Handcasting Polyacrylamide Gels

Protocol

Bulletin 6201

Handcasting Polyacrylamide Gels

Single-Percentage Gels

1 Prepare the resolving and stacking gel solutions without APS or TEMED. (Tables 1 and 2, consult the instruction manual for the system you are using for more details.)

Table 1. Volume of resolving gel solution required to fill a gel cassette. Volumes listed are required to completely fill a gel cassette. Amounts may be adjusted depending on the application (with or without comb, with or without stacking gel, etc.).

Spacer (Gel Thickness)	Mini-PROTEAN®* Cell	Criterion™ Cell	PROTEAN II xi Cell	
			16 cm	20 cm
0.5 mm	—	—	12.8 ml	16.0 ml
0.75 mm	4.2 ml	—	19.2 ml	24.0 ml
1.0 mm	5.6 ml	15.0 ml	25.6 ml	32.0 ml
1.5 mm	8.4 ml	—	38.4 ml	48.0 ml
3.0 mm	—	—	76.8 ml	96.0 ml

* 10 ml of monomer solution is sufficient for two stacking gels of any thickness.

Table 2. Recipes for stacking and resolving gels. Adjust amounts as needed for the format you are using (see Table 1).

	Stacking Gel	Resolving Gel		
	4%	7.5%	12%	X%
30% Acrylamide/bis	1.98 ml	3.75 ml	6.0 ml	0.5 × X ml
0.5M Tris-HCl, pH 6.8	3.78 ml	—	—	—
1.5M Tris-HCl, pH 8.8	—	3.75 ml	3.75 ml	3.75 ml
10% SDS	150 µl	150 µl	150 µl	150 µl
diH_2O	9 ml	7.28 ml	5.03 ml	11.03–(0.5 × X) ml
TEMED	15 µl	7.5 µl	7.5 µl	7.5 µl
10% APS	75 µl	75 µl	75 µl	75 µl
Total Volume	15 ml	15 ml	15 ml	15 ml

2 Degas the solution under a vacuum for at least 15 min. While solutions are degassing, assemble the glass cassette sandwich.

3 Place a comb into the assembled gel sandwich. With a marker, place a mark on the glass plate 1 cm below the teeth of the comb. This will be the level to which the separating gel is poured. Remove the comb.

GENERAL TIPS FOR HANDCASTING

Acrylamide and bisacrylamide are neurotoxins when in solution. Avoid direct contact with the solutions and clean up spills.

For casting multiple gels, use the Mini-PROTEAN 3 Multi-Casting Chamber (catalog #165-4110), PROTEAN II xi Multi-Gel Casting Chamber (catalog #165-2025), or PROTEAN Plus Multi-Casting Chamber (catalog #165-4160).

Use only high-quality reagents, especially acrylamide monomers, to avoid polymerization problems.

Proper degassing and filtering of the casting solution is critical for both reproducibility of the polymerization (oxygen removal) and the avoidance of problems related to mass spectrometry (keratin contamination).

A temperature of 23–25°C is best for degassing and polymerization; equilibrate the stock solutions to room temperature.

APS/TEMED-initiated reactions should proceed for at least 2 hr to ensure maximum reproducibility of pore size.

Make fresh APS solution every day for best performance.

Replace TEMED every three months because it is subject to oxidation, which causes the gradual loss of catalytic activity.

The glass plates must be clean and free of chips. Clean glass plates with ethanol and lint-free cloths before use.

The height of the stacking gel should be at least 2x the height of the sample in the well. This ensures band sharpness, even for diluted protein samples.

Store gels flat in the fridge at 4°C. Do not freeze. Wrap handcast gels tightly in plastic wrap with combs still inserted.

Run handcast gels with discontinuous buffer systems right after gel casting because the buffer discontinuity (pH and ionic strength) gradually disappear during storage. SDS-PAGE gels are not stable at pH 8.8 over a longer time period.

For more information about acrylamide polymerization, refer to Acrylamide Polymerization – a Practical Approach, Bio-Rad bulletin 1156.

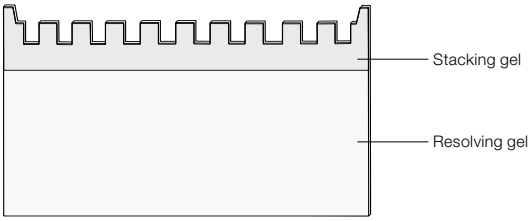
BIO-RAD

Pour the Resolving Gel

1. Add the APS and TEMED to the degassed resolving gel solution, and pour the solution to the mark, using a glass pipet and bulb.
2. Using a Pasteur pipet and bulb, immediately overlay the monomer solution with water-saturated *n*-butanol.
3. Allow the gel to polymerize 45–60 min. The gel is polymerized once you see a line form between the stacking and the resolving gel. Pour off the overlay solution and rinse the top of the gel with diH_2O.

Alternative Casting Procedure

It is possible to cast separation and stacking gels one after another, with no intermediate step requiring overlay solution (water-saturated n-butanol). Recalculate your gel casting recipes so that the separation gel solution contains 25% (w/v) glycerol. Due to the significant difference in density, the two solutions won't mix when the stacking gel solution is carefully poured on top of the resolving gel solution.



