Agarose Gel Casting Protocol

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[1]UCSC

Oct 20, 2020

1 *Works for me* This document is published without a DOI.

UCSC BME 22L

DOCUMENT CITATION

2020. Agarose Gel Casting Protocol. **protocols.io**
https://protocols.io/view/agarose-gel-casting-protocol-bnnzmdf6

CREATED

Oct 20, 2020

LAST MODIFIED

Oct 20, 2020

DOCUMENT INTEGER ID

43449

CASTING A 1% AGAROSE/0.5X TBE GEL IN THE BENTO LAB GEL BOX
BME 22L, FALL 2020, DRAFT

Before you begin, put on gloves, safety glasses and your lab coat.

1) **Adding Agarose to the Pyrex Erlenmeyer flask**

a) Use a felt tip marker to label the 1 mL mark on a high-quality Eppendorf tube.
b) Fold a crease in a slip of weigh paper. Gently add a small volume of dry agarose along the crease in the weigh paper. Gently tap the weigh paper and dispense the agarose into the marked Eppendorf tube. Repeat until the agarose reaches just below the 1 mL mark. This is equivalent to ~ 0.5 g agarose.

c) Dispense the agarose into the clean Erlenmeyer flask.

2) **Preparing 50 mL of 0.5X TBE buffer, and transfer to the Erlenmeyer flask**

a) Mark a 50 mL blue cap tube as '0.5 X TBE'.

b) Transfer 2.5 mL of 10X TBE to the 50 mL tube. The best way to do this is by using a pipetter to dispense 1 mL twice, and then 0.5 mL one time.

c) Carefully transfer distilled water to the blue cap tube until it reaches 50 mL. Seal with the cap and invert several times to mix the solution thoroughly. Transfer this solution to the Erlenmeyer flask that contains solid agarose. Gently swirl the solution to disperse the agarose.

3) **Melting the agarose**

a) Place the flask in the microwave and set it to high and 15 seconds. Run. Using a gloved hand or a washcloth, remove the flask from the microwave and gently swirl the contents. You will see

that some of the particles have melted, but there are still some suspended solids and solids stuck around the perimeter of the flask.
b) Repeat step (a) until there are no more suspended solids. The solution should be clear, with no suspended particles and very few bubbles if any. The flask will be hot so be careful when you handle it.

c) Set the flask down to cool for ~ 5 minutes. During this period, put the black rubber dams into the gel box.

d)When the gel solution is approximately 55 degrees C, you will be able to touch the flask at the level of the solution with a disposal-gloved hand and it will not feel too hot to touch.

e) Gently pour the agarose solution into the gel tray. Place the larger gel comb in the slots near the black electrode. Wait for the gel to solidify (~30 minutes).

4) **Running the gel**

a) Check that the gel has solidified. It should appear milky white.

b) Remove the rubber dams, and attach the electrodes to the copper contacts on the box. Red-to-red; black-to-black. Connevt the wires to the Bento Lab, and place the box on top of the transilluminator.

c) Fill the reservoirs at either end of the gel box with 0.5X TBE prepared as before in the 50mL blue cap tube labeled for this purpose. Continue to fill until there are a couple of millimeters of solution over the top of the gel.

d) Using the orange nob, set the Bento lab to the electrophoresis panel. Select 50V and 1 hour.

e) Click the check mark to begin the run. If the gel is running properly, a lightning bolt should appear on the control panel. If the gel is not running properly there will be an error message. Check that the electrodes are fully attached. Also check that dams have been removed and that you have filled the reservoirs with 0.5X TBE as instructed.