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Preparing and running a tricine-urea gel

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Abstract

In this protocol, the preparation and running of a tricine-urea gel will be explained.

Troubleshooting

1 Make 3X gel buffer. For the gel buffer, the following is needed:

	Ingredients	Gel Buffer (3x)
	Tris	3 M
	SDS	0.3 %

Table 1: contents of Gel Buffer (3x)

Then use HCl to set the pH of this solution to 8.45

2 Make AB-6 solution by dissolving 46.5 g of acrylamide and 3 g of bisacrylamide in 100 mL of dH2O.Store this solution at 4°C.

3 Make the separation gel. This is a 16% urea tricine gel. For 30 ml of separation gel, the following ingredients are needed:

	Ingredients	Separation gel
	Gel buffer (3x)	10 ml
	AB-6	10 ml
	Urea	10.8 g
	dH2O	To 30 ml

Table 2: contents of separation gel

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

4 In a small tube, get 10 ml of separation gel.

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

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

Watch out! The gel will quickly polymerize!

7 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.

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

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

9 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 3: contents of stacking gel

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

10 Get 5 ml of separation gel in a tube.

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

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

Watch out! The gel will quickly polymerize!

13 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.

14 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.

15 For running a Tricine gel, two buffers are needed: a cathode and an anode buffer. These buffers contain the following ingredients:

	Ingredients	Cathode buffer (10x)	Anode buffer (10x)
	Tris	1 M	1 M
	Tricine	1 M	
	SDS	1 %	

Table 4: contents of cathode and anode buffers

Adjust the pH of the anode buffer to 8.9 with HCl. Dilute the stock of the buffer 1:10 with dH2O

16 Pour the cathode buffer (1x) within the casket and the anode buffer in the electrophoresis cell.

17 Take the combs out of the separation gel

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

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

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

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

21 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.

22 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.

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

Pay attention: the tricine gel will increase in size when it is being stained and is way more elastic in comparison to an acrylamide gel.

24 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!

25 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.