Sequencing Gel Casters

Models SGC-1 and SGC-2 Operating and Maintenance Manual 7218313



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Important Read this instruction manual. Failure to read, understand and follow the instructions in this manual may result in damage to the unit, injury to operating personnel, and poor equipment performance.

Caution All internal adjustments and maintenance must be performed by qualified service personnel. ▲

<u>Statement of Proper Use:</u> Use this product only for its intended purpose as described in this manual. Do not use this product if the power leads are damaged or if any of its surfaces are cracked.

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Potential electrical hazards. Only qualified persons should perform procedures associated with this symbol.



Equipment being maintained or serviced must be turned off and locked off to prevent possible injury.



Hot surface(s) present which may cause burns to unprotected skin, or to materials which may be damaged by elevated temperatures.



Marking of electrical and electronic equipment, which applies to electrical and electronic equipment falling under the Directive 2002/96/EC (WEEE) and the equipment that has been put on the market after 13 August 2005.

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. It is marked with the WEEE symbol. Thermo Fisher Scientific has contracted with one or more recycling/disposal companies in each EU Member State European Country, and this product should be disposed of or recycled through them. Further information on Thermo's compliance with this directive, the recyclers in your country and information on Thermo products will be available at www.thermofisher.com.

- ✓ Always use the proper protective equipment (clothing, gloves, goggles, etc.)
- ✓ Always dissipate extreme cold or heat and wear protective clothing.
- ✔ Always follow good hygiene practices.
- ✓ Each individual is responsible for his or her own safety.

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Our **Sales Support** staff can provide information on pricing and give you quotations. We can take your order and provide delivery information on major equipment items or make arrangements to have your local sales representative contact you. Our products are listed on the Internet and we can be contacted through our Internet home page.

Our **Service Support** staff can supply technical information about proper setup, operation or troubleshooting of your equipment. We can fill your needs for spare or replacement parts or provide you with on-site service. We can also provide you with a quotation on our Extended Warranty for your Thermo Scientific products.

Whatever Thermo Scientific products you need or use, we will be happy to discuss your applications. If you are experiencing technical problems, working together, we will help you locate the problem and, chances are, correct it yourself...over the telephone without a service call.

When more extensive service is necessary, we will assist you with direct factory trained technicians or a qualified service organization for on-the-spot repair. If your service need is covered by the warranty, we will arrange for the unit to be repaired at our expense and to your satisfaction.

Regardless of your needs, our professional telephone technicians are available to assist you Monday through Friday from 8:00 a.m. to 6:00 p.m. Eastern Time. Please contact us by telephone or fax. If you wish to write, our mailing address is:

> Thermo Fisher Scientific 401 Millcreek Road, Box 649 Marietta, OH 45750

International customers, please contact your local Thermo Scientific distributor.

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Section 1 General Information

The Otter Sequencing Gel Caster provides the user with a simple and easyto-use method for casting sequencing gels. Relying on a horizontal sliding plate procedure, capillary action draws the gel between plates. Surface tension, not tape, keeps the gel from leaking. No taping or special assembly is required. The sliding casting method has been shown to require significantly less time to cast and the clean process decreases exposure to acrylamide and reduces clean up time. Gels can be cast in less than one minute. Any standard size sequencing gel can be cast using either the SGC-1 or SGC-2.

SGC-1

Gel Size: 20-42cm W x 48cm L Footprint: 21.6cm W x 71cm D (fully closed) x 12.2cm H

SGC-2

Gel Size: 20-42cm W x 65.5cm L Footprint: 21.2cm W x 102cm D (fully closed) x 12.2cm H

Unpack and Check Your Order

Before starting, unpack the unit and inventory your order. If any parts are missing, contact Technical Services immediately. Reference the order or catalog number on your invoice and check the corresponding parts list.





Section 2 Using the System

The following additional equipment may be required for casting.

