Instructions for use

Gel Multi Caster ROTIPHORESE®

12 x (for 12 gels) 3118.1

PACKING LIST

Art. No.	Bottom Gasket for Top Filling	Leveling Bubble	Clip	12 mm Saver Plate	5 mm Saver Plate	3 mm Saver Plate	Separation Sheets
3118.1	1	1	1	1	5	3	12

Please verify that you received the unit completely and without any damage. Any faults or losses have to be reported to ROTH immediately. ROTH can not accept responsibility for goods that were sent back without informing them.
Please take a look at the packing list and check whether all components and accessories are present.

CARE AND MAINTENANCE

Cleaning Gel Multi Caster Unit
Unit is best cleaned using warm water and a mild detergent.
Please note: Water at temperatures above 60 °C can cause damage to the unit and components.
The tank should be thoroughly rinsed with warm water or distilled water to prevent build up of salts.
Vigorous cleaning is not necessary or advised. Air drying is preferably before use.

The unit should only be cleaned with the following:
Warm water with a mild concentration of soap or other mild detergent. Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons. The unit should not be left in detergents for more than 30 minutes.
Please note: The unit should never come into contact with the following cleaning agents; these will cause irreversible and accumulative damage:
Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol, Isobutanol or any other alcohol. Alkalis.

RNase Decontamination
Clean the unit with a mild detergent as described above.
Wash with 3% hydrogen peroxide (H_2O_2) for 10 minutes.
Rinse with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,
Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using.
RNaseZAP™ (Ambion) can also be used. Please consult the instruction for use with acrylic gel tanks.

OPERATION

A. Top Filling – Standard Single Percentage Gels

Note: Use only glass plate sandwiches consisting of one notched glass plate and one plain glass plate with bonded spacers.

1. Use the levelling bubble provided to ensure that the surface is level upon which the caster to be set up.
2. Loosen the clamp screws, remove the caster door, and place the open casting chamber on the bench top so that the centre of the casting chamber is facing upwards.
   Please note: Make sure that the white silicone gasket, supplied for top filling, is in the bottom of the casting chamber. Ensure that the flat surface is pointing upwards. The V-shaped ridge on the underside of the white silicone gasket should also fit snugly in the V-shaped channel within the casting chamber.
3. Insert the glass and spacer plates as follows:
   There should be a separation sheet at the rear of the casting chamber followed by a glass plate sandwich (1 mm spacer). This combination of separation sheet and glass plate sandwich is repeated a further eleven times.
   Then a 5 mm spacer plate is inserted followed by a 3 mm spacer plate and a further two separation sheets. At this point the glass and spacer plate stack should be flush with the edges of the casting chamber.
4. Overlay the caster door and secure it to the casting chamber by tightening the screws.
   The front gasket should flatten all the way around which indicates a good seal.
5. Restore the casting chamber to its upright position without dislodging the glass plates. If, by tightening the screws, the glass plates have been pushed upwards push them downwards to ensure that they retain contact with the silicone mat. The spare 5 mm spacer plate may be used to push each glass plate sandwich back into position.
6. Make sure that the clip provided is used to seal the silicone hose at the front.
7. Pour pre-prepared acrylamide solution (including APS and TEMED initiators) into the top of the glass plate sandwich closest to the front of the casting chamber (i.e. in the cavity between the plain glass plate with bonded spacers and the notched glass plate). This should be performed evenly and smoothly to allow the gel level to equilibrate.
   It is much easier if the required volume has been determined previously and prepared accordingly (e.g. by filling the assembled gel caster - containing glass plates, spacer plates and separation sheets - with water and noting the volume required).
   If this is not the case, then fill the middle plates and look for when the end plates reach the desired level.
   Please note: The purpose of the white silicone mat is not to stop leakage as in a standard casting base, but to provide even gel equilibration and distribution between the individual gel sandwiches, in addition to minimizing the volume of acrylamide required. The acrylamide will migrate by capillary action from the front gel sandwich to the other gel sandwiches in the caster. This is entirely normal, and necessary to fill all of the sandwiches evenly.
   Please note: Once the acrylamide has set the separation sheets may be used to pry apart the individual gel sandwiches as described at the end of this protocol.
8. The gel will have to be allowed to set and a suitable overlay (e.g. distilled water) applied if a stacking layer is to be poured above the resolving layer.
9. Insert the combs and allow the gels to set.

