LT BIOTECH UAB
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Helios 50-HS

1. Basic product information

Helios 50-HS is a strong cation-exchange chromatography resin that takes advantage of differences in the nature and magnitude of charge between different molecules under specific conditions. The base frame of the chromatography resin is a porous polymer microsphere prepared by suspension polymerisation and emulsion polymerisation of styrene and divinylbenzene. Its unique through-hole structure is especially suitable for the separation and purification of large-scale samples such as recombinant proteins, antibodies, nucleic acids, viruses and virus-like particles, and polysaccharides.

Helios 50-HS has excellent scale-up performance:
(1) It has a polystyrene-divinylbenzene frame for high stability and resistance to aggressive CIP cleaning.
(2) The unique through-hole design achieves a high flow rate.
(3) It combines the performance of high capacity, high resolution and high flow rate to improve process efficiency.

2. Chromatography resin parameters

Resin type	Strong cation exchange
Functional group	$-(CH_2)_3SO_3^-$ (sulfopropyl)
Matrix	Polystyrene-divinylbenzene
Median particle size	50 μm
Dynamic binding capacity	≥50mg lysozyme/ml*
Recommended flow rate	200~600 cm/h
Maximum working pressure	70 bar
Working temperature	4–40°C

** Measurement conditions of dynamic capacity: column height, 5 cm; test flow rate, 300 cm/h; buffer pH 7; lysozyme sample 3 mg/ml, when lysozyme reaches 10% breakthrough of starting concentration.*

3. Chemical resistance

pH stability*	1–14
Chemical stability	All commonly used ion exchange buffers, 75% ethanol**, 2M acetic acid, 1M HCl, 1M NaOH, 8M urea, 6M Guanidine Hydrochloride
Avoid	Strong oxidising agent, oxidising acid, strong reducing agent, acetone, benzyl alcohol

** The physical and chemical properties and functions of the chromatography resin did not change significantly after being placed at 40°C and pH 1–14 for 7 days.*

*** v/v, volume ratio*

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4. Method of use

4.1 Chromatographic conditions
(1) Buffer selection: the buffer salts whose buffer groups do not interact with the chromatography resin should be selected. If the binding elution mode is used, the equilibration buffer should be a low-salt (less than 5 mS/cm) and low-pH (usually 1 pH unit lower than the isoelectric point of the target molecule) buffer to facilitate the binding of the target molecule. Consider the stability of the sample in the buffer; the elution buffer is usually a buffer with a high concentration of salt (such as 1M NaCl) added to the equilibration buffer. If the flow-through mode is used, the equilibration buffer should adopt conditions favourable for the binding of impurities. After the target molecule has completely flowed through, it is directly washed with high-concentration salt.
(2) Flow rate: according to the bed height of the column, a linear flow rate of 200~600 cm/h is generally selected.
(3) Sample pretreatment: to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous membrane before loading it, and desalting to adjust the pH and conductivity of the sample.

4.2 Chromatography steps
(1) Equilibration: use the equilibration buffer to fully equilibrate the column until the pH and conductivity are stable and basically consistent with the equilibration buffer. This step usually requires 3–5 column bed volumes (CV).
(2) Sample loading*: determine the loading volume and loading amount of the sample on the Helios 50-HS according to the binding capacity measured in the small test experiment.
(3) Impurity washing*: use equilibration buffer or other suitable buffer to wash the column until the UV stabilises and returns to the baseline.
(4) Elution*: elution is achieved by increasing the concentration of salt ions. The concentration of salt ions in the eluent can be gradually increased by linear gradient or step gradient to elute molecules with different binding strengths. Fraction collection was performed on the eluted sample. pH gradient elution or mixed elution can also be used.
(5) Regeneration: rinse the column with a buffer containing high salt (such as 2M NaCl).
(6) Re-equilibration: re-equilibrate the column with equilibration buffer.
* If the flow-through mode is used, the ‘sample loading’ step should be set to collect; the ‘impurity washing’ step, to ensure that all the target molecules flow through, the collection can be stopped; the ‘elution’ step should directly use high-salt buffer – impurities can be washed away.

5. Cleaning and regeneration

Contaminants (e.g. lipids, endotoxins and proteins) accumulate on the column as the number of uses of the chromatography resin increases. Determine the frequency of CIP according to the degree of contamination of the chromatography resin (if the contamination is considerable, CIP is recommended after each use to ensure repeatability of results and to prolong the working life of the chromatography resin). For different types of impurities and contaminants, the recommended cleaning conditions are as follows:
• Removal of strongly binding proteins: wash with 5 CV of 2M NaCl solution, or use a high salt buffer not lower than pH 2, such as 1M NaAc solution.
• Removal of strongly hydrophobic proteins and precipitated proteins: first wash with 5 CV of 1M NaOH solution, then wash the lye with 5–10 CV of ultra pure or pure water.
• Removal of lipoproteins and lipids: first wash with 5 CV of 70% ethanol or 30% isopropanol, then rinse with 5–10 CV of ultra pure or pure water.
Note: 70% ethanol or 30% isopropanol should be degassed before use; the flow rate should be 30–60 cm/h during CIP. Reverse cleaning should be used when the clogging is severe.

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To reduce the microbial load, it is recommended that 0.5~1M NaOH solution is used to treat the chromatographic resin. Treatment time is 15~30 minutes.

6. Storage

Keep the unopened chromatography resin in the original container and store at 4~30°C in a well-ventilated, dry and clean place. Do not freeze. Wash the used column with 2–3 CV of 20% ethanol solution and store at 2~8°C.

7. Destruction and recycling

Since Helios 50-HS chromatography resin is difficult to degrade in nature, it is recommended that the discarded chromatography resin is incinerated to protect the environment. For chromatography resin that has been in contact with biologically active samples such as viruses and blood, follow the local biosafety requirements before destroying or disposing of it.

8. Packing method

Detailed information on resin packaging is available on request. Please contact your local distributor.

9. Ordering information

Product name: Helios 50-HS

Product Cat. No	Package
801-00025	25 ml
801-00100	100 ml
801-00500	500 ml
801-01000	1 L
801-05000	5 L
801-10000	10 L
801-20000	20 L