

RNA GEL

Gel:

Medium Gel:

- 73.7ml dH₂O
- 10.0ml 10x MOPS
- 1.2g Agarose (for RNA gels)
 - *16.3ml Formaldehyde 37%

Small Gel:

- 36.8ml dH₂O
- 5.0ml 10x MOPS
- 0.6g Agarose (for RNA gels)
 - *8.1ml Formaldehyde 37%

1. Add water, MOPS, and Agarose to a 250ml flask
2. Boil the mixture in the microwave with a watch glass over the top of the flask
3. Spin the mixture on the stirrer until ~40-50°C
N.B: Meanwhile tape up ends of horizontal gel casting tray and position comb (10 lane typical)
4. Move cooled Agarose solution to the hood.
5. Add the formaldehyde and swirl.
6. Immediately pour into the gel caster and set for ~1 hour in the hood.
7. Remove tape from casting tray and place in electrophoresis chamber
8. Add sufficient 1x MOPS running buffer (~1 L) to the electrophoresis tank until gel is covered

Making the RNA Sample Loading Buffer:

- Add the following to a 1.5ml microfuge tube and vortex to mix:
 - 650ul Formaldehyde 37%
 - 200ul 10x MOPS
 - 150ul nuclease-free dH₂O

Preparing the Samples for the Gel:

1. Load 1-5ug of RNA
2. Add an equal volume of RNA sample loading buffer to each sample.
3. Calculate the new volume and add an equal volume of deionized formamide to each sample (in fridge 5S-19; after using, flush with nitrogen gas, recap tightly).
4. Heat Samples at 65°C for 15 minutes
5. Immediately place on ice for 5 minutes. Pulse spin.
6. Add 2ul of BPB and 5ul of EtBr (10 mg/ml stock).
7. Rinse each well of gel with transfer pipette; load samples

Run the gel at 80V for 1-2 hours. Remember RNA runs to red electrical lead!
Take a photo of gel with Dr. Shewchuk's gel box camera.