Non-denaturing Agarose Gel Electrophoresis

This protocol is for the Non-denaturing Agarose Gel Electrophoresis

Note

- Use a flask of at least three times larger volume than that of the solution to avoid boiling over.
- Use the same 1X electrophoresis buffer to prepare the gel and to run electrophoresis.
- Dilute 50X TAE Buffer or 10X TBE Buffer to a 1X concentration immediately before use.
- Use TBE buffer for analysis of DNA bands smaller than 1500 bp. For larger DNA, use TAE buffer.
- For intensified gel staining, add ethidium bromide to both the gel and the electrophoresis buffer at a final 0.5 µg/ml concentration. Alternatively, stain the gel after electrophoresis (see below).

Wear gloves when handling ethidium bromide.

- For reliable analysis of supercoiled/relaxed plasmid ethidium bromide should not be included in the electrophoresis buffer or gel. The gel should be stained only after electrophoresis is complete.
- Ethidium bromide and exposure to UV light may cause DNA alterations. Therefore, avoid UV exposure and do not stain DNA with ethidium bromide if the purified fragments will be used for cloning experiments.

1. Weigh out the required amount of agarose (depending on the gel percentage) into an Erlenmeyer flask.
2. Add the appropriate volume of either 1X TBE or 1X TAE buffer 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.

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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.

Optional: *for intensified gel staining add ethidium bromide to a final concentration of 0.5 µg/ml. Mix well and heat for 1 min without boiling.*

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. Low percentage LM agarose gels need to be solidified at 4°C.
8. Immerse the gel into the desired electrophoresis buffer. Load the samples onto the gel.
9. Run electrophoresis at 5-7 V/cm until the bromophenol blue runs approximately two-thirds of the way down the gel.
10. After electrophoresis the gel can be stained by immersing it into a 0.5 µg/ml ethidium bromide solution for 15-20 min, stained with SYBR® Green I or any other DNA staining technique.

Warning. Hot agarose solution should be handled very carefully.

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