

Helios 50-XS

1. Basic product information

Helios 50-XS is a strong cation-exchange chromatography resin with polystyrene-divinylbenzene as the base frame, and the surface of the base frame has been modified by hydrophilicity. The chromatographic resin has been greatly improved on the basis of traditional cation exchangers, which reduces the non-specific adsorption of the resin. It can withstand high pressure and operate at high flow rates, and high resolution improves the product yield. Robust salt tolerance enhances process flexibility and calibration accuracy, and simplifies workflow. In addition, the high dynamic binding capacity can reduce the packing volume of the column bed, saving buffer consumption and circulation. It is widely used in the separation and purification of monoclonal antibodies, recombinant proteins, vaccines, blood products, viruses, DNA and polypeptide products.

2. Chromatography resin parameters

Resin type	Strong cation-exchange
Matrix	Polystyrene-divinylbenzene
Functional group	Sulfopropyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$)
Median particle size	50 μm
Total ionic capacity	0.16–0.20 mmol/ml
Dynamic binding capacity	≥ 102 h-IgG/ml
Protein separation (conductivity/mS/cm)	Trypsin: 12.8–15.6 Cytochrome: 20.0–24.6 Lysozyme: 29.6–36.7
Pressure-resistant flow rate	300 cm/h

3. Chemical resistance

pH stability	2~12 (working range), 1~14 (CIP)
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** The physical and chemical properties and functions of the chromatographic resin have no obvious change after being placed in an environment of 40°C and pH 2–12 for 7 days.*

4. Method of use

4.1 Column packing

The slurry concentration is equal to the volume of the resting gel divided by the total volume after homogenisation. The best packing effect can be obtained by using 0.5M NaCl with a slurry concentration of 60–70%. Methods are:

- 1) The column bed volume (CV) of the chromatographic column is $V = A_c \times L$, where $A_c = \pi \times r^2$. (A_c : crosssectional area of the chromatographic column; L : length of the chromatographic column; r : radius of the chromatographic column.)
- 2) Agitate the resin to form a homogenous slurry, and measure the required mass or volume. It should be about

1.2 column bed volumes (CV) to prevent shrinkage.

- 3) Replace 20% ethanol with 0.5M NaCl solution and equilibrate overnight.
- 4) Before loading the column, use 0.5M NaCl solution to adjust the concentration of the slurry to 65–70%; pour the slurry into the chromatography column all at once and mark the height after settling, to balance.
- 5) Install the distributor, adjust the height so that the compression coefficient is 1.05~1.10. Then start the infusion pump, and use 1.5~2 working flow rate to stabilise the column bed.
- 6) Determination of column efficiency and symmetry according to SOP must meet predetermined standards.

4.2 Evaluation of column efficiency

After packing, the chromatographic column is washed with 3–5 CV of ultrapure or pure water. Balance at a flow rate of 100 cm/h and perform a column efficiency test.

Column efficiency test method for ion exchange chromatography column Sample: 2M NaCl solution
Loading volume: 1~5 % CV Eluent: 0.5M NaCl solution Linear velocity: 100 cm/h Detection: conductivity detector

4.3 Rinsing

Packed columns should be rinsed with at least 5 CV of ultrapure or pure water.

4.4 Equilibration

Equilibrate the column with an appropriate 5–10 CV buffer until the conductivity and pH of the effluent remain unchanged (consistent with the equilibration solution). For example, equilibration buffer could be 20 mM PBS, pH 7.0. Screening and optimisation should be carried out according to the stability and isoelectric point of the target protein, and the type of ion exchange resin.

4.5 Sample loading

Solid samples can be prepared by dissolving in equilibration buffer. Low-concentration sample solutions can be dialysed with equilibration buffer; high-concentration sample solutions can be diluted with equilibration buffer. To avoid column clogging, samples should be centrifuged or membrane filtered (preferably 0.45 or 0.22 μm).

The amount of feed is calculated according to the loading capacity of the resin and the content of the target protein in the feed solution. Before loading the sample, ensure that the sample buffer is as consistent as possible with the equilibration buffer.

4.6 Elution

After the sample is loaded, continue to rinse with the equilibration buffer until the baseline is stable. According to the situation, the method of increasing the salt concentration or changing the pH of the mobile phase can be used to elute the samples adsorbed on the chromatographic resin in sequence.

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 0.2–0.5M NaOH solution (contact time 1-2 hours), then wash 5-10 CV of equilibration solution and 5 CV of ultra pure or pure water.
- **Removal of lipoproteins and lipids:** first wash with 5 CV of 50% ethanol or 30% isopropanol (contact time 0.5-1 hour), then rinse with 5–10 CV of ultra pure or pure water. It can also be cleaned with alkaline or acidic solution containing non-ionic surfactant, such as 0.1~0.5% Triton X-100 + 0.1M acetic acid for 1–2 hours, and rinsed with 50% ethanol above 5 CV to remove the detergent. Rinse with 5 CV of ultrapure or pure water as above (when using high-concentration organic solvents, the method of gradually increasing the concentration of organic solvents should be adopted to avoid air bubbles).

Note: 50% 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.

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 chromatography resin is difficult to degrade in nature, it is recommended that the waste 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-XS

Product Code in Catalogue	Product Volume
205-00025	25 ml
205-00100	100 ml
205-00500	500 ml
205-01000	1 L
205-05000	5 L
205-10000	10 L

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