- One or more pairs of glass plates to fit any standard manual or automatic sequencing electrophoresis system.
- Matching spacer set and comb for each gel to be cast.
- Binder clamps 9 per gel cassette. Owl catalog # CL-12.
- Rain-X or Sigmacote (Sigma Chemical, St. Louis, MO)
- Ethanol for washing plates, combs and spacers.

Gel Casting Basic laboratory safety procedures should be followed when preparing sequencing gels. When working with acrylamide solutions, always wear proper protective clothing, gloves and eye protection.

Careful attention must be given during each step of sequencing gel preparation for consistent results. Begin by cleaning plates thoroughly:

- 1. To completely clean glass plates of any acrylamide residue, leftover detergents, and dust, wash plates with warm water and detergent.
- 2. Rinse plates completely with deionized water then wipe with 95% ethanol to eliminate any traces of detergent and water marks. A final wipe should be done using a dust free cloth such as Kimwipes.

Operating

1. The caster consists of two adjustable ends and two side pieces with a cut out railing. The railing is open at one end so that long glass can slide past the length of the caster. The opposite end has a stop cut into the sides so that the bottom plate is held in position. Loosen knobs on the ends of the caster and place glass into unit. Slide the sides of the caster together to tightly fit the glass plate. Tighten knobs.



Figure 2-1. Knob Location

- 2. Making sure that the casting area is level, place the blank, longer glass plate into the caster, pushing it to the end with the stops. The rail it is sitting on is slightly lower than the rail on the other end of the unit.
- 3. Moisten spacers with drops of water and place them flush to the edge of the glass against the side of the rail. Moistening helps to keep the spacers from moving while sliding the notched plate over them during casting.
- 4. Place the notched top plate on top of the blank plate so that the two overlap by about one inch. The side rail will hold the top plate above the bottom blank plate. This plate should slide freely up to the top of the blank plate. Have your gel solution ready at this point.
- 5. Slowly pour gel solution onto the bottom plate at the point where the two plates overlap. The acrylamide will flow into the space between the two plates by capillary action. Gently slide the top plate across the bottom plate while pouring gel solution along the leading edge of the glass. If a bubble forms, simply move the top plate backwards until you have passed the point where the bubble formed and then proceed



Figure 2-2. Pour Gel Solution

forward again until the gel is completely cast.

Section 2 Using the System

Operating (continued)

- 6. Clamp along the spacers in the spaces provided by the caster. Clamp in the middle of the spacer and not in the gel area.
- 7. Insert comb (see page 9 for Sharktooth and Well Comb instructions) at the top of the gel and clamp with two or three clamps as space allows. Clamp in the middle of the comb and not on the teeth or beyond the comb into the gel area.



Section 3 Technical Tips

The difference between a standard well comb and a sharktooth comb:

A well comb has rectangular teeth and is similar to combs used for protein gels. After polymerization, these combs must be removed carefully to avoid destroying the acrylamide walls that create the wells. A sharktooth comb has pointed teeth. It is placed with the flat side down during gel casting to create a flat surface on the top of the gel and





reinserted with the teeth down towards the gel for loading. Samples are loaded in the space between the teeth.

Sharktooth comb: Carefully insert the flat edge of the sharktooth comb between the plates to a depth of 2 to 3mm below the shorter plate. If the flat edge of the comb is inserted too deeply (more than 4 or 5mm) during gel casting, the resulting trough will be too deep to load samples easily with a pipet. It is wise to make marks on the comb to distinguish the right/left sides so the comb can be put back in the same orientation for sample loading later, in the event that the material has any slight variation in thickness. Place binder clamps over the comb and glass plates along the upper edge to force the glass plates against the comb. This will ensure a tight fit of the comb during and after polymerization. If top binder clamps are not used, the comb may not be tight enough to prevent sample leaks between wells following gel polymerization. Do not clamp the outer edges over the side spacers. Allow the gel to completely polymerize.

