Protocol

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Low Melt Agarose Gel Preparation Protocol

Introduction

Low melt agarose is often used for gel agarose preparation for PCR, restriction enzyme ligation and sequencing. Dissolution of agarose involves dispersion and hydration. First, dispersion is the separation of the particles by the buffer without clumping. On the other hand, during hydration, individual particles are surrounded by the solution (water, buffer). Hydration is determinative for a good and easy dissolution process; it is advisable to allow hydration time before heating to allow melting and dissolution (solid particles get into a liquid state).

It is important to note that different agarose types behave differently and that pore size is determined by the concentration and agarose type used. In addition, appropriate agarose and concentration should be chosen for each application. Here, we describe a general protocol for the preparation of an agarose gel utilizing Low Melt Agarose.

Materials

- Low Melt Agarose (GoldBio Catalog # A-204)
- Buffer (TAE or TBE) (TAE Stock Solution preparation protocol, TBE Stock Solution preparation protocol)
- Beaker
- Microwave

Storage and Handling

- Store Low Melt Agarose at room temperature.

Method

1. Add low melt agarose powder slowly into rapidly stirring buffer solution to avoid clumping.

   Note: The buffer solution should be cool for a good dispersion; if the buffer is warm the possibilities of clumping increase.

2. 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.

   Note: Adjust time and power settings according to your microwave output strength.

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3. To prevent overheating: reduce microwave power, remove beaker from the microwave after 1 minute and swirl it very gently and carefully. Place it back in the microwave and continue for the remaining 1 minute or so.

4. Then, for total agarose melting: boil the solution only enough to affect total dissolution. Check for "fish eyes" (incomplete dissolution).

   Note: Overboiling can cause agarose hydrolysis and lower gel strength.

5. Cool to 60°C and pour carefully into the gel cassette. Cooling prevents bubble formation.

6. After pouring, allow the gel to cool gradually; rapid cooling will cause irregular gel matrix and band distortion during electrophoresis.

   Note: Low melt agarose gels need to sit for an additional 30 minutes or overnight at 4-8°C to allow a total gelling process.

   Note: Low melting or low percentage gels require that the electrophoresis is run in cold buffer. High voltages can cause overheating of the buffer, which can melt the gel.

7. Once the gel is set, flood with the buffer. The gel can be stored refrigerated for several days.

   Note: 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.

Tips

- Always use a beaker 2-4 times the volume of the solution.
- Always wear appropriate protection: the microwaved solution can become overheated and foam when disturbed.
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- To prevent overheating: reduce microwave power, remove beaker from the microwave after 1 minute and swirl it very gently and carefully. Place it back into the microwave and continue for the remaining 1 minute or so.
- 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.

Associated Products

- Tris Acetate (GoldBio Catalog # T-090)
- Boric Acid (GoldBio Catalog # B-030)