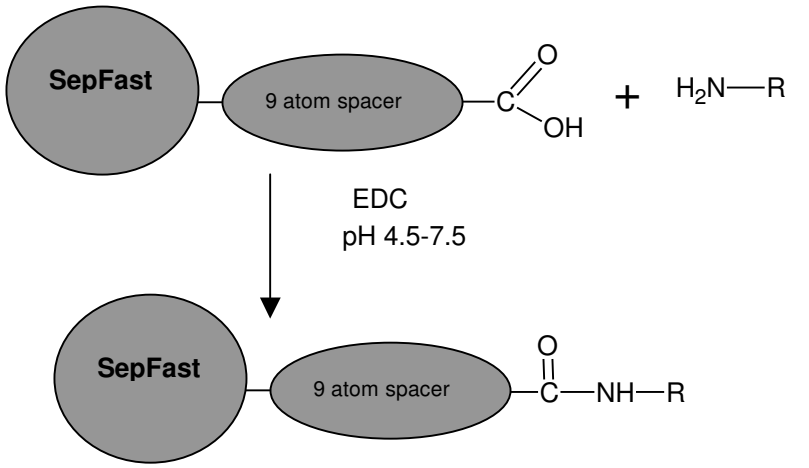
Data & Instructions

Carboxyl-activated SepFast

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

Carboxyl-activated SepFast forms chemically stable amide linkages with ligands containing primary amine groups. It has a long spacer (9 atoms). This pre-activated agarose base matrix can be readily employed to make various custom affinity chromatography media for both small scale and large scale purification applications.



1. Properties

Carboxyl activated SepFast 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 spacer arm to the backbone. It reacts directly with the primary amine groups in molecules to be immobilized in the presence of a carbodiimide agent. Carboxyl-activated SepFast is supplied as an aqueous suspension in 20% ethanol. The main characteristics are summarized in Table 1.

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

Group to be coupled	$-NH_2$
Matrix	Highly cross-linked 4% agarose beads (for 4HF)
Particle size	50 – 150 μm
Spacer arm	9 atoms
Activation level	8 - 19 μmol carboxyl / 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 ligand coupling procedure.

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 Carboxyl-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 with 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 carboxyl 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

Carboxyl-activated SepFast 4HF should be stored under 8^{o}C. The coupled wet medium should be stored in the presence of a bacteria-proof agent (e.g. 20% ethanol) at 4-8^{o}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.
Carboxyl-activated SepFast 4HF	5 ml	380101
	50 ml	380102
	1 litre	380103

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