

Preparation of Urea gel

Aim of the experiment

This is an explanation for preparing a 12 % Urea gels for RNA purification or analysis. You always can prepare more than one gel in a single tube. Always use 50 ml tubes for preparing a gel.

Materials

- Urea
- 10X TBE
- H₂O
- 29:1Acrylamite
- APS
- TEMED
- 1 µl DNA ladder
- 1 µl Ribo ladder

Procedure

1. Weigh 4.8 g Urea in a small beaker.
2. Add 1 ml 10x TBE and 2 ml H₂O.
3. Mix this at about 50 °C on magnetic stirrer until the Urea is diluted, in the meantime prepare 50 ml tube with 3 ml Acrylamite 29:1.
4. Add the diluted Urea solution to the Acrylamite and vortex.
5. Add 28 µl APS, then vortex.
6. Add 8 µl TEMED vortex.
7. Pour solution into cassette let it harden.
8. Transfer into floating chamber, fill with TBE 1x buffer, let it the heating at 60°C run for 30 minutes, low voltage about 40 V can be used as well, "wash" pocket by pipetting up and down with 100 µl pipette (Urea is in the pockets).
9. Before loading the gel "wash" pockets as before.
10. Load with 1 µl sample with loading dye, use low range DNA ladder, and riboruler (use 0,5 µl of ladder) → sample volume 3 µl, denaturing buffer added.