

PROCEDURE FOR USE AGAROSE

AGAROSE DISSOLUTION

1. Dispersion:

Dispersion: separation of the particles by the buffer without clumping.

2. Hydration:

individual particles are surrounded by the solution (water, buffer). Determinative for good and easy dissolution process; it is advisable to allow hydration time before heating for melting and dissolution.

1. Melting and dissolution:

solid particles get into liquid state.

- Different agarose types behave differently: no universal protocol for heating and dissolving agarose.
- Pore size is determined by concentration and agarose type used. Appropriate agarose and concentration should be chosen for each application.

GEL PREPARATION TIPS

1. Always use a beaker 2-4 times the volume of the solution.
2. Add agarose powder slowly into rapidly stirring buffer solution to avoid clumping.
3. The buffer solution should be cool for a good dispersion; if the buffer is warm, possibilities of clumping are great.
4. Allow agarose powder to hydrate in the solution for a few minutes before heating - this allows for a quicker and easier dissolution and reduces foaming.
5. Adjust time and power settings according to your microwave output strength.
6. Always wear appropriate protection: the microwaved solution can become overheated and foam when disturbed.
7. To prevent overheating: reduce microwave power, remove beaker after 1 min from the microwave and swirl it very gently and carefully. Place it back into the microwave and continue for the remaining 1 minute or so.
8. For total agarose melting: boil the solution only enough to affect total dissolution. Check for "fish eyes" (incomplete dissolution). Overboiling can cause agarose hydrolysis and lower gel strength.



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9. To avoid bubble formation: cool to 60°C and pour carefully into the gel cassette.
10. After pouring, allow the gel to cool gradually; rapid cooling will cause irregular gel matrix and band distortion during electrophoresis.
11. Low melt agarose gels need to sit for an additional 30 min or overnight at 4-8°C to allow a total gelling process.
12. Low melting or low percentage gels: it is important to run electrophoresis in a cold buffer. High voltages can cause overheating of the buffer which can melt the gel.
13. Buffer composition can be determinative in the gelling process, if agents that disrupt hydrogen bond formation are added to the buffer, melting temperature and gel strength will decrease, or even inhibit gel formation.
14. Once the gel is set, flood with the buffer. The gel can be stored refrigerated for several days.
15. Agarose gels can be remelted and repoured several times without damage so that a large volume of agarose can be prepared and smaller portions taken from time to time.

For laboratory use only. Not for use in diagnostic or therapeutic procedures.

ABT INST RESINS Rev. 2008/B