

# 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:

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

2. Mix and add 10  $\mu$ l 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  $\mu$ g/ml ethidium bromide in 1X TBE solution for 15 min.

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