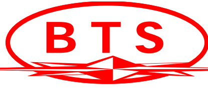

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BTS Provides the Best tools for Protein Purification!

PLAIN & CROSSLINKED AGAROSE BEADS

GENERAL DESCRIPTION

Agarose is a very inert polysaccharide which forms hydrophilic and high gel strength gels at low concentrations.

Agarose Beads are microspheres of agarose gels with different particle diameters and concentrations. Small spherical particles of agarose act as a porous gel to filter or separate a mixture of molecules according to their individual sizes. Due to its chemical structure (easy to activate), the agarose beads may be prepared to bind biomolecules in a reversible or irreversible manner.

Plain and crosslinked agarose beads are used in Gel Filtration Chromatography (or Molecular Exclusion Chromatography) as well as for activating beads for biomolecule purification or immobilization.

Gel Filtration Chromatography allows biomolecule separation depending on its size (molecular volume). It is widely used to fractionate proteins and water-soluble polymers. This technique is normally combined with other techniques based on different characteristics (acidity, alkalinity, charge, affinity by certain compounds, etc).

Gel Filtration Chromatography can be used for:

a) Group separation: separating big proteins from small ones or for de-salting a certain sample.
b) Fractionation of a biomolecule mixture: paying attention to the size of the components of that mixture.
c) Molecular volume determination.

The importance of the resolution is different depending on the application.

Agarose gels are composed of spherical particles with different pore sizes. Small and medium molecules pass in and out of the gel pores, slowing down as they pass through the column. Big molecules do not pass through gel pores, eluting in the so-called "dead space volume" of the column. Fractionation interval is the range of molecular weights a gel is able to separate. In this range molecules are separated depending on their size, eluting by molecular weight decreasing order. The volume of each protein when is eluted is called the elution volume, and it decreases in line with the molecular weight logarithm. So performing a previous calibration with 4-5 pure protein samples (with known molecular weight) it is possible to determine the molecular weight of a given protein by comparison to the molecular standards.

Plain and cross-linked agarose beads can also be used for activating processes, generating active groups inside its pores capable of reversible or irreversible biomolecule bonds. Due to the bead's large internal surface and to its composition (inert polysaccharide), agarose is an ideal medium for the preparation of activated beads.

ABT offers a wide range of plain and crossslinked agarose beads with different agarose concentrations (2, 4, 6, 8% & 10%) in different particle size distributions.

CHROMATOGRAPHY THEORY

SELECTIVITY CURVE

The selectivity curve of a matrix is a plot of Kav versus the logarithm of molecular weight for selected standards. Kav is a function of the elution volume of a molecule and is defined as (Ve-Vo)/(Vt-Vo) where Ve is the elution volume, Vo is the void volume and Vt is the total column volume. The term Kav indicates the ratio between the elution volume of a given molecule and the total available volume of the column. Its always has a value between 0 and 1.

If a molecule has a Kav of 0, the elution volume = the void volume.
If the Kav is 1, the elution volume = the total liquid column volume.

Kav = 0 if Ve = Vo
Kav = 1 if Ve = Vt

If a Kav is calculated to be less than 0, the column likely has developed channels and should be repacked.
If a Kav is calculated to be greater than 1, some kind of adsorption is indicated. Consider a buffer composition change to avoid the adsorption effect. If a peak elutes with a volume larger than the total column volume, the peak is not separated according to a gel filtration mechanism alone. Adsorption has occurred and one should consider changing the buffer conditions.

An advantage of calculating Kav instead of using elution volume to plot calibration curves, is that Kav allows one to compare different media. For a well packed, high performance column if the Kav values of two molecules differ by 0.2 or more, baseline separation can be achieved. For purification purposes, it is best to choose a gel with a selectivity range where the Mr of the molecule of interest is located near the middle of the linear range of the curve (e.g. Kav around 0.5).

A selectivity curve is usually fairly straight over the range Kav=0.1 to Kav=0.7. The steeper the linear portion of this curve, the greater the difference in elution volume for two molecules of different sizes.

LINEAR FLOW RATE

Linear flow rate is related to volumetric flow rate through the column diameter. It is useful when comparing columns of different dimensions.

$$\text{Linear flow rate (cm/hr)} = \frac{\text{volumetric flow rate (cm}^3\text{/min) X 60min}}{\text{Cross sectional area of column (cm}^2\text{)}}$$

LOADING CAPACITY OF A GEL FILTRATION COLUMN

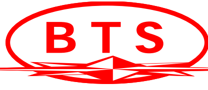
Volume is the limiting factor in gel filtration. Unless the concentration of the protein begins to affect the viscosity of the sample (this concentration varies from one protein to another), the amount of protein is not limited. For fractionating and sample analysis, we recommend no more than 1-2% of the bed volume. For desalting or group separations, the volume can be somewhat larger – 10 to 20% of the bed volume.

EXCLUSION LIMIT

The exclusion limit is the molecular weight of the smallest molecule which cannot enter the pores of the matrix. All molecules bigger than the exclusion limit elute in the void volume. The exclusion limit means that the sample is no longer fractionated and will be excluded from entering the pores. This limit will be just above the upper limit of the fractionating range.

FRACTION RANGE OF THE GEL

A selectivity curve is usually fairly straight over the range Kav=0.1 to Kav=0.7
The molecular weight range, which lies between these values, is defined as the useful fractionation range of the medium.



Tips for selecting an appropriate gel filtration media

Look for:

- Correct separation range
  (Since the selectivity curves of the various gels do overlap, more than one gel type may seem appropriate for a particular separation. In this case it is normally best to use the gel with the lower exclusion limit, because the substances of interest will be eluted sooner, possible improving the recovery of active molecules. Furthermore, considering gels with the same chemical composition, one with a lower exclusion limit will have greater rigidity allowing greater flexibility in the choice of flow rate.)
- Steep selectivity curve
- Small, uniform bead sizes
- Low non-specific absorption properties
- Scale-up possibilities (if necessary)
- Reliable, consistent supply.