

## 1. Description

Seplife® SP Large Scale HP/40 is a strong cation exchanger agarose resin for intermediate and polishing steps in proteins and mAbs downstream processing.

- High stability to CIP (cleaning in place) up to 1M NaOH.

The agarose matrix of Seplife® SP Large Scale HP/40 is functionalized with sulphopropyl groups

- and ensures high dynamic binding capacity and high flow rates leading to high productivity.
- Hydrophilic base matrix ensures very low levels of non-specific adsorption and high recovery rate
- Regulatory Support File (RSF) and food compliance documentation is available for Seplife® SP Large Scale HP/40.

Seplife® SP Large Scale HP/40 is based on highly cross-linked agarose and has a particle size suitable for high capacity and high resolution applications ((30-50 micron).

## 2. Properties

Product	Seplife® SP Large Scale HP/40
Appearance	White spherical beads
Type	Strong acid cation - Sulfopropyl
Matrix	Highly crossed linked 6% agarose
Ion exchange capacity (mmol/ml)	0.13-0.16 (H <sup>+</sup> )
pH ligand fully charged	Negatively charged at pH>2
Particle size range (µm)	30-50
pH stability	4-12 (operational), 3-14 (CIP)
Chemical Stability	Stable in all common aqueous buffers; 1M sodium hydroxide; 8M urea; 6M guanidine hydrochloride; 70% ethanol.
Flow rate* (cm/h)	Max 400 cm/h, 0.3MPa
10% Dynamic binding capacity (mg /ml)**	≥60 (lysozyme)
Shipped as	Slurry in 20% ethanol solution containing 0.2M sodium acetate

\*Testing conditions: Chromatography column 26mm×200mm; column bed height 20cm; temperature 25° C; mobile phase water.

\*\* Testing conditions: Binding buffer: 20mM PB, pH 6.7 Elution buffer: 20mM PB+1M NaCl, pH 6.7 Sample : Lysozyme. Column 8mm\*100mm, room temperature, Retention time 2 minute.

### 3. Instructions

#### 3.1 Column packing

Column packing should be done according to standard operating procedures. It is important to ensure that each material is at its working temperature, and when possible, the chromatography media may be degassed before column packing.

#### 3.2 Equilibration

Equilibrate the column with an appropriate 2-5 column volume buffer. Ensure the conductivity and pH of the effluent are exactly the same as the buffer. The equilibration solution should be a low concentration (20-50mM) buffer such as Tris or PBS.

#### 3.3 Sample feeding

1. The sample is prepared in the equilibration buffer; turbid sample should be centrifuged and filtered before loading. Samples with high conductivity (too high salt concentration) should be processed before loading.
2. Generally, the target product is bound to the media, the impurities are washed away with the equilibration buffer solution, and then an eluent is selected and used to wash off the target product.
3. The extent to which the media adsorbs sample components depends on the charged nature of the sample, the ionic strength and pH of the mobile phase. The lower the salt concentration, the stronger the adsorption of the sample components by the media.

#### 3.4 Elution

Elution can be carried out by increasing the salt concentration or changing the pH value. The method of increasing the salt concentration is often used for elution.

#### 3.5 Regeneration

Generally, use high salt concentration buffer (containing 1-2mol/L NaCl) or lower the pH to wash more than 10 times the volume of the column. Then wash with the equilibration solution that was used for binding proteins until the equilibrium is reached.

If there are inactivated proteins or lipids that cannot be washed away during regeneration, they can be removed by cleaning in place (CIP).

#### 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, and then wash with equilibrium buffer solution 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 should be noted that the concentration of the organic solvent should gradually increase to avoid bubbles.

### 4. Storage

Sealed and stored at 4-30°C (preservation solution is 20% ethanol in 0.2M sodium acetate) 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** Column selection: Theoretically, as long as the column is long enough, the ideal resolution can be obtained, but since the flow rate of the column is related to the pressure gradient, the increase of the column length will slow down the flow rate, broaden the peak, and reduce the resolution. As the column diameter increases, the inhomogeneity of liquid flow increases and the resolution decreases significantly.

**6.2** During the purification process, the pH and ionic strength of the elution buffer must be strictly controlled. The chromatography media must be thoroughly equilibrated with equilibration buffer before column chromatography.

**6.3** Column loading: The loaded column bed must have a flat surface, with no channel flow or air bubbles, otherwise it should be reloaded.

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

**6.5** The sample volume should be small and the concentration should not be too high.

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

## 7. Ordering information

Product Name	Product Code	Pack Size
Seplife® SP Large Scale HP/40	A2066402	25ml
	A2066403	100ml
	A2066404	500ml
	A2066405	1L
	A2066406	5L
	A2066407	10L

*Production date: See label*

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

**Manufacturer: Sunresin New Materials Co. Ltd.**

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