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Making and running an acrylamide protein gel V.2

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ABSTRACT

In this protocol, the preparation and running an acrylamide gel is explained. Using this gel, proteins with a size of >15 kDa can be visualized. For the visualization of proteins with a size of 15 -30 kDa, a 12% acrylamide gel can be used. For the visualization of proteins with a size of 30-50 kDa, a 10% acrylamide gel can be used.

1 Make the separation gel. In the separation gel, the proteins are separated by size. The following is needed for 10 ml of separation gel (add in the following order):

Ingredients	10% acrylamide gel	12% acrylamide gel
dH2O	4.1 ml	3.4 ml
Acrylamide (30%, 37.5:1; Bio-Rad)	3.3 ml	4 ml
Tris-HCl (1.5 M, pH 8.8)	2.5 ml	2.5 ml
SDS	100 µl	100 µl

Table 1: contents of an acrylamide separation gel

Pro tip: Make the separation gel in larger volumes and store it at 4°C

2 Set up the Spacer plate (either 0.75 mm, 1 mm or 1.5 mm) with a cover plate in a clamp and make sure with water and ethanol that there is no leakage.Pour off ethanol and water and remove traces using filter paper.

3 In a small tube, get 10 ml of separation gel

4 Add 100x APS (100µl)and mix the solution by inverting the tube a few times.

5 Add 1000X TEMED(10 µl)and mix the solution by inverting or by pipetting up and down.

Watch out: the gel will quickly polymerize

30m

6 Pipette the separation gel between the glass plates till approximately 2 cm below the top of the cover plate. Add isopropanol on top of the gel to remove bubbles and keep the gel from drying out. Wait till the gel is completely polymerized (usually takes about 30 minutes).

Pro tip: keep the tube with the remaining gel solution next to the casted gel to easily see when the gel is completely polymerized.

7 Pour off the isopropanol and remove remaining isopropanol usingH2O. Remove traces of H2O using filter paper.

Pro tip: use a marker to indicate the height of the separation gel on the glass.

8 Prepare a 4% stacking gel. The following ingredients are needed for 10 ml of stacking gel (add in following order):

Ingredients	4% Polyacrylamide
dH2O	6.1 ml
Acrylamide (30%, 37.5:1; Bio-Rad)	1.3 ml
Tris-HCl (0.5 M, pH 6.8)	2.5 ml
SDS	100 µl

Table 2: contents of a stacking gel

Pro tip: Make the stacking gel in larger volumes and store it at 4°C

9 Get 5 ml of separation gel in a tube.

10 Add 100x APS (50µl) and mix the solution by inverting the tube a few times.

11 Add 1000X TEMED (5 µl)and mix the solution by inverting or by pipetting up and down.

Watch out! The gel will quickly polymerize!

30m

12 Pipet the stacking gel on top of the polymerized separation gel. Insert a comb (corresponding to the gap between the glass plates) to create either 10 or 15 wells. Wait till the stacking gel is completely polymerized.

Pro tip: keep the tube with the remaining gel solution next to the casted gel to easily see when the gel is completely polymerized.

13 Once the stacking gel has been polymerized, take the plates with the gel out of the clamps. Put them in a casket together with either another gel or a buffer dam to generate a compartment. Place the casket with the gel in a vertical electrophoresis cell.

Pro tip: a cast gel can be made one day before running the gel. Wrap the gel in wet paper towels and keep the gel in a plastic bag at 4°C.

14 Add SDS-PAGE running buffer till the top of the casket. The following is needed to make 1L SDS-page running buffer:

Ingredients	1x SDS-PAGE running buffer
Glycine	14.4 g
Tris	3.02 g
SDS	1 g
dH2O	0.9 L

Table 3: contents of the SDS-PAGE running buffer

Pro tip nr 1: make 10x stock SDS-PAGE running buffer and dilute 100 ml till a final volume of 1 L before use.

Pro tip nr 2: add the SDS-PAGE running buffer till the top of the casket and wait a few minutes to make sure that the casket is not leaking. If the buffer volume in the casket drops too low, current will stop.

15 Take the combs out of the separation gel

16 Load the samples, together with a protein ladder. Small wells (15 wells) can hold approximately 15 µl of sample, big wells (10 wells) can hold approximately 35 µl of sample.

Pro tip: make sure to not add too much sample to avoid samples flowing into adjacent lanes. Best is to load 10 µl for small wells and 25 µl for big wells

17 Once the samples have been loaded, fill up the electrophoresis cell with running buffer to the line for the corresponding number of gels. For 1 gel, fill up to the line for 2 gels. For 3 gels, fill up to the line for 4 gels

Pro tip: Fill the electrophoresis cell with running buffer by overflowing the casket to make sure that the casket is completely filled.

18 Put on the lid and set up the power supply. Set the current to 40V.

19 When the samples enter the separation gel, increase the current to 120V.

20 When the samples have travelled far enough through the gel, turn off the current and take out the casket. Throw away the running buffer, or reuse it the next time. Use a tool to open the two glass plates and take out the gel.

21 Put the gel in a big petridish and wash it 5 times with water (while gently shaking) or incubate it for 30 minutes on a rocking shaker.

22 Pour off the water and add CoomassieBlue staining. Let it stain for 1 hour (or overnight) on the rocking shaker.

23 Pour off the Coomassie Blue staining (not down the drain) and add dH2O to destain the gel. Change the dH2O approximately every 60-90 minutes. Place the petridish on a rocking shaker.

Pro tip: The Coomassie blue staining can be used up to 3 times, so do not throw it away after using it once!

24 When the gel is destained, use a gel imager (with a white screen) and use a Coomassie Blue visualization protocol. Save (and print) the image.