

RNA DENATURING MOPS GEL PROTOCOL

Materials Needed:

Embi Tec Precast MOPS gels (Cat. No. GE-6000, 6010, 6020, 6030)

Embi Tec Sample Buffer (Cat. No. EC-1021)

Embi Tec Running Buffer (Cat. No. EC-1020)

Embi Tec Red Well Visualization Plate (Cat. No. EP-1047, 1048)

Protocol for Using Embi Tec's RNA Denaturing Sample Buffer

1. Mix *Embi Tec Denaturing Sample Buffer (Cat. No. EC-1021)* with the RNA sample in a 2:1 v/v ratio.
2. Heat at 65°C for 15 min.
3. Immediately chill on ice. At this point the sample is ready to be loaded.

Gel Running Condition

1. Dilute **Embi Tec 10X MOPS Running Buffer (Cat. No. EC-1020)** to 1X with deionized water. You will need 450 ml of running buffer per gel.
2. Place appropriate Red Well Visualization Plate in running tank, and place MOPS gel on top of plate.
3. Add the 1X MOPS buffer in the running tank. **(IMPORTANT: Use 450 ml running buffer in the RunOne Electrophoresis Unit)**
**Note – this volume is 35% higher than the normal recommended volume for TAE/TBE because the MOPS buffer system is susceptible to buffer discontinuity if adequate buffer mixing is not allowed. The higher buffer volume allows for free circulation of buffer between the two chambers.*
4. Place Embi Tec ¹Precast RNA gel in the running tank. Carefully remove any trapped air bubbles using a disposable pipette.
5. Load samples
6 well portrait – 30 ul
12 well portrait – 11 ul
12+1 well medium – 30 ul
16+2 well medium – 11 ul
6. Run at 100V (Approximate run time is 1 hr and 30 min).

Staining Protocol

1. Stain gel using 0.1 ug/ml of EtBr.
2. Place on shaker and allow to stain for 10 min.
3. Visualize under UV transilluminator. If bands appear faint, allow to stain for an additional 10 min.
4. Destain for 15 min in water.