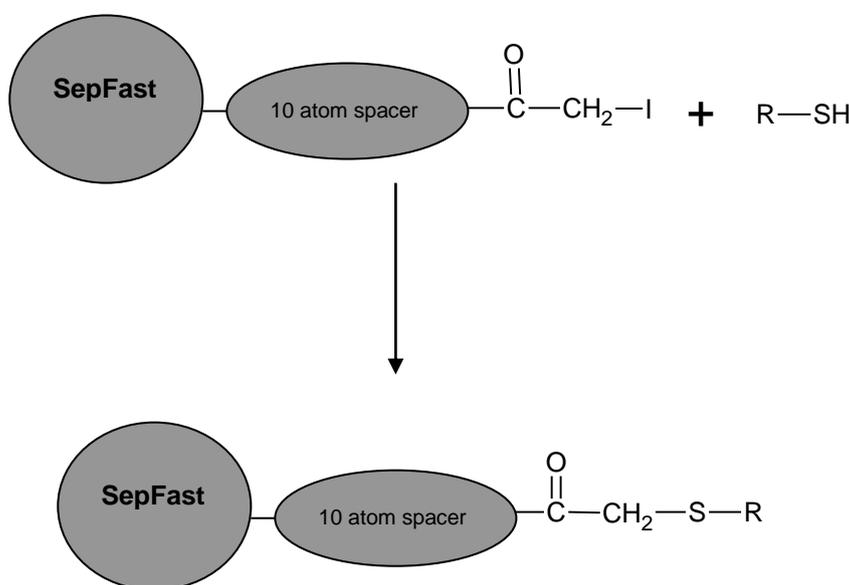


Data & Instructions

Irreversible Thiol-Coupling SepFast

Irreversible Thiol-coupling SepFast can be used for the specific immobilization of thiol containing molecules. The coupling chemistry is based on a haloacetyl activation method that is a successful and well-documented technique. The reaction to the thiol group is highly specific and the formed bond is irreversible. The coupling reaction is rapid and easy to carry out. No toxic chemicals or equipment is required.

Irreversible Thiol-coupling SepFast media forms an extremely stable thioether bond with ligands containing thiol (i.e. sulphhydryl) groups. It has a long spacer arm that facilitates much improved ligand accessibility. This pre-activated agarose based resin can be readily employed to make various custom affinity chromatography media for both small scale and large scale purification applications.



1. Properties

There is a choice of two different activated base matrices that suit ligands of various molecular weights.

- Irreversible Thiol-Coupling 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.
- Irreversible Thiol-Coupling SepFast 6HF is made of highly cross-linked 6% 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 by attaching a haloacetyl group through a long hydrophilic spacer arm. It reacts rapidly with molecules containing thiol groups. Irreversible Thiol-coupling SepFast media is supplied as an aqueous suspension. The main characteristics are summarized in Table 1.

Table 1: Characteristics of Irreversible Thiol-coupling SepFast media:

Group to be coupled	-SH
Matrix	SepFast 4HF: highly cross-linked 4% agarose SepFast 6HF: highly cross-linked 6% agarose
Particle size	50 – 150 μm
Coupling level	At least 3 mg BSA / 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 $^{\circ}\text{C}$ - +8 $^{\circ}\text{C}$

2. Ligand immobilization

The following is a general ligand coupling procedure.

2.1 Dissolve the target ligand in coupling buffer, 50 mM Tris / HCl, 0.15 M NaCl, 5 mM EDTA, pH 8.5. In general, for protein ligands, make a gel concentration of 5 – 20 mg/ml. For small ligands, make a 10 – 100 $\mu\text{mol/ml}$ gel.

The volume of the coupling buffer should be the same as or half that of the settled gel.

2.2 Wash the Irreversible Thiol-coupling 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 Mix the slurry at room temperature for 1 hour.

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

2.6 Mix the resin with 50 mM cysteine in the coupling buffer for 30 mins at room temperature to block any non-reacted sites.

2.7 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.

2.8 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 8.0 to pH 8.5. However, the coupling pH may be optimized 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 buffer solution containing sulphhydryls should be avoided, as these will compete with ligand coupling.

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 Reaction time and temperature

The reaction between a ligand and the activated medium is fast. The typical time is 1 hour at room temperature. However, time and temperature may be optimized to maintain the biological activity of the ligand.

The activated medium is very light sensitive. The coupling reaction should be protected from light for a prolonged coupling process.

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 free thiols at pH 8 to 9, such as cysteine. The impact of the immobilized blocking agent on the affinity of the target ligand should be considered.

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 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

Irreversible Thiol-coupling SepFast media should be protected from light and 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.biotooolomics.com for further information or contact the technical team or sales representatives.

7. Ordering information

Product	Quantity	Code no.
Irreversible Thiol-coupling SepFast 4HF	5 ml	390101-5ML
	50 ml	390101-50ML
	1 litre	390101-1L
Irreversible Thiol-coupling SepFast 6HF	5 ml	390104-5ML
	50 ml	390104-50ML
	1 litre	390104-1L



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