After polymerization, wash out the trough with buffer gently in order to remove any unpolymerized acrylamide and excess urea. Place the comb between the glass plates in the correct left to right orientation (the same orientation the trough was cast in) with the comb teeth pointing down toward the gel. Slowly and carefully slide the comb until it just makes contact with the gel surface, without piercing it. Well comb: Carefully insert the comb. Place binder clamps over the comb and glass plates along the upper edge to force the glass plates against the comb. After polymerization, flood the comb with 1X TBE and remove gently. Rinse the wells out with deionized water or TBE in order to remove excess urea or unpolymerized acrylamide.

The difference between notched and offset glass:

Notched or eared glass plates face in towards the upper buffer chamber during a gel run. The "ears" are glass tabs on each side of the plate, which prevent buffer from running out of the upper buffer chamber.

Offset glass plates serve the same purpose as notched plates. Offset glass is usually about 2 cm shorter than the front, or blank, glass plate. Instead of glass ears, pieces of adhesive sponge are adhered either to the top of the spacers or to the apparatus itself. These sponge tips wear out and may leak. Notched glass plates are more fragile and expensive than offset glass and sponge tips, but many researchers prefer notched glass because it is easier to use.

The third spacer: This is a bottom spacer, and can be cut to the desired length. It is optional. If you use a bottom spacer when casting, remember to remove it before running the gel.







OFFSET

Figure 3-2. Glass



Figure 3-3. Spacer

3-2 Sequencing Gel Caster

Section 4 Troubleshooting

Problem

Bubbles continually form between glass plates while casting.

Solution

Be sure plates are clean of any acrylamide debris and are dust free. If bubbles do occur even after cleaning plates, simply move the top plate backwards until the bubble is cleared and then proceed forward. From time to time a more thorough cleaning of plates is required: 5 minutes in 1N HCl, rinse in hot water, then rinse in deionized water. Repeat if necessary.

Problem

Gel solution appears to be leaking while pouring.

Solution

Be sure the caster is set up on a level surface. Leveling platforms are available from Owl, catalog # B-LP.

If gel will not stay between the plates at the bottom of the cassette, this may be caused by bowed plates. Try another set of glass or flip the top piece so that the other side is facing the gel solution. Dirty glass can also cause slight leaking. See cleaning instructions above and in the Gel Casting section of this manual.

Problem

When loading samples, the samples seem to be running into other well

Solution

This may be due to a slight thickness variation between the comb and the spacers used to pour the gel. Clamping the plates over the comb as well as over the spacers will allow the gel to polymerize evenly and should alleviate this problem. Using glass plates that are not completely flat may also cause this problem, try flipping the plate over and using the other side for casting or try a new plate.

Section 5 Care and Cleaning

Caution Organic solvents cause acrylic to "craze" or crack. Clean all acrylic systems with warm water and a mild detergent. Do not use ethanol or other organic solvents to clean these products. Do not autoclave, bake, or microwave your unit. Temperatures over 50°C can damage the acrylic. ▲

Note If an RNase free electrophoresis system is desired, there are various methods to rid the system of RNA contamination. For fast and easy decontamination, use RNase AWAY^{®*}. Spray, wipe or soak labware with RNase Away, then wipe or rinse the surface clean; it instantly eliminates RNase. RNase Away eliminates the old methods that include treatment with 0.1% Diethyl Pyrocarbonate (DEPC) treated water and soaking in dilute bleach. DEPC is suspected to be a carcinogen and should be handled with care. This electrophoresis system should never be autoclaved, baked, or placed in a microwave.

To order RNase AWAY, contact Technical Services:

Number
1 liter bottle

*Rnase AWAY[®] is a registered trademark of Molecular BioProducts

Care of Acrylic

The following chemical compatibility chart is supplied for the convenience of our customers. Although acrylic is compatible with most solvents and solutions found in the biochemical laboratory, some solvents can cause substantial damage. Keep this chart handy to avoid harm to your apparatus by the use of an inappropriate solvent.

