

Denaturing Polyacrylamide/Urea Gel Electrophoresis

This protocol is for the Denaturing Polyacrylamide/Urea Gel Electrophoresis

Note

Double stranded DNA ladders are not recommended for denaturing electrophoresis as they may form an atypical pattern. However these usual discrepancies are normally acceptable for analysis of cDNA or other ssDNA in denaturing PAGE.

1. For a denaturing 10% polyacrylamide gel solution of 40 ml, mix the following:

10X TBE Buffer	4 ml
20% acrylamide/bisacrylamide	10 ml
UREA	19.2 g (to 8 M final concentration)
Deionized water	to 40 ml

2. Vigorously agitate the solution by magnetic stirring to ensure complete mixing and solving of UREA powder.
3. Add 40 μ l TEMED and swirl the flask to ensure thorough mixing.
4. Immediately add 400 μ l of fresh 10% (w/v) APS and mix thoroughly.
5. Pour the acrylamide between the gel plates and insert the comb.
6. Clamp the comb in place at the top of the gel to avoid separation of the gel from the plates as the acrylamide polymerizes. Allow the gel to polymerize for 30 min.

Important note: polymerization begins as soon as APS is added to the mixture, so all succeeding actions must be performed promptly.

7. After polymerization is complete, remove the comb and any bottom spacers from the gel. Fill the lower reservoir of the electrophoresis tank with 1X TBE buffer. Initially, place the gel into the lower tank at an angle to avoid the formation of air bubbles between the plates and the gel bottom. Clamp the gel plates to the top of the electrophoresis tank and fill the upper reservoir with 1X TBE so that the wells are covered.
8. Pre-run and warm the gel for at least 30 min at 5 V/cm (constant voltage).

Note

Heat the gel (buffer) during the whole run at 60-70°C.

9. Wash the wells with 1X TBE buffer to remove UREA and gel pieces.
10. Load the samples.
11. Run the gel at 6 V/cm till the lower dye front reaches the three thirds of the gel.
12. Soak the gel for about 15 min in 1X TBE to remove the urea prior to staining.
13. Stain the gel in a 0.5 μ g/ml ethidium bromide aqueous solution for about 30 min.
14. Examine the gel under the UV light.

Caution: acrylamide is a neurotoxin; always wear gloves, safety glasses, and a surgical mask when working with acrylamide powder.

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