Product Data Sheet

# Seplife® 6AG Phe/HL/90

## 1. Description

Seplife® 6AG Phe/HL/90 is aromatic hydrophobic agarose resin designed for hydrophobic interaction chromatography (HIC) resin for capture and intermediate purification of biomolecules.

- The aromatic HIC resins is based on Seplife® 6AG/90 base matrix covalently functionalized with chemically-stable ether linkages.
- The agarose backbone ensures minimal levels of non-specific binding.
- The binding of biomolecules on Seplife® 6AG Phe/HL/90 is a HIC resin based on 4% cross-linked agarose and has a large particle size (45-165 micron) is performed at high salt concentration followed by elution with a low salt concentration solution.
- High stability to CIP (cleaning in place) up to 1M NaOH.
- Regulatory Support File (RSF) is available for Seplife® 6AG Phe/HL/90.

Seplife® 6AG Phe/HL/90 is a HIC resin based on highly cross-linked agarose (6%) and has a particle size range 45-165 micron.

## 2. Properties

| Product | Seplife® 6AG Phe/HL/90 |
|---|---|
| Appearance | White spherical beads |
| Type | Strong hydrophobic chromatography media |
| Matrix | 6% cross-linked agarose |
| Ligand | Phenyl |
| Particle size range (μm) | 45-165 |
| pH stability | 3-13 (operational), 2-14 (CIP) |
| Chemical Stability | Stable in all common aqueous buffers, 1.0M NaOH; 70% ethanol; 30% isopropanol; 0.5% SDS; 10% ethylene glycol; 6.0M guanidine hydrochloride; 8.0 M urea; 3.0M $(NH_4)_2SO_4$ |
| Flow rate* (cm/h) | Max 750 cm/h |
| Shipped as | 20% ethanol slurry |

*Testing conditions: Chromatography column 16mm×400mm; column bed height 25cm; temperature 25° C; mobile phase water.

## 3. Instructions

### 3.1 Column packing

Column loading should be performed in accordance with standard operating procedures. It is important to ensure that each material is at its working temperature, and when possible, the media should be degassed before column packing.

### 3.2 Equilibration

Equilibrate the column with an equilibration buffer solution of 2 to 5 times the volume of the column bed until the conductivity and pH of the effluent are completely consistent with those of the sample feeding buffer.

### 3.3 Sample feeding

1. Samples are prepared with an equilibration solution, and cloudy samples need to be centrifuged and filtered before loading.

2. Typically, the resin is used in bind-elute mode: let the target product bind to the column, wash off the impurities with the equilibration solution, and then choose an eluent to wash off the target product.

3. The extent to which the media adsorbs sample components depends on the hydrophobic nature of the sample, the ionic strength of the mobile phase, and the temperature. When the salt concentration is high, or the temperature is high, or the sample components are highly hydrophobic, the media will adsorb the components firmly.

### 3.4 Elution

The adsorbed biomolecules can be eluted by reducing the salt concentration. The elution can be enhanced by adding surfactants or organic solvents, the most commonly used is a low-salt concentration buffer, such as 0.02-0.05 mol/L PBS.

### 3.5 Regeneration

1. First wash with 3 to 5 CV of distilled water at the operating flow rate,. Then wash with 3 to 5 CV of the equilibration solution.

2. In case of inactivated proteins or lipids that cannot be washed away during regeneration, they can be removed by cleaning-in-place.

### 3.6 Cleaning-In-Place (CIP)

1. For proteins bound by ionic bonds, 0.5~1 BV of 2M NaCl can be used to remove them.

2. For precipitated proteins, hydrophobically bound proteins or lipids, first wash with 1 BV of 0.1M NaOH. Then wash with the equilibration buffer until the pH is neutral.

3. For proteins and lipids with strong hydrophobic binding, wash with 4-10 BV of 70% ethanol or 30% isopropanol.

It is important to note that the concentration of the organic solvent should gradually increase to avoid bubble formation.

## 4. Storage

Sealed and stored at 4~30°C (preservation solution is 20% ethanol) in a ventilated, dry and clean place, do not freeze.

## 5. Transportation

Avoid sunlight, rain, and heavy pressure during transportation. It is strictly forbidden to transport with toxic and hazardous materials.

## 6. Precaution

6.1 The order of the binding strength of typical ligands and proteins from strong to weak is the following: phenyl, octyl, butyl, isopropyl, ether. If the sample is tightly combined with the media, it can be replaced with a weaker media for easier elution.

6.2 In HIC, the hydrophobic chromatography media and hydrophobic ligands have a great influence on selectivity. Also, the influence of the following factors cannot be ignored: solubility of the sample, and the purification scale.

6.3 Since strong hydrophobic proteins and hydrophobic ligands are very tightly combined, when screening hydrophobic media, if the sample is a strong hydrophobic protein, start with low hydrophobic media and choose a media that obtains the highest resolution and capacity at low salt concentrations.

6.4 The sample and chromatography media Seplife®6AG Phe/HL/90must be thoroughly equilibrated with equilibration buffer before column chromatography can be performed.

6.5 Optimized separation can be obtained during gradient elution when using 1/5 of the total binding capacity of the chromatographic column.

6.6 The loaded column bed must have a flat surface, with no channel flow or air bubbles, otherwise it should be reloaded.

**6.7** For large-scale purification and capture of proteins, step elution can reduce separation time and buffer consumption.

**6.8** During the elution process, the flow rate should be strictly controlled.

**6.9** During sample loading and the entire elution process, prevent the column surface from drying out.

## 7. Ordering information

| Product Name | References | Pack Size |
| --- | --- | --- |
| Seplife® 6AG Phe/HL/90 | A3023202 | 25ml |
| | A3023203 | 100ml |
| | A3023204 | 500ml |
| | A3023205 | 1L |
| | A3023206 | 5L |
| | A3023207 | 10L |

*Production date: See label*

*Expiry date: 5 years, under proper storage conditions*

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