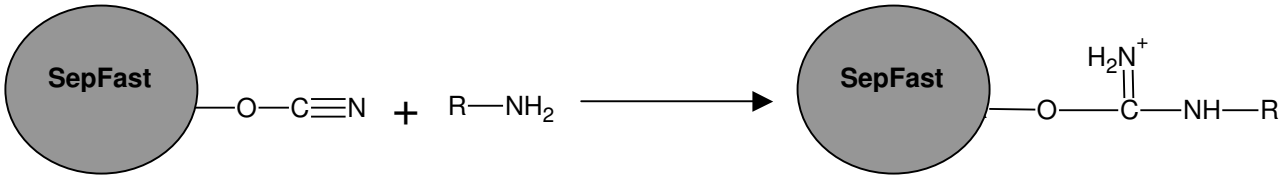
Data & Instructions

CNBr-activated SepFast

CNBr-activated agarose beads have a well-proven track record for the preparation and use of custom affinity chromatography media. Coupling biospecific ligands to CNBr-activated agarose is a widely used, successful, and well-documented technique. The coupling reaction is spontaneous, rapid and easy to carry out. No toxic chemicals or special equipment is required.

This pre-activated SepFast beads can be readily employed to make various affinity chromatography media for both small scale and large scale purification applications. The application areas cover immobilization of proteins, peptides and nucleic acids.



1. Properties

CNBr 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 reacted with cyanogen bromide (CNBr) to create active groups that react with the primary amine groups in the molecules to be immobilized. CNBr-activated SepFast media is supplied as a freeze-dried powder stabilized with additives. The main characteristics are summarized in Table 1.

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

Group to be coupled	$-NH_2$
Matrix	Highly cross-linked 4% agarose beads
Particle size	50 – 150 µm
Swelling factor	3 – 5 ml / g powder
Coupling level	> 5 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	$+4^{O}C$ - $+8^{O}C$

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. 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 that of the settled gel.

2.2 Weigh out the required amount of powder (1 g dry powder gives about 9 - 12 ml of settled medium) and suspend it in cold 1 mM HCl (use 20 ml cold 1 mM HCl for 1 g dry powder). Leave the medium to swell at a cold temperature for 2 to 3 mins.

2.3 Remove the liquid in a filtration device quickly. The rehydrated medium should be used immediately in the following coupling steps.

2.4 Transfer the washed and suction dried gel to the coupling solution.

2.5 Mix the slurry at 4°C overnight or at room temperature for 3-4 hrs.

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

2.7 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.

2.8 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.

2.9 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 10 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 format may be introduced to improve the solubility of the ligand. The suitability of such solvents should be tested in advance.

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 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 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.

3.6 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

Lyophilized CNBr-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.
CNBr-activated SepFast 4HF	1 g	310101
	5 g	310102
	500 g	310103
	We also provide bulk quantities, please contact us for more information	

BioToolomics Ltd
Unit 30A,
Number 1 Industrial Estate
Consett
County Durham,
DH8 6TJ
United Kingdom

www.biotoolomics.com