OPERATION

B. Bottom Filling – Gradient Gels

Note: Use only glass plate sandwiches consisting of one notched glass plate and one plain glass plate with bonded spacers.

1. Use the levelling bubble provided to ensure that the surface is level upon which the caster to be set up.
2. Loosen the clamp screws, remove the caster door, and place the open casting chamber on the bench top so that the centre of the casting chamber is facing upwards.
   Please note: Remove the large bottom white gasket for top-filling, to reveal a V-shaped channel. This channel is necessary for acrylamide gradient equilibration when the gel is poured.
3. Insert the glass and spacer plates as follows:
   There should be a separation sheet at the rear of the casting chamber followed by a glass plate sandwich (1 mm spacer). This combination of separation sheet and glass plate sandwich is repeated a further eleven times.
   Then a 5 mm spacer plate is inserted followed by a 3 mm spacer plate and a further two separation sheets. At this point the glass and spacer plate stack should be flush with the edges of the casting chamber.
4. Overlay the caster door and secure it to the casting chamber by tightening the screws.
   The Front gasket should flatten all the way around which indicates a good seal.
5. Restore the casting chamber to its upright position without dislodging the glass plates. If, by tightening the screws, the glass plates have been pushed upwards push them downwards to ensure that they retain contact with the silicone mat. The spare 5 mm spacer plate may be used to push each glass plate sandwich back into position.
6. The first time a certain number of gels of a certain thickness are cast, it is necessary to determine the required volume of acrylamide so as to ensure a complete gradient. This can be done by calculating the volume of gel in each plate and multiplying by the number used. There is also 8 ml of gel which will not fill the glass plates. This will fill the bottom gap and is necessary for even filling of the plates. This should be taken into consideration and added to the lowest gradient.
7. Place the Gradient Mixer (Art. Nr. N857.1 / N858.1) on a magnetic stirring plate and attach the outlet to the port on the Gel Multi Caster. Ensure the outlet valve on the Gradient Mixer is closed.

The following steps need to be performed, smoothly sequentially and rapidly to ensure correct and complete pouring. It is usually necessary to practice these steps beforehand to ensure that the polymerization time of the gel is not exceeded by the pouring time. If this is the case the initiators can be adjusted to allow polymerization over a longer time period.

8. Determine the volume of both high and low concentrations of gel necessary and immediately prior to inserting, add APS and TEMED. Then quickly pour the solutions into the correct chambers of the Gradient Mixer as per the manufacturer's instructions.
9. Turn on the stirring bar in the mixing chamber of the Gradient Mixer, open the tubing clamp of the Gradient Mixer and allow the Gel Multi Caster to fill. This may need a peristaltic pump if the flow is not fast enough.
10. Insert the combs and allow the gels to set.

REMOVAL AND STORAGE OF GELS

Note: Wear rubber gloves while performing the following procedure to prevent accidental exposure to unpolymerized acrylamide.

1. Unscrew the clamp screws and remove the front plate.
2. Remove the gels one at a time front first from the stack. Carefully separate the gel sandwiches from the separation sheets and any acrylic blocks that were used as space-fillers.
3. Rinse off the tops of all the gels thoroughly with distilled water.
4. Store the gels upright in a tightly sealed container. Add a few millilitres of 1X gel buffer (identical to the buffer in the gel) to the bottom of the container and to the tops of the gels to prevent them from drying out.
5. Store tightly sealed at 4 °C.
6. Clean the entire casting chamber thoroughly with distilled water. Residual acrylamide in the stopcock valve and inlet port can be removed using a paper clip or a syringe needle.

ACCESSORIES

Gradient Mixer, MINI	N857.1
Gradient Mixer, MIDI	N858.1

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