Codes:

- S Safe (no effect, except possibly some staining)
- A Attacked (slight attack by, or absorption of, the liquid)
 - (slight crazing or swelling, but acrylic has retained most of its strength)
- U Unsatisfactory (softened, swollen, slowly dissolved)
- D Dissolved (in seven days, or less)

Chemical	Code	Chemical	Code	Chemical	Code
Acetic acid (5%)	S	Ethyl alcohol (50%)	А	Naptha	S
Acetic acid (Glacial)	D	Ethyl alcohol (95%)	U	Nitric acid (10%)	S
Acetic Anhydride	А	Ethylene dichloride	D	Nitric acid (40%)	А
Acetone	D	Ethylene glycol	S	Nitric acid concentrate	U
Ammonia	S	2-Ethylhexyl Sebacate	S	Oleic acid	S
Ammonium Chloride (saturated)	S	Formaldehyde (40%)	S	Olive oil	S
Ammonium Hydroxide (10%)	S	Gasoline, regular, leaded	S	Phenol 5% solution	U
Hydroxide (10%)	S	Glycerine Heptane (commercial grade)	S	Soap solution (Ivory)	S
Ammonium Hydroxide concentrate	S	Hexane	S	Sodium carbonate (2%)	S
Aniline	D	Hydrochloric acid (10%)	S	Sodium carbonate (20%)	S
Benzene	D	Hydrochloric acid concentrate	S	Sodium chloride (10%)	S
Butyl Acetate	D	Hydro uoric acid (40%)	U	Sodium hydroxide (1%)	S
Calcium chloride (saturated)	S	Hydrogen peroxide (3% solution)	S	Sodium hydroxide (10%)	S
Carbon tetrachloride	U	Hydrogen peroxide (28% solution)	U	Sodium hydroxide (60%)	S
Chloroform	D	Isooctane	S	Sodium hydrochlorite (5%)	S
Chromic acid (40%)	U	Isopropyl alcohol (100%)	А	Sulfuric acid (3%)	S
Citric acid (10%)	S	Kerosene (no. 2 fuel oil)	S	Sulfuric acid (30%)	S
Cottonseed oil (edible)	S	Lacquer thinner	D	Sulfuric acid concentrate	U
Detergent Solution (Heavy Duty)	S	Methyl alcohol (50%)	А	Toluene	D
Diesel oil	S	Methyl alcohol (100%)	U	Trichloroethylene	D
Diethyl ether	U	Methyl Ethyl Ketone	U	Turpentine	S
Dimethyl formamide	U	Methylene chloride	D	Water (distilled)	S
Dioctyl phthalate	А	Mineral oil (white)	S	Xylene	D
Ethyl acetate	D				

	Table 5-1.	Chemical	Compatibili	ty for Aci	rylic-Based	Products
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This list does not include all possible chemical incompatibilities and safe compounds. Acrylic products should be cleaned with warm water, a mild detergent such as Alconox[™], and can also be exposed to a mild bleach solution (10:1). In addition, RNAse removal products are also safe for acrylic. Contact Technical Services with any questions.

Section 6 Optional Equipment

Contact Technical Services to order replacement parts.

Optional Accessories	Catalog No.	
Binder Clamps (pkg of 12)	CL-12	
Stainless Steel Clamps (pkg o	f 12)	CL-12S
Glass Plate Dimensions	Туре	Catalog No.
20cmW x 45cmL x 3/16"T	Notched Glass	S1S-45R
	Blank Glass	S1S-45G
20cmW x 43cmL x 3/16"T	Offset Glass	S1S-43G
35cmW x 45cmL x 3/16"T	Notched Glass	S2S-45R
	Blank Glass	S2S-45G
35cmW x 43cmL x 3/16"T	Offset Glass	S2S-43G
35cmW x 65cmL x 3/16"T	Notched Glass	S2SL-65R
	Blank Glass	S2SL-65G
35cmW x 63cmL x 3/16"T	Offset Glass	S2SL-63G
Spacers (set includes 2 sid	e and 1 bottom)	Catalog No.
45cmL	1.0cmW x 0.4mm thick	S2S-SA4
	1.0cmW x 0.2mm thick	S2S-SA2
65cmL	1.0cmW x 0.4mm thick	S2SL-SA4
	1.0cmW x 0.2mm thick	S2SL-SA2

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