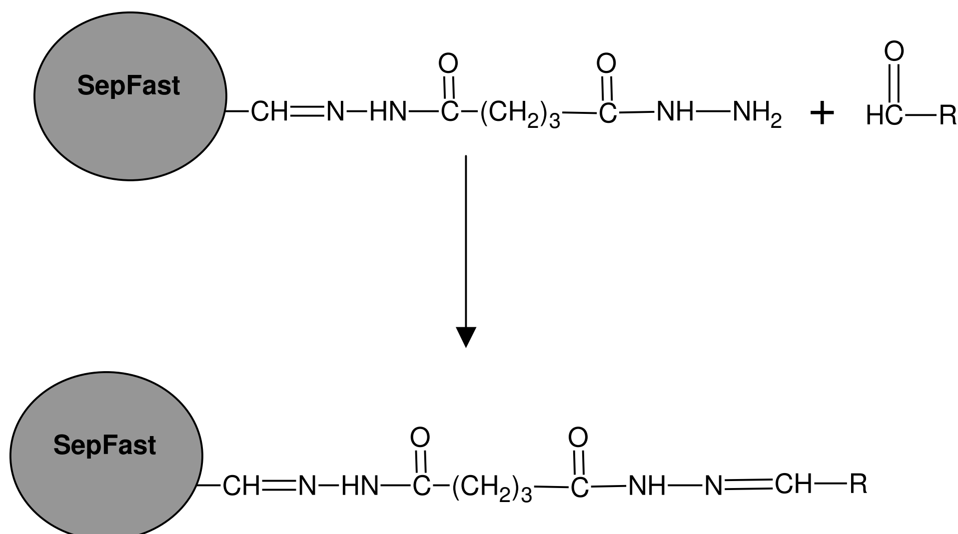
Data & Instructions

Hydrazide-activated SepFast

Hydrazide-activated agarose beads permit the coupling of aldehyde- or ketone-containing ligands through the formation of stable hydrazone linkages. It is a particularly powerful way to immobilize glycoproteins via their carbohydrate chains, while leaving critical active sites intact. It is a method so called “site-directed immobilization”. For example, antibodies can be immobilized to an agarose solid support through a linkage to their glycosylated side chain, which leaves their Fab region free for antigen binding.

Glycoproteins can be readily oxidized with sodium periodate to generate formyl groups on their carbohydrate chains before the coupling reaction with Hydrazide-activated agarose beads. It is a successful and well-documented technique. The coupling reaction is mild and easy to carry out. No toxic chemical or special equipment is required.

Hydrazide-activated SepFast resins can be readily employed to make custom affinity chromatography media for both small scale and large scale purification applications.



1. Properties

Hydrazide 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 with hydrazide through a long hydrophilic spacer arm. Hydrazide-activated SepFast media is supplied as an aqueous suspension. The main characteristics are summarized in Table 1.

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

Group to be coupled	-HC=O (aldehyde), -C=O (ketone)
Matrix	Highly cross-linked 4% agarose beads
Particle size	50 – 150 µm
Coupling level	>2 mg hIgG / ml medium
pH stability	3-11 (ligand dependent)
Chemical stability	Compatible with all commonly used aqueous chemicals, provided the ligand to be coupled can withstand
Storage	+4°C - +8°C

2. Ligand immobilization

2.1 Oxidation of glycoproteins

Glycoproteins need to be oxidized with sodium periodate to generate formyl groups on their carbohydrate chains first.

2.1.1 Dissolve or dilute glycoprotein to a concentration of 1 – 10 mg / ml in coupling buffer, 0.1 M sodium phosphate, pH 7.0, in an amber container.

2.1.2 Add sodium meta-periodate (5 mg / ml coupling buffer). Gently mix to dissolve the oxidizing agent.

2.1.3 Incubate the sample in the dark at room temperature for 30 minutes. The reaction is light sensitive.

2.1.4 Stop the reaction by desalting and buffer exchange in a suitable gel filtration column.

2.2 Coupling reaction

The following is a general ligand coupling procedure.

2.2.1 Wash Hydrazide-activated SepFast medium with at least 5 gel volumes of coupling buffer in a filtration device.

2.2.2 Transfer the washed and suction dried gel to the oxidized glycoprotein solution. The volume ratio between protein solution and gel should be 1 : 1.

2.2.3 Mix the slurry at room temperature overnight.

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

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

3. General considerations over the immobilization efficiency

3.1 pH

A buffer with neutral pH is most frequently used for protein immobilization. However, the coupling pH may be optimized between 6 to 10 to get the best result (e.g. high coupling yield with high ligand activity).

3.2 Coupling solution

A solution containing ketone groups should be avoided.

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.

3.3 Further reductive amination

The stability of the hydrazone bond formed between hydrazide and formyl groups can be further improved by mild reductive amination. Sodium cyanoborohydride can be added to convert the hydrazone bond to a secondary amino bond.

3.4 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 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

Hydrazide-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.
Hydrazide-activated SepFast 4HF	5 ml	450104
	50 ml	450105
	1 litre	450106

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