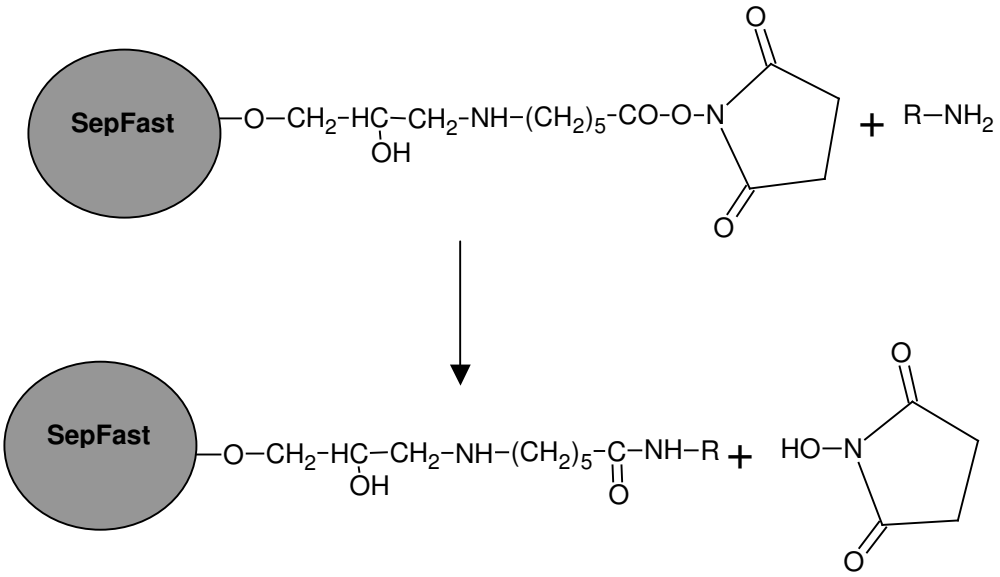
Data & Instructions

NHS-activated SepFast

NHS (N-hydroxysuccinimide)-activated agarose has a well-proven track record for the preparation and use of custom affinity chromatography media. Coupling biospecific ligands to NHS-activated agarose is a successful and well-documented technique. The coupling reaction is spontaneous, rapid and easy to carry out. No toxic chemicals or special equipment is required.

NHS-activated SepFast media forms chemically stable amide bonds with ligands containing primary amine groups. The activated NHS group has a long spacer arm (11 atoms) that is particularly helpful in the immobilization of structurally restricted ligands such as proteins and peptides. This 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

NHS 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 in a packed column.

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 base matrix is activated through a long hydrophilic spacer arm (11 atoms) with N-hydroxysuccinimide at the end. It reacts directly with the primary amine groups in molecules to be immobilized to form stable amide bonds. NHS-activated SepFast media is supplied as a suspension in 100% isopropanol. The main characteristics are summarized in Table 1.

Table 1: Characteristics of NHS-activated SepFast media:

Group to be coupled	$-NH_2$
Matrix	SepFast 4HF: highly cross-linked 4% agarose
Particle size	50 – 150 μm
Activation level	5 - 19 μmol NHS / ml medium
pH stability	3 -13 (ligand dependent)
Chemical stability	Compatible with all commonly used aqueous chemicals, provided the ligand to be coupled can withstand
Storage	$+2^{O}C$ - $+8^{O}C$

2. Ligand immobilization

The following is a general ligand coupling procedure.

2.1 Dissolve the target ligand in coupling buffer, 0.1 M $NaHCO_3$, pH 8.3 containing 0.5 M NaCl. In general, for protein ligands, make a gel concentration of 5 – 20 mg/ml. For small ligands, make a 1 – 10 μmol/ml gel. The volume of the coupling buffer should be the same as or half that of the settled gel.

2.2 Wash the NHS-activated gel with cold 1 mM HCl in a filtration device as quick as possible. The total volume of acid required is 10 to 15 gel volumes. The washed medium should be used immediately in the following coupling steps.

2.3 Transfer the washed and suction dried gel (from step 2.2) to the solution prepared in step 2.1. Adjust the pH if it has shifted.

2.4 Mix the slurry at $4^{o}C$ overnight or at room temperature for 2-4 hrs.

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

2.6 Re-suspend the gel to the same volume of blocking solution, 0.1 M Tris/HCl, pH 8.0 or 1 M ethanolamine pH 8.0, for 2-3 hrs.

2.7 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.

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

3. General considerations over the immobilization efficiency

3.1 pH

A buffer at pH 8.3 is most frequently used for protein immobilization. However, the coupling pH may be optimized between 6 to 9 to get the best result (e.g. high coupling yield with high ligand activity).
Always remember to adjust the coupling pH after a ligand has dissolved.

3.2 Coupling solution

A solution containing amino groups should be avoided.

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.

The volume of coupling solution to the volume of activated gel should be consistent. The ideal ratio of 0.5-1 : 1 v/v is recommended. The presence of less water will reduce the chance of hydrolysis of the activated NHS.

3.3 Salt

The presence of salt in the coupling buffer may improve the immobilization efficiency.

3.4 Activated groups in the base matrix

For certain ligands or applications, the activation level in the base matrix may be too high and the activity of the coupled ligand could be reduced.

Coupling at a reduced pH may reduce the points that a ligand molecule is attached by. It may improve the activity of the coupled ligand. Controlled hydrolysis of the activated groups, such as incubating the gel with coupling buffer a few hours before a ligand is added, could also reduce the over-coupling issue.

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.

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 8 to 9, such as Tris or ethanolamine.

3.7 Washing of the final medium

The non-attached or weakly attached ligand needs to 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 media 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

NHS-activated SepFast media should be stored in 100% isopropanol 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.
NHS-activated SepFast 4HF	5 ml	330104
	50 ml	330105
	1 litre	330106

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