Cell GT System**
1704486	**Mini-Sub Cell GT System**, with 7 x 7 cm tray, casting gates, mini-gel caster
Sub-Cell GT Systems with PowerPac Basic Power Supply, 100–120/220–240 V	
1640302	**Sub-Cell GT Cell and PowerPac Basic Power Supply**
1640301	**Wide Mini-Sub Cell GT Cell and PowerPac Basic Power Supply**
1640300	**Mini-Sub Cell GT Cell and PowerPac Basic Power Supply**

6.2 Sub-Cell GT System Accessories

Catalog #	Description
Sub-Cell GT Systems	
1704390	**Sub-Cell GT Base**
1704391	**Sub-Cell GT Replacement Lid with Cables**
1704412	**Sub-Cell GT Gel Caster**, full size
1704392	**Sub-Cell GT QuickSnap Electrode Assembly (Anode)**, red
1704393	**Sub-Cell GT QuickSnap Electrode Assembly (Cathode)**, black
1704415	**Sub-Cell GT Gel Casting Gates**, pkg of 2
1704416	**Sub-Cell GT UV-Transparent Gel Tray**, 15 x 10 cm
1704417	**Sub-Cell GT UV-Transparent Gel Tray**, 15 x 15 cm
1704418	**Sub-Cell GT UV-Transparent Gel Tray**, 15 x 20 cm
1704419	**Sub-Cell GT UV-Transparent Gel Tray**, 15 x 25 cm
Wide Mini-Sub Cell GT Systems	
1704370	**Wide Mini-Sub Cell GT Base**
1704371	**Wide Mini-Sub Cell GT Replacement Lid with Cables**
1704422	**Mini Sub-Cell GT Gel Caster**
1704372	**Wide Mini-Sub Cell GT QuickSnap Electrode Assembly (Anode)**, red
1704373	**Wide Mini-Sub Cell GT QuickSnap Electrode Assembly (Cathode)**, black
1704425	**Wide Mini-Sub Cell GT Gel Casting Gates**, pkg of 2
1704416	**Sub-Cell GT UV-Transparent Gel Tray**, 15 x 10 cm
1704426	**Wide Mini-Sub Cell GT UV-Transparent Gel**, 15 x 7 cm

Catalog # Product Description

Mini-Sub Cell GT Systems

1704360 **Mini-Sub Cell GT Base**
1704361 **Mini-Sub Cell GT Replacement Lid with Cables**
1704422 **Mini Sub-Cell GT Gel Caster**
1704362 **Mini-Sub Cell GT QuickSnap Electrode Assembly (Anode)**, red
1704363 **Mini-Sub Cell GT QuickSnap Electrode Assembly (Cathode)**, black
1704434 **Mini-Sub Cell GT Gel Casting Gates**, pkg of 2
1704435 **Sub-Cell GT UV-Transparent Mini-Gel Tray**, 7 x 10 cm
1704436 **Sub-Cell GT UV-Transparent Mini-Gel Tray**, 7 x 7 cm

Sub-Cell GT System Combs
Fixed Height Combs for Sub-Cell GT and Wide Mini-Sub Cell GT Systems

Catalog #	Well Number	Thickness, mm	Well Width, mm	Well Volume Capacity,* µl
1704449	30	1.50	2.69	20.2
1704447	20	0.75	4.84	18.2
1704448	20	1.50	4.84	36.3
1704445	15	0.75	5.52	20.7
1704446	15	1.50	5.52	41.4
1704443	10	0.75	9.87	37.0
1704444	10	1.50	9.87	74.0

Adjustable Height Combs for Sub-Cell GT and Wide Mini-Sub Cell GT Systems**

Catalog #	Well Number	Thickness, mm	Well Width, mm	Well Volume Capacity,* µl
1704344	30	1.50	2.69	20.2
1704321	20	0.75	4.84	18.2
1704322	20	1.50	4.84	36.4
1704323	15	0.75	5.52	20.7
1704324	15	1.50	5.52	41.4
1704325	10	0.75	9.87	37.0
1704326	10	1.50	9.87	74.0

Preparative Combs for Sub-Cell GT and Wide Mini-Sub GT Systems

Catalog #	Well Number	Thickness, mm	Well Width, mm	Well Volume Capacity,* µl
1704442	4	1.50	26.42	198.2
1704441	2	1.50	50.29	377.2
1704440	1	1.50	106.43	798.23
1704328**	1	3.00	106.43	1,596.5

* Well volume capacity was determined based on a well depth of 0.5 cm.
** Adjustable height combs require a comb holder (catalog #1704320).

