

# Most frequent questions with REGARDS TO AGAROSE

## 1. What factors do I have to take into account at the time of choosing the type of agarose?

There are numerous types of agarose that may be used. The right choice will determine the results of the work performed.

## 2. What agarose concentration should be used in the preparation of a gel?

The agarose concentrations which are most commonly used to separate nucleic acids are found in the range of 0.5% - 4% (even at 0.3% if agarose D5 is used).

The appropriate concentration in each case depends on the type of agarose and the size of the fragments needing to be separated. As a general rule you have to take into account that, the greater the concentration of agarose the smaller the fragments that are separated.

## 3. Can TAE or TBE buffers be used as alternatives in the preparation of the gels and following electrophoresis?

The two buffers are similar and may be used as alternatives with any type of agarose; however they have different properties which make them appropriate for different applications.

TAE buffer has a low ionic strength and low buffering capacity which makes it necessary to do buffer recirculation when electrophoresis time periods are long.

TBE buffer has a high ionic strength and high buffering capacity which allows working without recirculation in long electrophoresis time periods. It is recommended for the separation of small fragments and with small differences in sizes between them.

## 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 as alternatives. You have to take into account that buffer TBE has less mobility and gives out better resolution for fragments < 1kb, while for fragments > 10kb a better resolution is obtained with buffer TAE.

## 5. What is the recommended buffer in preparatory electrophoresis?

In the case of a preparatory gel, where the DNA from the gel needs to be recovered for later manipulation, a TAE buffer is recommended. If TBE is used, the bore present in this buffer interacts with the hydroxyl groups of the polysaccharide forming complexes which may hinder the recovery of the DNA.

## 6. Is it important to work with buffer recirculation during long electrophoresis time periods?

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

## **7. At what voltage should an agarose gel be run?**

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.

## **8. Sometimes the bands appear to undulate wavy in some lines - 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 4°C for 30 minutes is recommended, or dipping in buffer before withdrawing the comb.

## **9. What quantity of DNA is to be loaded per well?**

The quantity is variable, because what is important is the quantity of DNA in the bands which are to be separated.

The minimum quantity of DNA that may be detected by means of EtBr staining is 10 ng. 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 and a thickness of 3-4 mm is recommended.

The thickness of the comb is also important and significantly affects the resolution. A thin comb (1 mm) gives very well defined bands, while a thick comb gives thick bands leading to reduced resolution.

## **11. Which is the most recommended dissolving method?**

Any method is appropriate. The most convenient and fastest method is dissolving in a microwave.

For high concentrations where dissolving is more difficult due to the viscosity and formation of foam, dissolving in a boiling water bath is easier.

Dissolving by means of an autoclave is very good for very high concentrations and when a sterile solution is desired.

## **12. How can the formation of foam be prevented during the dissolving process?**

It is best to hydrate the agarose powder in the buffer for 10-15 minutes before heating to complete the dissolution. The hydration period reduces the amount of foam formed and makes the dissolution easier.