Alkaline Agarose Gel Electrophoresis

Protocol

This protocol is for the Alkaline Agarose Gel Electrophoresis

Note

- Double stranded DNA ladders are not recommended for denaturing electrophoresis as they may form an atypical pattern. However, these discrepancies are normally acceptable for analysis of cDNA or other ssDNA in alkaline gels.
- Use a flask of at least three times larger volume than that of the solution to avoid boiling over.
- Wear gloves when handling ethidium bromide.

1. Weigh out the required amount of agarose (depending on the gel percentage) into an Erlenmeyer flask.
2. Add the appropriate volume of the buffer (30 mM NaCl, 2 mM EDTA, pH 7.5) and swirl to mix.
3. Weigh the flask with the solution.

For high percentage gels (3-5%): *add an excess amount of distilled water to increase the weight by 10-20%.*

4. Boil the mixture in a microwave oven (at medium power) until the agarose melts completely; swirl the flask several times while boiling. To prepare the highest quality agarose gels of any percentage, an additional 3-5 min of boiling after completely melting the agarose is recommended. A significant amount of water evaporates during this procedure and therefore restoring of the initial weight (in step 5) is required to obtain the desired percentage gel.
5. Weigh the flask again and if necessary, add hot distilled water to restore the initial weight.

For high percentage gels (3-5%): *check (by weighing) that the excess 10-20% of water has evaporated and, if needed, continue boiling to remove any excess, or add hot distilled water to restore the initial weight.*

6. Cool the solution to 65-70°C. Pour carefully on a clean casting plate with an appropriate comb. If bubbles appear, push them carefully away to the sides with a pipette tip.
7. Solidify the gel for approximately 30 min before use.
8. Immerse the gel for at least one hour into the alkaline electrophoresis buffer (30 mM NaOH, 2 mM EDTA). Dilute 5 volumes of the DNA sample or ladder with one volume of 6X alkaline electrophoresis loading buffer (180 mM NaOH, 6 mM EDTA, 18% Ficoll 400, 0.05% bromcresol green).
9. Heat samples and ladder at 70°C for 5 min, then chill on ice for 3 minutes. Load onto the gel.
10. Run electrophoresis at 3 V/cm in alkaline electrophoresis buffer (30 mM NaOH, 2 mM EDTA) until the dye runs approximately two-thirds of the way down the gel.

After electrophoresis the gel should be immersed for 30 min in 100-300 ml of 0.5 M Tris-HCl buffer, pH 7.5 and later stained in a 0.5 µg/ml ethidium bromide solution for 30 min. If staining is not enough, the whole procedure can be repeated.

Warning. *Hot agarose solution should be handled very carefully.*

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