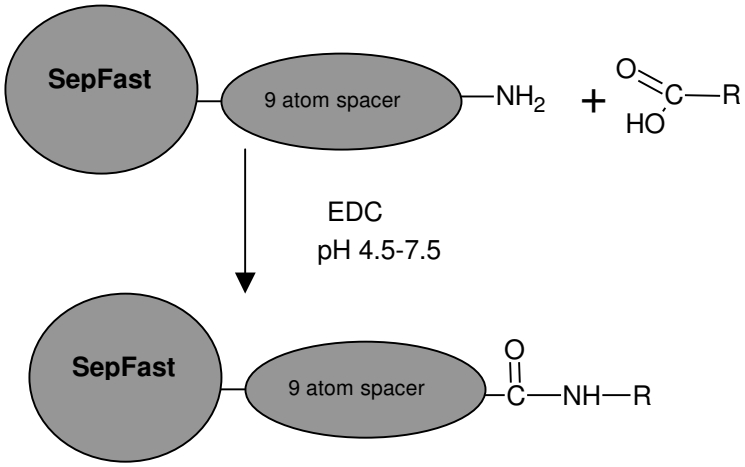
Data & Instructions

Amine-activated SepFast

Amine-activated agarose beads can be used for the immobilization of carboxyl containing or aldehyde containing molecules. The coupling chemistry is carbodiimide-based and is a successful and well-documented technique.

Amine-activated SepFast forms a chemically stable amide linkage with ligands containing carboxyl groups. It has a long spacer (9 atoms). The pre-activated agarose base matrix can be readily employed to make various custom affinity chromatography adsorbents for both small scale and large scale purification applications.



1. Properties

Amine-activated SepFast 4 High Flow (4HF) is made of highly cross-linked 4% beaded agarose. It shows high mechanical rigidity allowing high flow throughput with reduced back pressure.

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 large protein molecules.

The base matrix is activated by attaching a long hydrophilic chain with an amine group in the end. They react with either carboxyl containing ligands in the presence of a carbodiimide agent, or aldehyde containing ligands in the presence of a reducing agent. Amine-activated SepFast is supplied as an aqueous suspension in 20% ethanol. The main characteristics are summarized in Table 1.

Table 1: Characteristics of Amine-activated SepFast 4HF:

Group to be coupled	-COOH, CHO
Matrix	Highly cross-linked 4% agarose beads (4HF)
Particle size	50 – 150 µm
Spacer arm	9 atoms

Activation level	10 - 25 μmol amine / ml medium
pH stability	2 -13 (ligand dependent)
Chemical stability	Compatible with all commonly used aqueous chemicals, provided the ligand to be coupled can withstand
Storage	$+4^{O}C$ - $+8^{O}C$

2. Ligand immobilization

The following is a general coupling procedure for carboxyl containing ligands.

2.1 Dissolve the target ligand in coupling buffer. Adjust pH to 4.5. The volume of the coupling buffer should be the same as or half that of the settled gel.

2.2 Wash the Amine-activated gel with at least 5 volumes of coupling buffer in a filtration device.

2.3 Transfer the washed and suction dried gel (from step 2.2) to the solution prepared in step 2.1.

2.4 Add carbodiimide as a dry powder or a pre-dissolved solution to the slurry, to a final concentration of 0.1 M. If carbodiimide is pre-dissolved in water, adjust the pH to 4.5.

2.5 Mix the slurry at room temperature or at $+4^{O}C$ - $+8^{O}C$.

2.6 Monitor and adjust the reaction pH to 4.5 during the first 1 hr by adding 0.1 M sodium hydroxide.

2.7 Leave the coupling reaction from 2 hrs to 24 hrs.

2.8 Wash the gel with 5 volumes of 0.1 M acetate buffer + 0.5 M NaCl, pH 4.0, followed by 5 volumes of 0.1 M Tris/HCl + 0.5 M NaCl, pH 8.0. Repeat the above washing cycle 2 more times.

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

3. General considerations over the immobilization efficiency

3.1 pH

The coupling reaction proceeds quite efficiently between pH 4.5 to pH 7.5. However, the coupling pH may be optimized to get the best result (e.g. high coupling yield with high ligand activity).

The pH value decreases during the first hour of the coupling reaction. Always remember to adjust the coupling pH by adding 0.1 M sodium hydroxide. The pH value will become stable afterwards.

3.2 Coupling solution

Deionized water can be used directly as a coupling solution. A buffer solution containing amino groups, sulphhydryls or carboxyl groups should be avoided, as these will compete with ligand coupling.

Certain organic solvents in diluted form may be introduced to improve the solubility of the ligand. The suitability of such solvents should be tested in advance.

3.3 Choice of carbodiimide

A water soluble carbodiimide should be used, for example 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) or 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide (CMC) (usually synthesized as the metho-p-toluene sulphonate salt). EDC is the most frequently used carbodiimide agent.

3.4 Concentration of carbodiimide

The amount of carbodiimide added should exceed the activated amine groups in a stoichometric way. 0.1 M in the final reaction slurry is recommended as a starting point.

3.5 Reaction time and temperature

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.
The typical reaction time is overnight at room temperature or in a cold room.

3.6 Blocking remaining activated groups

If an excess amount of ligand is added, blocking of the residual activated groups may not be necessary.

The activated groups that haven't reacted with the ligand should be capped by adding extra small molecules containing primary amines at pH 8 to 9, such as 1 M ethanolamine, in the presence of carbodiimide.

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

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

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.
Amine-activated SepFast 4HF	5 ml	350101
	50 ml	350102
	1 litre	350103

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