Pour the Stacking Gel

1. Dry the area above the separating gel with filter paper before pouring the stacking gel.
2. Place the comb in the cassette and tilt it so that the teeth are at a slight (~10°) angle. This prevents air from becoming trapped under the comb while the acrylamide solution is being poured.
3. Add the APS and TEMED to the degassed resolving gel solution, and pour the solution down the spacer nearest the upturned side of the comb. Pour until all the teeth are covered by the solution.
4. Realign the comb in the sandwich and add monomer to fill the cassette completely. An overlay solution is not necessary for polymerization when a comb is in place.
5. Allow the gel to polymerize 30–45 min.
6. Remove the comb by pulling it straight up slowly and gently. Rinse the wells completely with diH_2O.

TIPS

When pouring the resolving gel solution, pour the solution down the middle of the outside plate of the gel sandwich or down the side of one of the spacers. Pour smoothly to prevent it from mixing with air.

For the overlay solution, water, n-butanol, or t-amyl alcohol can also be used. With n-butanol or t-amyl alcohol, the overlay solution can be applied rapidly because very little mixing will occur. If using water to overlay, use a needle and syringe and a steady, even rate of delivery to prevent mixing.

Do not allow alcohols to remain on the gels for more than 1 hr or dehydration of the top of the gel will occur. It is sometimes convenient to cast the separating portion of the discontinuous gel the afternoon before casting the stacking gel and running the gel. If the stacking gel is to be cast the following day, place approximately 5 ml of 1:4 diluted running gel buffer on top of each separating gel after rinsing with deionized water to prevent dehydration of the separating gel.

Casting Gradient Gels

Gradient Gels

This protocol is for preparing 12 mini-format linear gradient gels. It requires the Model 485 Gradient Former and Mini-PROTEAN 3 Multi-Casting Chamber. For other protocols, refer to the instruction manual for the gradient former you are using.

1 Determine the volume of acrylamide to prepare (≥40 ml is required for the Model 485 Gradient Former). Assemble the stack of Mini-PROTEAN 3 Cassettes as described in the Mini-PROTEAN 3 Multi-Casting Chamber instruction manual. Then, flow water through the stopcock and measure the volume required to fill the cassettes. Disassemble the chamber and dry all components. Prepare the required volume (+5 ml) of acrylamide. Table 3 provides estimated volumes for the casting of 12 mini-format gels.

Table 3. Volume of acrylamide required for 12 mini-format gels. Prepare the amount listed below plus an additional 5 ml.

Spacer Plates	Volume (required to fill 12 gels)	Volume to Prepare
0.75 mm	80 ml	85–90 ml
1.0 mm	100 ml	105–110 ml
1.5 mm	140 ml	145–150 ml

2 Determine the chamber volumes. To create a linear gradient, the volume in each chamber is half the total gel volume required (or 20 ml, whichever is greater). As an example, casting twelve 1.0 mm gels requires 100 ml, so prepare 105 ml (step 1). Divide that volume by two to determine the volume required for each chamber of the gradient former (52.5 ml each for the light and heavy chambers).

3 Determine the heavy and light acrylamide formulations using the chart on the right. Reassemble the multi-casting chamber.

4 Place the gradient former on a magnetic stir plate and add a magnetic stir bar to the mixing chamber labeled “light”. Attach the luer fitting to the stopcock valve on the inlet port. Run a piece of Tygon Tubing (1/8” ID Tygon Tubing works well) from the gradient former to the luer fitting on the multi-casting chamber.

5 Combine all reagents except the initiators, and degas the solution for 15 min.

6 Just prior to pouring, add TEMED and APS to both solutions and mix gently. Pour the appropriate monomer solutions into the gradient chambers. (Consult the gradient former instruction manual for complete instructions.) Pour the light solution into the mixing chamber labeled “light”, and the heavy solution in the reservoir chamber labeled “heavy”.

7 Turn on the stirring bar in the mixing chamber, open the tubing clamp of the gradient maker and the stopcock valve of the casting chamber, and pour the gels.

TIPS

If gravity flow isn’t fast enough, use a peristaltic pump to pump the entire set of gradients within 10 min. If it is not possible to complete the operation in 10 min from the time initiators are added, then it might be necessary to reduce the amount of initiators (use half the amount of TEMED) to slow polymerization. The gradient should be poured as quickly as possible, without mixing the gradient solution in the casting chamber.

Light Solution (4%)

30% Acrylamide stock (30%)(X ml) = (4%)(55 ml)	X = 7.3 ml
1.5M Tris-HCl stock buffer, pH 8.8 (1.5 M)(X ml) = (0.375 M)(55 ml)	X = 13.8 ml
Water (55 ml) – (7.3 ml + 13.8 ml) = X	X = 34 ml
10% APS (500 µl)/(100 ml) = (X µl)/(55 ml)	X = 275 µl
TEMED 10% APS volume; (275 µl)/10 = X	X = 27.5 µl

Heavy Solution (20%)

30% Acrylamide stock (30%)(X ml) = (20%)(55 ml)	X = 36.7 ml
1.5 M Tris-HCl stock buffer, pH 8.8 (1.5 M)(X ml) = (0.375 M)(55 ml)	X = 13.8 ml
Water (55 ml) – (36.7 ml + 13.8 ml) = X	X = 4.5 ml
APS (500 µl)/(100 ml) = (X µl)/(55 ml)	X = 275 µl
TEMED 10% APS volume; (275 µl)/10 = X	X = 27.5 µl

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (bulletin 6040).

Tygon is a trademark of Norton Company.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 3065 7550*
Canada *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *1800 88 22 88* ***United Kingdom*** *020 8328 2000*

Bulletin 6201 Rev B US/EG *14-1332 0714 Sig 1213*