

Hoefer SG15, SG30, SG50 and SG100

gradient makers





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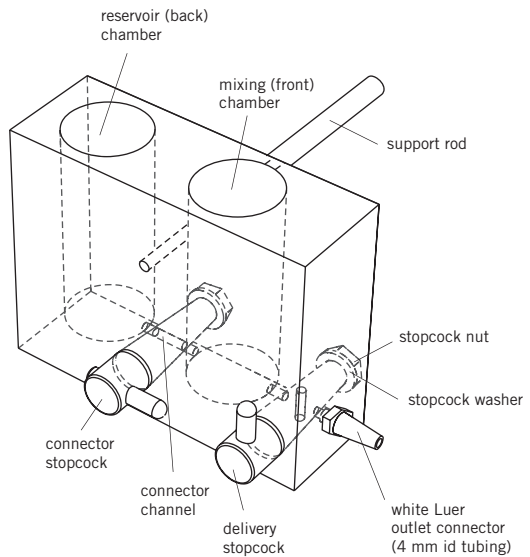
Introduction: Hoefer SG15, SG30, SG50, and SG100 gradient makers

The Hoefer® SG gradient makers are designed for producing linear gradients of aqueous solutions ranging in volume from 15–100 ml. The gradient makers are machined from solid blocks of acrylic plastic. They are suitable for casting acrylamide pore gradient gels, casting immobilized pH gradient gels, pouring density gradients for centrifugal sedimentation separations and delivering salt gradients for low pressure chromatography systems.

Fig 1. Hoefer SG gradient maker.

Included but not shown:

- Adaptor barbed fitting (1)
- 22-gauge needle (1)



The gradient makers have a white Luer outlet fitting that can take either tubing with female Luer connectors or tubing of 4 mm inside diameter (ID). Also included with the gradient maker is a support rod (that can be screwed into the body of the gradient maker, allowing it to be clamped onto a vertical stand), an adapter barbed fitting (to convert the end of a 2 mm ID tubing into a female Luer connector) and a 22-gauge needle (that can be attached to the white Luer outlet fitting so that it can accept 22-gauge tubing).

1. Generating linear gradients

To generate a linear gradient between two concentrations, equal volumes of solutions of the two concentrations are measured into the two chambers of the gradient maker. As solution is delivered out of the mixing chamber, an equal volume flows in from the reservoir chamber where it is rapidly diluted and mixed to uniformity by a magnetic stir bar. The initial concentration delivered will be that of the solution in the mixing chamber, the final concentration will be that of the reservoir chamber. For the most consistent delivery of gradients, a peristaltic pump is recommended.

Table 1. Operating ranges for linear gradients

model	max total vol (ml)	min vol/chamber (ml)	max stir bar length (mm)
SG15	15	3	12
SG30	30	5	15
SG50	50	8	15
SG100	100	16	20



1

Make sure all parts are clean and liquid flows freely through all channels, stopcocks and tubing.

2

Add a magnetic stir bar of the appropriate dimensions to the mixing chamber (Table 1) and place the unit on a magnetic stirrer. If volumes will be less than half the capacity of the unit, an identical stir bar should be placed in the reservoir chamber as well to balance the displacement and prevent backflow into the reservoir when the chambers are first connected. Connect tubing to the outlet connector and pump, and adjust pump speed, if used. Position or connect the tubing to the receiving vessel (gel casting unit, centrifuge tube, etc.)

3

Close both stopcocks (handles up) and add the required volume of the final solution to the reservoir (back) chamber.

4

Carefully open the connector stopcock and allow just enough solution to flow through the connector channel to fill it to the edge of the mixing chamber, then close the stopcock. Be sure no large bubbles remain to obstruct flow through the channel.

5

Add the required volume of the starting solution to the mixing chamber and start the magnetic stirrer.

6

Open the delivery stopcock.

7

Simultaneously open the connector stopcock and start the pump.

Note: If there is a substantial difference in densities between the two solutions, there will be a sudden flow from the denser chamber to the lighter chamber to bring the two into hydrostatic balance. This will result in the gradient not being fully linear. To avoid this, add equal *weights*, rather than volumes, of the solutions to the appropriate chambers.



8

If it is important that no bubbles disturb the gradient, watch the delivery carefully and as soon as the last of the solution has entered the pump head, stop the pump and remove the tubing from the receiving container.

9

Flush and rinse all parts thoroughly with distilled water after use.

1.1 Pouring gradients from the top

Filling a container with a gradient (*e.g.* casting acrylamide pore gradient gels) can be done either dense solution first (“from the top”), or light solution first (“from the bottom”).

To fill from the top:

1

Proceed as described above, putting light (final or top) solution in the reservoir chamber (step 3) and dense solution in the mixing chamber (step 5).

2

Place the delivery outlet against the upper edge of the receiving container. Adjust the pump rate so that the solution flows evenly down the side in a smooth, continuous stream. The delivery speed should be slow enough that the newly arriving solution does not mix with the underlying solution. Alternatively, using a rigid cannula at the end of the delivery tubing, hold the tip of the cannula just above the surface of the solution, raising it smoothly as the container fills.

1.2 Pouring gradients from the bottom

This technique is commonly used in filling multiple gel casting chambers.

1

Proceed as described on page 4, putting dense (final or bottom) solution in the reservoir chamber (step 3) and light solution in the mixing chamber (step 5).

2

Connect the delivery tubing to the bottom inlet of a gel casting unit or to a cannula long enough to reach the bottom of the receiving container. Adjust the pump rate so that the solution is not forced up in a “fountain” that mixes with the overlying solution.

3

If all of the gradient solution must be delivered to the container, a displacement solution may be used. Just as the last of the gradient mix is pumped out of the mixing chamber, and before any air enters the tubing, add an appropriate volume of a denser displacement solution to the mixing chamber and pump it through until all of the gradient mix has been delivered. It is convenient to include a dye in the displacement solution to visually track the boundary between the gradient mix and the displacement solution.

2. Care and maintenance

The gradient maker should be cleaned thoroughly with distilled water after use to prevent polymerization or crystallization of solutions in the chambers and stopcocks. Do not use abrasives, acetone, pure alcohols or organic solvents to clean this unit.

3. Ordering information

	qty.	product no.
SG15 gradient maker, 15 ml total volume	1	SG15
SG30 gradient maker, 30 ml total volume	1	SG30
SG50 gradient maker, 50 ml total volume	1	SG50
SG100 gradient maker, 100 ml total volume	1	SG100
White Luer outlet fitting, (4 mm) SG15, SG30, SG50, SG100	1	SG100-6
Stopcock, standard size, for SG15, SG30, SG50	1	SG100-1
Long stopcock, for SG100, connector stopcock	1	SG100-9
Short stopcock, for SG100, delivery stopcock	1	SG100-10
SG500 gradient maker, 500 ml total volume	1	SG500



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