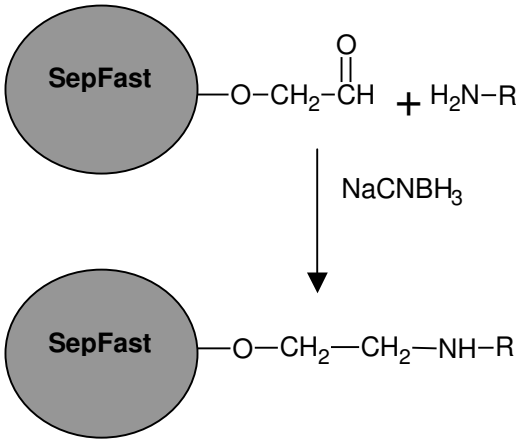
# Data & Instructions

## Aldehyde-activated SepFast

Aldehyde-activated agarose beads have a well-proven track record for the preparation and use of custom affinity chromatography media. Coupling biospecific ligands to aldehyde-activated agarose is a successful and well-documented technique.

Aldehyde-activated SepFast media forms chemically stable secondary amine linkages with ligands containing primary amine groups through reductive amination reaction. 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

There is a choice of 2 different base matrices that suit various molecules to be coupled or purified.

- Aldehyde-activated SepFast 4 High Flow (4HF) is made of highly cross-linked 4% beaded agarose. Its large pore size is suitable for coupling or purifying large molecules. It also shows high mechanical rigidity allowing high flow throughput with reduced back pressure.
- Aldehyde-activated SepFast 6 High Flow (6HF) is made of highly cross-linked 6% beaded agarose. Its moderate pore size is suitable for coupling smaller molecules. It also 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 various molecules.

The base matrix is activated through oxidation to generate aldehyde groups. It reacts directly with the primary amine groups in molecules to be immobilized in the presence of reducing agents. Aldehyde-activated SepFast media is supplied as an aqueous suspension in 20% ethanol. The main characteristics are summarized in Table 1.

***Table 1: Characteristics of Aldehyde-activated SepFast 4HF / 6HF:***

| | |
|---|---|
| Group to be coupled | $-NH_2$ |
| Matrix | SepFast 4HF: Highly cross-linked 4% agarose beads<br>SepFast 6HF: Highly cross-linked 6% agarose beads |
| Particle size | 50 – 150 μm |

| | |
|---|---|
| Activation level | > 5 μmol aldehyde / 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 | $+4^{O}C$ - $+8^{O}C$ |

**2. Ligand immobilization**

The following is a general ligand coupling procedure.

2.1 Dissolve the target ligand in coupling buffer, 0.1 M sodium phosphate, pH 7.0. In general, for protein ligands, make a concentration of 5 – 20 mg/ml gel. For small ligands, make a 10 – 100 μmol/ml gel. The volume of the coupling buffer could be the same as, or half that of, the settled gel.

2.2 Wash the Aldehyde-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. Adjust the pH if it has shifted.

2.4 Mix the slurry in a well-ventilated fume hood at room temperature for 2-4 hrs.

2.5 Add sodium cyanoborohydride (0.5 g / 100 ml activated gel) to the slurry. Leave the reaction overnight. Note: sodium cyanoborohydride is toxic and should be handled with extreme care.

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, in the presence of sodium cyanoborohydride, 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***

Reductive amination proceeds quite efficiently between pH 4 to pH 10. However, the coupling pH may be optimized get the best result (e.g. high coupling yield with high ligand activity).

Always remember to adjust the coupling pH after a ligand is 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.

### *3.3 Salt*

The presence of salt in the coupling buffer (e.g. 0.5 M or saturated) 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 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 blocking of the activated groups, such as incubating the gel with 5 – 10 mM blocking 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 Choice of reducing agent*

Other reducing agents such as sodium borohydride could be used in the coupling reaction. Cyanoborohydride is a milder reductant that only reduces the intermediate Schiff base formed between the aldehyde group and the primary amine group to give a stable secondary amine linkage. Borohydride is a stronger reductant that reduces both Schiff base and un-coupled aldehyde groups.

### *3.7 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.

### *3.8 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

Aldehyde-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. |
|---|---|---|
| Aldehyde-activated SepFast 4HF | 5 ml | 340101 |
| | 50 ml | 340102 |
| | 1 litre | 340103 |
| Aldehyde-activated SepFast 6HF | 5 ml | 340104 |
| | 50 ml | 340105 |
| | 1 litre | 340106 |

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

www.biotoolomics.com