Data & Instructions

Epoxy-activated SepFast MAG

Epoxy-activated SepFast MAG is a magnetic agarose resin that can be readily employed to make various magnetic affinity chromatography adsorbents for both small scale and large scale purification applications.

Epoxy-activated agarose can be used for the immobilization of sugars and other carbohydrates via stable ether linkages with hydroxyl groups. It can also be used for the coupling of primary amine-containing or thiol-containing molecules. Coupling biospecific ligands to Epoxy-activated agarose is a successful and well-documented technique. The reaction is very easy to conduct. No toxic chemical or special equipment is required. All the chemical bonds formed are very stable.

Epoxy activated SepFast is useful in coupling both small molecules and very large molecules. This pre-activated agarose base matrix can be readily employed to make various magnetic affinity chromatography media for both small scale and large scale purification applications.

1. Properties

There is a choice of two different Epoxy activated magnetic resins that suits various ligands and applications.

- Epoxy activated SepFast MAG 4HF is made of highly cross-linked 4% agarose containing magnetic material. It shows high mechanical rigidity and allows harsher operational conditions, such as concentrated organic solvents, to be applied. It is useful for the coupling of protein molecules.

- Epoxy activated SepFast MAG 6HF is made of highly cross-linked 6% agarose containing magnetic material. It shows high mechanical rigidity and allows harsher operational conditions, such as concentrated organic solvents, to be applied. It has high levels of epoxy groups for applications that require high ligand densities. It is useful for the coupling of small molecules or peptides etc.

Agarose has long been used for chromatographic separations due to its excellent hydrophilic and low non-specific-binding nature. The particles have an open pore structure with excellent mass transfer properties to various molecules.

The material is activated through attaching a hydrophilic carbon chain with epoxy group in the end. The main characteristics are summarized in Table 1.

Table 1: Characteristics of Epoxy-activated SepFast MAG:

Group to be coupled	$-NH_2$, -OH, -SH
Matrix	SepFast MAG 4HF: Highly cross-linked 4% magnetic agarose SepFast MAG 6HF: Highly cross-linked 6% magnetic agarose
Particle size*	50 – 150 μm
Activation level	>30 μmol epoxy / ml for SepFast MAG 4HF >80 μmol epoxy / ml for SepFast MAG 6HF
pH stability	4 -13 (ligand dependent)
Chemical stability	Compatible with all commonly used aqueous chemicals, provided the

	ligand to be coupled can withstand. Certain organic solvents could be used.
Storage	+4^{O}C - +8^{O}C

Other particle sizes available on request.

2. Ligand immobilization

2.1 Pre-treatment of the medium

Epoxy-activated SepFast MAG resin (slurry format) is stored in 20% denatured ethanol. It needs be washed with deionised water first to remove the ethanol.

2.2 Ligand coupling

The following is a general ligand coupling procedure.

2.2.1 Dissolve the target ligand in the coupling buffer. For small ligands, at least 100 μmol/ml gel is recommended. For coupling via hydroxyl groups, a higher ligand concentration (e.g. 1000 to 3000 μmol/ml for small ligands and 10% to 30% w/v for polymers) may be required.

Organic solvents may be used.

The volume ratio of coupling buffer to activated medium should be 0.5 : 1 to 1 : 1.

2.2.2 Transfer the washed and suction dried gel (from step 2.1.2) to the above solution.

2.2.3 Mix the slurry at 20^{o}C - 40^{o}C overnight (at least 16 hrs).

2.2.4 Wash the gel with at least 5 gel volumes of the coupling buffer.

2.2.5 Re-suspend the gel to the same volume of blocking solution, such as 0.1 M to 0.5 M ethanolamine, pH 13, at 40^{o}C to 50^{o}C for 4 hr or overnight at room temperature.

2.2.6 Wash the gel with 5 volumes of 0.1 M Tris/HCl + 1 M NaCl, pH 8.0, followed with 5 volumes of 0.1 M acetate buffer + 1 M NaCl, pH 4.0. Repeat this washing cycle 2 more times.

2.2.7 Wash the gel with working / equilibration buffer before use.

3. General considerations over the immobilization efficiency

3.1 pH

Typically, pH 9 to pH 13 is required to couple most ligands. Coupling of hydroxyl groups requires high pH such as pH 13 or over.

The stability of the ligand in the above pH range should be considered.

3.2 Coupling solution

A solution containing amino groups or other nucleophilic components should be avoided.

Distilled water, carbonate or phosphate buffers can be used. Sodium hydroxide can be used to generate a high pH.

Certain organic solvents in diluted format may be introduced to improve the solubility of the ligand. The suitability of such solvents should be tested in advance. The volume of coupling solution to the volume of activated gel should be consistent.

3.3 Temperature
Reaction at 20°C - 40°C under temperature control is desirable for reproducible results.

3.4 Ligand concentration
For certain ligands or applications, a vast excess of ligand should be added to achieve the desired immobilization level.

3.5 Reaction time
The contact time between a ligand and the activated medium during a coupling process may be optimized to maintain the biological activity of the ligand and the coupling efficiency. Higher reaction temperature or higher pH will reduced the required reaction time.

3.6 Blocking remaining activated groups
The activated groups that haven't reacted with the ligand should be capped by adding extra small molecules containing primary amines at pH 12 to 13, such as Tris or ethanolamine.

3.7 Washing of the final medium
The non-attached or weakly attached ligand needs be fully washed away after the coupling reactions. A washing method employing alternating high pH and low pH can ensure an efficient removal of the unwanted species.

4. Use of the immobilized affinity medium

The ligand coupled medium can be used for purifications using batch stirred tank mode or packed column mode. Handling of this material follows the same principles as handling of other agarose-based media.

5. Storage

Epoxy-activated SepFast MAG media should be stored under 8°C. The coupled wet adsorbents should be stored in the presence of a bacteria-proof agent (e.g. 20% ethanol) at 4-8°C. Never freeze the coupled media.

6. Further information

Visit www.biotoolomics.com for further information or contact the technical team or sales representatives.

7. Ordering information

Product	Quantity	Code no.
Epoxy-activated SepFast 4HF	50 ml	410201
	300 ml	410202
	1 litre	410203
Epoxy-activated SepFast 6HF	50 ml	410204
	300 ml	410205
	1 litre	410206

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