FAQ agarose

MOST FREQUENT QUESTIONS ABOUT AGAROSE GEL ELECTROPHORESIS

Most frequently asked questions about agarose gel electrophoresis

1 What factors do I have to take into account when selecting the type of agarose?

The application will determine the proper agarose to choose. Gel electrophoresis techniques use standard /low melting regular and GQT (Genetic Quality Tested) grades and sieving agaroses, and finally sieving agaroses that separate large and small size DNA fragments which also have many uses including blotting, cloning, PCR amplification techniques, etc.

2 What agarose concentration should be used in the gel preparation?

The agarose concentrations that are commonly used to separate nucleic acids are found in the range of 0.5 % - 4 % (even as low as 0.3 % , if using D-5 agarose). The appropriate concentration depends on the type of agarose and the size of the fragments to be separated, as a general rule, higher concentrations must be used if smaller size fragments need to be separated.

3 What buffer should be used to optimize gel electrophoresis?

TAE and TBE are the typical buffers of choice. The two buffers are similar and may be used with any type of agarose; however, they do have different properties which make them appropriate for different applications. The 1X TAE buffer has a low ionic strength and low buffering capacity which makes it necessary to recirculate the buffer solution when electrophoresis run times are long, whereas the 1X TBE buffer has a high ionic strength and high buffering capacity allowing for extended run times ; this buffer is also recommended for smaller fragments, especially with small differences in sizes between them. Lastly, the 1X TAE buffer enhances separation of large DNA fragments.

4 What is the recommended buffer to be used in analytical electrophoresis?

Both buffers, TAE (1X) and TBE (1X or 0.5X), may be used. TAE provides better resolution for large fragments (> 10 kb), while TBE has less mobility and provides better resolution for smaller fragments (< 1 kb).

5 What is the recommended buffer in preparative electrophoresis?

If DNA need to be recovered from the gel for later manipulation, the TAE buffer is recommended. Where the TBE buffer to be used, the borate in the buffer would nteract with the hydroxyl groups of the agarose polysaccharide forming complexes that may hinder recovery.

6 Is it important to work with buffer recirculation during extended electrophoresis time periods?

Recirculation prevents the formation of a pH gradient and buffer depletion, therefore recirculation may be necessary for extended (> 5 hours) electrophoresis when TAE buffer is used because of its low buffering capacity.

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7 What voltage should be used for a proper electrophoresis conditions?

The recommended voltage is 4-10 volt/cm (distance between the anode and cathode, not the length of the gel) in horizontal electrophoresis. If the voltage is too low, the mobility of the bands is reduced, and band broadening will occur due to diffusion. If the voltage is too high, the resolution diminishes mainly due to the gel overheating.As a rule lower voltage (1-2V/cm) larger sizes (>10 Kb) and higher voltage (4-10 V/cm) smaller sizes (< 1Kb)

8 Sometimes the bands appear wavy - What could be the cause?

The most frequent cause of wavy bands is dried gel residues stuck to the teeth of the comb. To prevent this, the comb should be well cleaned and possible residues eliminated. Care is needed when withdrawing the comb from the gel to avoid dragging part of the gel with the comb. Maintaining the gel at 4ºC for 30 minutes is recommended, or dipping it in the buffer before withdrawing the comb.

9 What quantity of DNA should be loaded per well?

The quantity is variable, however, what is important is the quantity of DNA in the bands of interest. The minimum quantity of DNA that may be detected by means of EtBr staining is 10 ng. The amount of DNA depends on well volume, fragment size and distribution of fragment sizes. The maximum quantity of DNA that you may have in a band, which can still be clear and well defined, is approximately 100 ng. These quantities may vary if another staining system is used. To determine the appropriate quantity, various lines with different quantities may be loaded.

10 How should the gel be prepared to obtain the best resolution?

The thickness of the gel is very important so a 3-4 mm thick comb is recommended. The thickness of the comb is also important and signficantly affects the resolution. A thin comb (1 mm) produces very well dried bands, while a thick comb produces thick bands leading to reduced resolution.

11 Which agarose dissolution method is recommended?

Any method is appropriate. The most convenient and fastest method is dissolving the agarose in a microwaving. For high concentrations where viscosity and foaming make a difficult dissolution, dissolving in a boiling water bath is easier. Similarly, dissolving by autoclaving is very good option when working at very high concentrations, and when a sterile solution is desired.

12 How can foaming be avoided during the dissolving process?

It is recommended to hydrate the agarose powder in the buffer for 10-15 minutes before heating to complete the dissolution. The hydration time minimizes foaming and makes the dissolution easier. When microwaving, it is important not to superheat the agarose. As an option, if the microwave power is too high, foaming can be minimized by heating the flask for 1 minute, removing it from the microwave oven, gently swirl the flask to resuspend any settled powder, put again in the microwave and heat for another minute or untill total solution.