

RNA gels: Formaldehyde method

Note: Formaldehyde is toxic and a potential carcinogen. Handle it in the fume hood.

A. Preparation of the gel

1. Use RNase-free gel units only.
2. Pour and run the gel in a hood to avoid formaldehyde vapors.
For 200 ml of 1.5% gel (large tray with 25 samples):
 - i. melt 3 g agarose in 162 ml autoclaved water. (Using DEPC-treated solutions is not essential here since the formaldehyde will inactivate RNase.)
 - ii. Cool the agarose in the hood to approx. 60°C by mixing with magnetic stirrer.
 - iii. Add 10X MOPS running buffer to 1X final concentration, and formaldehyde to 0.7 M. NO Ethidium Bromide added at this stage!
 - a. 20 ml 10X MOPS
 - b. 11.6 ml of 37% formaldehyde

10X MOPS: 0.2 M MOPS pH 7.0, 10 mM EDTA, 50 mM NaOAc.

Autoclave and store in the dark at 4C. It is normal for 10x MOPS to turn a little yellow after it is autoclaved (Don't overautoclave!), and the yellowing will increase over time. Do not use it once it becomes strongly yellow, and do not "overautoclave" since this will increase the yellowness.

B. Sample preparation (work fast and clean on ice)

1. Put 5 µg RNA (your samples and RNA standards, if desired) to RNase-free tube. Adjust volume to 6 µl with DEPC-treated water; if the original volume of RNA is too high, lyophilize or ethanol precipitate.

Add (can be prepared as master mix, then add 17 µl/sample):

- 10 µl formamide (deionized by passage through mixed bed resin)
 - 4 µl formaldehyde
 - 2 µl 10 X MOPS buffer
 - 1 µl bromophenol blue dye mix (commercial 6x Loading dye is fine)
- The total volume is 23 µl.

2. Heat at 65C for 10 min, cool on ice and spin briefly to collect condensate.
3. Place on ice and quickly load the gel.

C. Electrophoresis

1. The gel should be cast, loaded, and run in the hood. 1X MOPS is used as running buffer.
2. Cover the gel with 1-2 mm of 1X MOPS buffer (no formaldehyde). Try to load the gel within an hour, to avoid dilution of the formaldehyde in the gel.
3. Load the gel and run for at least ten minutes at 5 V/cm in 1X MOPS buffer. After about 10-20 minutes, once the samples have entered the wells increase the voltage and run until the dye has run 9-10 cm (2/3 to 3/4 of the way down the gel).
4. Stain in 200 ml 1X MOPS with 15 µl Ethidium Bromide by shaking for ca 20 min. Wash in 1X MOPS for 10 min to remove unbound Ethidium bromide. Cover the tray by aluminium foil to protect your gel.
5. Photograph the gel on cleaned Gel-doc station to document the concentration and integrity of the RNA. Note that formaldehyde gels are weaker than normal agarose gels, particularly when warm (cooling will "firm them up" a bit). So handle the gel carefully!!
6. If fine, proceed to blotting

DEPC-water

1 L deionized water
1 ml DEPC (diethylpyrocarbonate)

- Add few yellow tips and a stirring bar and stir overnight.
- Autoclave to inactivate DEPC.

10x MOPS (1 L)

41 g MOPS
6.8 g Na acetate (mw 136.08)
20 ml 0.5 M EDTA
800 ml H₂O
pH to 7.0 w/ NaOH,
fill vol. to 1L