

## SDS Polyacrylamide Gel Electrophoresis

Clark Lab

Kay Finn

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Criterion Cassettes from BioRad (cat.# 3459902). Ready to fill: 18 well format hold 25 ul samples.

Mark 1 cm below the wells with lab marker, so that filling maximum of the lower separating layer is know. ID the cassettes if using more than 1 at a time.

Mix the separating gel solution (by swirling) in a 125 ml flask, according to the chart, adding good water, acrylamide, and buffer with pipets. Then add the N,N-TEMED (from shelf) and 10% APS solution (thawed from freezer stock aliquot) when ready to pour. Swirl to mix again. Tilt the cassette toward yourself and angle to one side to fill from the bottom without bubbles. Using a pipet, pour 10ml in one time gently righting the cassette as you pour. Check the level when set in stand. Level will be slightly above the mark but will shrink down on polymerization. Gently add good water with pasteur pipet to layer over the acrylamide to give a smooth top line. Allow to set for 1 hour. Tilt the flask with remaining sample to check for polymerization before continuing.

Mix the stacking layer: good water, acrylamide, and upper buffer (using pipets and/or pipettors for 1 ml or less, swirl as before. Remove the water layer from the separating gel by pouring it into the sink and using a kimwipe to wick out the remaining water from the one side that is lowest.

Add the N.N-TEMED and 10% APS (kept on ice from use before) to the buffers in flask. Swirl, add 3ml by pipet to the top of the other layer to overfill it. Then carefully add the comb, making sure that no bubbles are caught in the wells. Let cure for at least 45 minutes. Put 10% APS back in the freezer after marking the lid to note how many times that particular tube has been thawed and refrozen. Do not use a tube more than 5 times. Wells become sloppy on the sides or worse after 5 cycles.

After curing gently rock the comb to pull it out, checking that the wells are formed correctly, write out the plan for loading wells.

Remove the tape from the lower edge of the cassette and place in the gel box, fill upper reservoir to fill line (about 60ml) and fill the lower reservoir to the fill line with running buffers. Use Hamilton syringe or pipetter with long tips to load 25 ul max in wells gently, careful not to get any out of the wells.

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| Run 1.2 hrs, at 150 volts. Stain #2 for 30 minutes minimum, Stain#3 for 1 hr min. Destain. Can stop in Stain #2 or #3 for overnight, shaking. |
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Scan on Densitomer in Huber Lab or the Scan Wizard if desired, then dry between Cellophane for storage.