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TECHNICAL INFORMATION

Catalog Number: 191495, 191496, 193452
Agarose Beads

Description: Beaded agarose for fractionating molecules of high molecular weight by gel filtration. Cross-linked beaded agarose is more resistant to denaturing conditions, and thus offers more versatility in the choice of sample buffer and eluent.

The term gel filtration (GF) refers to the ability of a porous gel to "filter" or separate a mixture of macromolecules according to their individual hydrated sizes. The hydrated size of a molecule is typically a function of its molecular weight, branching, the ambient pH, ionic strength, and some other factors (ambient detergents, urea or equivalents, chelators, or chaotropic agents). The latter parameters can, therefore, be chosen to facilitate certain molecular separations by GF. Scaled-up or preparative GF is often used as a molecular purification technique.

GF is done under low, medium, and high pressure conditions. Most agarose beads, however -- even after epichlorhydrin crosslinking -- can only be used under low pressure. "Low Pressure" means < 2 PSI or 140 cm of fluid "head pressure". Fluid head pressure refers to the distance from the liquid surface of the eluant reservoir to the point at which the eluant discharge becomes discontinuous (i.e. forms drops).

Fractionation Ranges:

Agarose Bead Concentration	Protein Fractionation Range (MW in daltons)	Polysaccharide Fractionation Range (MW in daltons)	DNA Fractionation Range (base pair)
2%	80,000 to 40,000,000	90,000 to 20,000,000	1340
4%	50,000 to 15,000,000	40,000 to 5,000,000	860
6%	10,000 to 5,000,000	10,000 to 1,000,000	180

Crosslinking: None

Slurry Concentration: 80% settled beads; 20% supernatant

Preservative: 0.03% sodium azide

Gel Exclusion Limit: Regardless of the agarose concentration chosen, there is always a molecular size for proteins, polysaccharides and DNA, which will not enter the pores of the gel. This is the smallest molecule which is excluded from the gel and is therefore called the exclusion limit. All molecules larger than the exclusion limit, expressed in either daltons (for proteins and polysaccharides) or base pairs (for DNA) will also be excluded from the gel and will pass through the column in the "void volume".

Chromatographic Suggestions & Tips[1]

1.**Sample volume:** Typically, about 1-4% of the total bed volume.
2.**Sample viscosity:** The sample viscosity should not be more than twice the viscosity of the column eluant.
3.**Sample application:** The sample should be evenly applied to the gel bed so as to minimize bed disturbance and sample

dilution.
4.**Need for crosslinked gel beads:** If urea, guanidine, KSCN, DMSO or similar reagents are to be used in the eluant, then a crosslinked gel will avoid significant bead softening. Similarly, if the gel beads are to sterilized either with alkali or by autoclaving, crosslinked beads must be used to avoid dissolution of the agarose gel.
Note: Contrary to other agarose bead suppliers' information, *non-crosslinked agarose gels will not remelt above 40ºC.* They gel at about 40ºC but exhibit hysteresis (GT does not equal Melt temp) and will not remelt until about 90-100ºC. Thus, it is possible to safely "pasteurize" the beads by incubation at 55ºC for about 5 minutes.
5.**Cold sterilization:** As an alternative to the pasteurization method above, it is also possible to cold sterilize slurries of beads using a 0.01 % solution of diethyl pyrocarbonate.

Analytical vs. Preparative Chromatography Applications[1]

Note how the choice of "the best" chromatographic beads and other conditions will vary as a function of whether your goals are analytical or preparative:

Analytical Method (Top priority: Maximum Resolution)

– smallest possible beads
– longest possible column
– optimum gel pore size
– small sample loading
– slowest practical flow rate (column pressure)

Preparative Method (Top priority: Maximum Thruput)

– largest possible beads
– shortest (and widest) column
– optimum gel pore size
– largest permissible sample loading
– fastest flow rate (i.e. highest permissible pressure)

How to Select the Appropriate Pressure Range[1]

1.**Step #1:** Note the agarose concentration and the bead size. In general, the gel strength will increase as a semilog function of increases in the agarose concentration. Similarly, the flow rate is directly proportional to the size of the beads.

2.**Step #2:** Using the recommended guide for column pressure as a function of the concentration and size of the media being used is the easiest way to begin.

To determine the practical upper pressure limit for a given sample of beaded media, the flow rate should be monitored at intervals as the head pressure is gradually increased either by increasing the height of the eluent reservoir or increasing the pump pressure (i.e. flow rate). When the plot of flow rate (y axis) vs. head pressure is no longer linear (i.e. begins to plateau) the upper practical pressure limit has been reached. The leveling off of flow with increased pressure results from bead compression which to occlude the interbead channel and therefore begins to restrict the flow of eluent through it.

3.**Alternative Units for Measuring Column Head Pressure**

Relative Pressure Term	PSI (lbs/sq. in.)	Height of Water	Atmospheres (Atm)	Bar	kilo-Pascals (kPa)
"Low"	< 2	< 140 cm	< 0.137	< 0.137	< 13.7
"Medium"	2 - 5	140 -350 cm	0.137 - 0.345	0.137 - 0.345	13.7 - 34.5
"High"	> 5	> 350 cm	> 0.345	> 0.345	> 34.5

"Atmospheric"	14.7	33.9 **Feet**	1.0	1.01	101

4.**Low, Medium, and High Pressure Liquid Chromatography Terminology**

Low Pressure: generally refers to operating pressures below 2 PSI (or 140 cm of water; most agarose bead usage is in this range)

Medium Pressure: generally from 2-5 PSI

High Pressure (i.e. HPLC): any pressure above 5 PSI.

Important: No matrix should be subjected to a pressure above the point where flow rate has begun to plateau with increasing pressure. To do so could irreversibly compress or deform the chromatography beads.

Availability:

Catalog Number	Description	Size
191495	Agarose Beads 2% Particle Form: > 95% spherical Exclusion Limit 30,000,000 daltons 100% smaller than 250 microns 80% smaller than 210 microns 90% larger than 62 microns 100% larger than 37 microns	100 ml 250 ml 500 ml 1 liter
193452	Agarose Beads 4% Particle Form: > 95% spherical Exclusion Limit 15,000,000 daltons 100% smaller than 250 microns 80% smaller than 210 microns 90% larger than 62 microns 100% larger than 37 microns	100 ml 250 ml 500 ml 1 liter
191496	Agarose Beads 6% Particle Form: > 95% spherical Exclusion Limit 5,000,000 daltons 100% smaller than 250 microns 80% smaller than 210 microns 90% larger than 62 microns 100% larger than 37 microns	100 ml 250 ml 500 ml 1 liter

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