

GRADIENT GELS FOR SDS-PAGE

OVERVIEW

This protocol is for pouring 4-10% gradient SDS-PAGE gels with the Hoefer Multi-Gel system. If a different range of gel concentration is needed the stock solutions should be adjusted accordingly. See notes on pouring standard SDS-PAGE gels for additional information. **Remember:** acrylamide is neurotoxic! Wear gloves, work on a blue pad, and use disposable tubes and pipets.

REFERENCES

- Amersham Biosciences Hoefer system information

MATERIALS

- 12 sets of front glass and back white plates, 24 spacers and 12 18-space combs
- buffers:
 - Separating gel buffer: 1.5 M tris (Trizma base), 0.4% SDS, pH 8.8
 - Stacking gel buffer: 0.5 M tris, 0.4% SDS, pH 6.8
 - 10 % ammonium persulfate (APS); fresh or from a frozen aliquot
 - Electrophoresis buffer: 0.3% Tris, 1.5% glycine, 0.1% SDS, pH 8.3
 - 50% sucrose, colored for final plug solution

METHODS

1. Scour plates with detergent, rinse well, rinse with acetone and dry.
2. Set up gradient system
 - Assemble multi-caster with plates (do not use the rubber plug in the bottom and open the filling tube), mark height of separating gel.
 - Elevate gradient apparatus and set up stirrer height
 - Connect tubing from gradient apparatus to multi-caster, through a pump if desired
 - Run water through the apparatus to test flow between chambers and no leaks (important), then remove water, turn stopcock to close opening between two chambers (lever vertical = closed)
 - Attach a 3-way valve to caster to push 50% sucrose in quickly
3. For 80 ml multi-caster system and 4-10% gels, combine the following in 50-ml conical tubes and invert a few times to mix well:

Separating gel	4% acrylamide (light)	10% acrylamide
Acrylamide (30%)	5.3 ml	13.5 ml
Water	24 ml	16.4 ml
Separating gel buffer	10 ml	10 ml (+ 6g sucrose to help with gradient mixing)
10% APS (last thing)	200 μ l	(all in light, since stirring)
TEMED (last thing)	100 μ l	(all in light, since stirring)

4. Place the lighter solution (here 4%) in the caster chamber that is closer to the outlet port.
5. Prime the valve by opening the connection between chambers and letting a small amount of light solution go through. Then close, transfer whatever ran through back, and put the heavy solution in the empty chamber.
6. With everything in place, begin stirring the light (mixing) chamber, open gradient valve, and begin pump. Allow the line to fill before attaching to multi-caster. Attach so the gel pouring apparatus fills from the bottom up, using the metal inlet.
7. When the acrylamide solutions are almost used up, move the tubing to colored 50% sucrose so this enters the casting chamber to plug the bottom and move the acrylamide to the desired height in the gels. When this is done clamp the tubing.
8. Run buffered n-butanol (100 μ l) gently into the top of each gel in multi-caster to cover the surface.
9. Draw up any remaining acrylamide into a Pasteur pipet to allow easy testing of when gel has solidified.
10. Thoroughly wash pump and gradient lines, and apparatus.
11. Once gels are solidified pour off the butanol, rinse tops of gels with distilled water, drain off (can dry by inserting filter paper). Then mix up stacker:

Separating gel	4% acrylamide
Acrylamide (30%)	5.3 ml
Water	24 ml
Separating gel buffer	10 ml
10% APS (last thing)	100 μ l
TEMED (last thing)	50 μ l

12. Working from the back, immediately insert all the combs. Helps to insert tips, bend top back slightly, then carefully slide in. Wear safety glasses (and gloves, of course), it can squirt!
13. When polymerized remove from casting system by inserting a razor blade or spatula in back of the stack and gently prying out. Wrap well in a moist paper towel and saran wrap, and mark with your initials, the % range of the gel, and the date.