Multichannel Pipet Compatible Fixed Height Combs for Sub-Cell GT and Wide Mini-Sub Cell GT Systems

Catalog #	Well Number	Thickness, mm	Well Width, mm	Well Volume Capacity,* µl
1704456	26	0.75	2.91	10.9
1704457	26	1.50	2.91	21.8
1704454	18	0.75	2.91	10.9
1704455	18	1.50	2.91	21.8
1704452	14	0.75	5.82	21.8
1704453	14	1.50	5.82	43.7
1704450	10	0.75	5.82	21.8
1704451	10	1.50	5.82	43.7

Fixed Height Combs for Mini-Sub Cell GT System

Catalog #	Well Number	Thickness, mm	Well Width, mm	Well Volume Capacity,* µl
1704464	15	0.75	2.59	9.7
1704465	15	1.50	2.59	19.4
1704462	8	0.75	5.54	20.8
1704463	8	1.50	5.54	41.6
1704461	2	1.50	20.32	152.4
1704460	1	1.50	43.43	325.7

Adjustable Height Combs for Mini-Sub Cell GT System**

Catalog #	Well Number	Thickness, mm	Well Width, mm	Well Volume Capacity,* µl
1704332	15	1.00	2.59	13.0
1704333	8	1.00	5.54	27.7

* Well volume capacity was determined based on a well depth of 0.5 cm.
** Adjustable height combs require a comb holder (catalog #1704331).

6.3 Related Bio-Rad Products

Catalog #	Product Description
Power Supplies	
1645050	**PowerPac Basic Power Supply**, 100/120–220/240 V
1645052	**PowerPac HC Power Supply**, 100/120–220/240 V
Semi-Dry Transfer Cells	
1703940	**Trans-Blot™ SD Semi-Dry Electrophoresis Transfer Cell**
Agarose	
1613101	**Certified Molecular Biology Agarose**, 125 g
1613105	**Certified PCR Agarose**, 500 g
1613107	**Certified Low Range Ultra Agarose**, 125 g
Molecular Rulers	
1708200	**AmpliSize DNA Molecular Ruler**, 50–2,000 bp; 250 µl
1708201	**20 bp Molecular Ruler**, 20–1,000 bp, 50 bands
1708351	**EZ Load 20 bp Molecular Ruler**, 20–1,000 bp, 50 bands
1708202	**100 bp Molecular Ruler**, 100–1,000 bp, 10 bands
1708352	**EZ Load 100 bp Molecular Ruler**, 100–1,000 bp, 10 bands
1708206	**100 bp PCR Molecular Ruler**, 100–3,000 bp, 30 bands
1708353	**EZ Load 100 bp PCR Molecular Ruler**, 100–3,000 bp, 30 bands
1708203	**500 bp Molecular Ruler**, 500–8,000 bp, 16 bands
1708354	**EZ Load 500 bp Molecular Ruler**, 500–8,000 bp, 16 bands
1708204	**1 kb Molecular Ruler**, 1–15 kb, 15 bands
1708355	**EZ Load 1 kb Molecular Ruler**, 1–15 kb, 15 bands
1708205	**2.5 kb Molecular Ruler**, 2.5–35 kb, 14 bands
Electrophoresis Buffers and Reagents	
1610733	**10x Tris/Boric Acid/EDTA (TBE)**, 1 L
1610743	**50x Tris/Acetic Acid/EDTA (TAE)**, 1 L
1610719	**Tris**, 1 kg
1610404	**Bromophenol Blue**, 10 g
1610423	**Xylene Cyanole FF**, 25 g
1610433	**Ethidium Bromide Solution**, 10 ml, 10 mg/ml
Imaging Systems	
12009077	**GelDoc Go Gel Imaging System with Image Lab Touch Software**
12003154	**ChemiDoc MP Imaging System with Image Lab Touch Software**
12018025	**ChemiDoc Go Imaging System with Image Lab Touch Software**

Section 7
References

1. Sambrook J et. al. (1989). Molecular Cloning, A Laboratory Manual, Second Edition (Cold Spring Harbor Laboratory Press).
2. Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, 1989.

Additional Reading

3. Kopchick, J. J., Cullen, B. R. and Stacey, D. W., *Anal. Biochem.*, **115**, 419 (1981).
4. Southern, E., *Methods in Enzymol.*, **68**, 152 (1979).
5. The Bio-Rad Silver Stain - Bulletin 1089, Bio-Rad Laboratories, Hercules, CA.
6. Bittner, M., Kupferer, P. and Morris, C .F., *Anal. Biochem.*, **102**, 459 (1980).
7. Bio-Rad Trans-Blot Cell Operation Instructions, Bulletin 1082, Bio-Rad Laboratories, Hercules, CA.
8. Winberg, G. and Hammarskjold, M. L., *Nucleic Acids Res.*, **8**, 253 (1980).
9. Jytatekadze, T. V., Axelrod, V. D., Gorbulev, V. G., Belzhelarskaya, S. N. and Vartikyan, R. M., *Anal. Biochem.*, **100**, 129 (1979).
10. Dretzen, G., Bellard, M., Sassone-Corsi, P. and Chambon, P., *Anal. Biochem.*, **112**, 295 (1981).

Bio-Rad
Laboratories, Inc.

Life Science
Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800*
Austria *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23*
Finland *00 800 00 24 67 23* ***France*** *00 800 00 24 67 23*
Germany *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300*
Hungary *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373*
Luxembourg *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280*
Norway *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23*
Portugal *00 800 00 24 67 23* ***Russian Federation*** *00 800 00 24 67 23*
Singapore *65 6415 3188* ***South Africa*** *00 800 00 24 67 23*
Spain *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189*
Thailand *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150*
United Kingdom *00 800 00 24 67 23*

D052339 Ver D (10016027) US/EG *24-0298 0524 Sig 0124*