

AGAROSE GELS FOR GLYOXATED RNA.

Preparation of RNA:

1. The RNA pellet should be SALT FREE. Wash at least twice with ice cold 70% EtOH. If you still observe a sizable salt pellet, re-precipitate with EtOH and wash thoroughly with ice cold 70% EtOH.
2. Air-dry pellet at least 30 minutes at room temperature.
3. Resuspend RNA pellet in 3.7 μ l H₂O (up to 20 μ g).
Then add: 2.7 μ l 6 M glyoxal
 8.0 μ l DMSO
 1.6 μ l 0.1M NaH₂PO₄
(The glyoxal should be de-ionized over any kind of ion-exchange resin, such as BioRad Chelex. Store aliquots at -20°C in tightly capped tubes.)
4. Incubate at 50°C for 1 hour.
5. Add 5g of a 50% glycerol, 0.01% bromophenol-blue, 0.01% xylene cyanol FF solution.

Preparing the Gel: (directly from McMasters and Carmichael PNAS #4 4835-4838, 1977)

1. Prepare a 1.4% agarose gel in 10mM NaP, pH 6.8. After solubilizing the agarose, allow to cool to 70°C, and then add SDS to 0.1% final concentration.
2. The gel can be run at 125V for 3 hours. The buffer must be circulated during the gel run.

Visualizing the RNA:

1. Soak the gel in 50mM NaOH for 20 minutes, shaking gently.
2. Soak gel in TBE or TAE + 1 μ g/ml EtBr for 20 minutes, shaking gently.
3. Photograph as usual under UV light. The gel can be dried, blotted, or processed as you like at this stage.