Casting SDS-polyacrylamide mini-gels

# Materials needed:

DistilledH2O

Tris-HCl, 1.5 M, pH 8.8

Tris-HCl, 0.5 M, Ph 6.8

SDS, 20% (W/V)

Acrylamide/Bis-acrylamide (30%/0.8% w/v), (in fridge by ice machine)

Ammonium persulfate, 10% (w/v)

TEMED (in fume hood)

SDS Running Buffer or TGS Running Buffer (same thing different names)

BioRad short plates (2), spacer plates (2), combs (2), green clamps (2), casting stand (1)

# Protocol:

Create a fresh 10% ammonium persulfate solution by mixing 950 µl of dH20 with 0.1 g of ammonium persulfate in a microcentrifuge tube. The final volume will be approximately 1 ml.

Prepare to cast gels by assembling 2 short plates, 2 spacer plates (make note of the spacer thickness, noted on the plate), one casting stand, and 2 green casting frames (see BioRad Mini-PROTEAN 3 cell Instruction Manual if necessary).

1. Insert combs (of the same thickness as the spacer plates) into the assembled plates, and use a sharpie to place a mark 5 mm below the teeth of the comb, then remove the comb.
2. Prepare acrylamide solution for separating gel by mixing the following reagents in a small beaker, in the following order:

| Item | Amount | Amount | Amount | Amount |
| --- | --- | --- | --- | --- |
| Gel Percentage | 7% | 10% | 12% | 15% |
| dH2O | 5.1 ml | 4.1 ml | 3.4 ml | 2.4 ml |
| 1.5 M Tris-HCl, ph 8.8 | 2.5 ml | 2.5 ml | 2.5 ml | 2.5 ml |
| 20% (w/v) SDS | 50 µl | 50 µl | 50 µl | 50 µl |
| Acrylamide/Bis-acrylamide | 2.3 ml | 3.3 ml | 4.0 ml | 5.0 ml |
| 10% (w/v) APS | 50 µl | 50 µl | 50 µl | 50 ul |
| TEMED | 5 µl | 5 µl | 5 µl | 5 µl |

Note: Use lower percentage gels to resolve bigger proteins, and higher percentage gels to resolve smaller proteins.

1. Mix the acrylamide monomer solution well, then transfer solution to casting plates using 1 ml pipette (blue tips) or transfer pipette. Transfer enough solution to reach the mark placed in step 3, then immediately add approximately 0.5 ml of dH2O or EtOH (70% or 95%) to the top of the gel.
2. After the separating gel has polymerized (about 30 minutes), remove the water or EtOH overlay by inverting the gel over the sink then blot with a Kim wipe.
3. Prepare acrylamide monomer solution for the stacking gel by mixing the following reagents in a small beaker, in the following order

|  |  |
| --- | --- |
| Item | Amount |
| Gel Percentage | 4% |
| dH2O | 3.0 ml |
| 0.5 M Tris-HCL, pH 6.8 | 1.25 ml |
| 20% (w/v) SDS | 25 µl |
| Acrylamide/Bis-acrylamide | 670 µl |
| 10% (w/v) APS | 25 µl |
| TEMED | 5 µl |

Note: The stacking gel monomer can be prepared while the separating gel is polymerizing, but ammonium persulfate and TEMED should not be added until immediately before casting/pouring the stacking gel.

1. Transfer acrylamide monomer solution to casting plates using 1 ml pipette or transfer pipette, filling the casting plates within 0-2 mm of the top of the short plate.
2. Immediately insert combs into space between plates and allow the gel to polymerize.

10. If not used within a few hours, store gels in the refrigerator. Keep moist by immersing in 1x TGS Running Buffer, or wrapping in a paper towel moistened with water and stored in a plastic bag or tupperware dish.

11. To run an SDS-PAGE gel, mix sample with appropriate amount of loading buffer in a microcentrifuge.

12. Place in 95°C heat block for 5 minutes, remove and let cool for 5 min. at room temperature, then spin briefly to collect condensate.

13. Prepare gels for electrophoresis, following the diagrams below.

Note: Before clamping the gels in place, raise glass plates slightly to create seal between top of the short plate and the bottom of the notch in the gasket.

14. Fill upper/middle chamber with SDS Running Buffer until completely full and fill the outer chambers with the running buffer about ¼ full (enough to cove the bottom electrode).

15. Place the loading guide in the middle chamber (optional) and load your samples in the wells of the gel.

16. Run at 150-200 volts until the dye reaches the bottom, which is about 40-60 min.

Hint: Check your gel about every fifteen minutes to ensure that the middle chamber is completely full, otherwise the gel may not run if it gets below the short plate.

1. Once gel is done running, remove the lid from gel box and empty out extra running buffer in the sink. Disassemble electrophoresis apparatus, and separate the two plates (using the green wedge if necessary).
2. Remove the stacking gel from the gel using the wedge or razor blade. Transfer gel to a container with Coomassie stain.
3. Allow the gel to stain for a minimum of two hours, then recycle the stain by placing it back into the “used stain” container. Rinse gel with water, then cover in “Destain” solution for one hour to overnight, add 1-2 wadded up Kim wipes to the Destain solution to absorb stain.
4. Rinse all equipment used with tap water, then distilled water. Resorvoir and electrodes can finish drying in the cabinets, plates and combs should be dried with Kim wipes and put away as well.

# Protocol Source/Reference: BioRad Mini-Protean 3 Instruction Manual