

1. Description

Seplife® 4AG Bu/90 is aliphatic hydrophobic agarose resin designed for hydrophobic interaction chromatography (HIC) resin for capture and intermediate purification of biomolecules.

- The aliphatic HIC resins is based on Seplife® 4AG/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® 4AG Bu/90 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® 4AG Bu/90.

Seplife® 4AG Bu/90 is a HIC resin based on 4% cross-linked agarose with particle size in the range 45-165 micron.

2. Properties

Product	Seplife® 4AG Bu/90
Appearance	White spherical beads
Type	Hydrophobic chromatography media
Matrix	4% cross-linked agarose
Ligand	Butyl
Particle size range (µm)	45-165
pH stability	3-13 (operational), 2-14 (CIP)
Chemical Stability	Stable in all common aqueous buffers, 1 mM HCl, 1.0 M NaOH, 30% isopropanol, 70% ethanol, 6 M guanidine hydrochloride
Flow rate* (cm/h)	Max 420 cm/h
Shipped as	20% ethanol slurry

*Testing conditions: chromatography column 16mm×300mm; *column bed height 15cm; temperature 25°C; mobile phase 0.1mol/L NaCl.

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 consistent with those of the sample feeding buffer. The equilibration buffer is generally a buffer with high salt concentration, such as 0.02-0.05mol/L PBS plus 1-2.5mol/L $(\text{NH}_4)_2\text{SO}_4$, etc.

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

Generally, first wash with more than 10 BV of a buffer solution of low salt concentration. Then, wash with the equilibration solution previously used for binding proteins until the equilibrium is reached. If there are inactivated proteins or lipids that cannot be washed off, 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 pH is reached.

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 increase gradually, otherwise bubbles may be created.

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. Precautions

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

6.2 The sample and chromatography media Seplife®4AG Bu/90 must be thoroughly equilibrated with equilibration buffer before column chromatography can be performed.

6.3 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.

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

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

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

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

7. Ordering information

Product Name	References	Pack Size
Seplife® 4AG Bu/90	A3033102	25ml
	A3033103	100ml
	A3033104	500ml
	A3033105	1L
	A3033106	5L
	A3033107	10L

Production date: See label

Expiry date: 5 years, under proper storage conditions

Manufacturer: Sunresin New Materials Co. Ltd.

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