Protocol

Denaturing Polyacrylamide/Urea Gels in TBE Buffer

This protocol is for the Denaturing Polyacrylamide/Urea Gels in TBE Buffer

1. Prepare 20 ml of a 5% polyacrylamide gel containing 7 M urea by adding:

47.5% acrylamide: 2.5% bis-acrylamide solution	2 ml
10 M urea	14 ml
10X TBE Buffer	2 ml
10% freshly prepared ammonium persulfate	0.2 ml
Deionized water	1.8 ml

2. Mix and add 10 µl TEMED. Mix again and pour the gel carefully avoiding the formation of air bubbles.
3. Insert the comb into the acrylamide and allow the gel to polymerize for at least 1 hour.
4. Fill the electrophoresis apparatus with 1X TBE buffer.
5. Heat the RNA samples and ladder at 70°C for 10 min, and chill on ice for 3 min.
6. Load onto the gel.
7. Run electrophoresis at 8 V/cm for about 1 hour.
8. Soak the gel for about 15 minutes in 1X TBE to remove urea prior to staining.
9. Stain the gel in 0.5 µg/ml ethidium bromide in 1X TBE solution for 15 min.

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