

## Formaldehyde Gel Protocol

Use all the material designated for RNA including the gel casting and running assembly.

1. Freeze dry the mRNA sample, without heat until they are dry. May use freeze dryer or use directly (for CsCl method)
2. Melt 1gm of agarose in 85mL of deionized water.
3. Add 12.5 mL of 10X MOPS buffer to the agarose solution. Allow the gel solution to cool to 55<sup>0</sup>C while preparing an electrophoresis gel mold. Place the gel mold in **fume hood** with formaldehyde solution. Then add 6.75 mL of **37% formaldehyde** to the cooled agarose. Mix well and quickly pour the agarose into the gel mold. If you wish to transfer the mRNA on the gel to a membrane, the gel should only be thick enough to handle easily (0.5-0.75 cm). ALLOW THE GEL TO SOLIDIFY IN THE FUME HOOD.
4. While the gel is solidifying, start a boiling water bath and prepare 5  $\mu$ L of loading buffer for each sample. Make the loading buffer by mixing (for 10 samples):  
24  $\mu$ L of deionized formamide  
17  $\mu$ L of loading dye  
9  $\mu$ L of 37 % formaldehyde solution  
Note: This solution is not stable do not use after 24 hrs.
5. Cover the solidified gel with 1X MOPS buffer.
6. Resuspend the freeze dried mRNA sample (or use 2 $\mu$ L RNA sample directly) in 5  $\mu$ L of loading buffer. Boil for 2 mins. Centrifuge the sample to collect condensation and immediately load onto the gel.
7. Electrophorese the gel at 100V for 20 minutes (until dye reaches to the bottom).
8. Examine the gel with UV illuminator.

### Solutions:

#### 10X MOPS Buffer:

	For 200 mL
0.2M MOPS	8.37
0.05M Sodium acetate	1.36 gm or 5.2 mL of 2M NaAc

0.01M EDTA

0.74 gm or 4 mL of 0.5 M EDTA

Bring the final pH of 5.5-7.0 with NAOH. Do not autoclave.

**Loading Dye:**

160  $\mu$ L of 10X MOPS Buffer

100  $\mu$ L sterile water

100  $\mu$ L ethidium bromide

80  $\mu$ L sterile glycerol

80  $\mu$ L saturated bromophenol blue in sterile water