Uni®SP-50XS

Strong Cation Exchange Chromatography Resin

PRODUCT INSTRUCTION MANUAL

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Strong Cation Exchange Chromatography Resin

NanoMicro Uni® SP-50XS strong cation exchange chromatography resin is based on a polymer matrix (which is hydrophilically surface-modified and then bonded with ion exchange groups) and provides good biocompatibility and physicochemical stability for bioactive macromolecules, greatly improving the purification efficiency. It is very suitable for the purification of biomolecules with low molecular weight, e.g. proteins, peptides, nucleic acids and antibiotics.

Uni®SP-50XS adopts hydrophilic coating for surface modification, characterized by low non-specific adsorption, small pore size, high resolution, and high loading capacity for small molecules, with the specific technical parameters listed as follows:

Table 1. Specifications of Uni®SP -50XS

Product	Uni®SP-50XS
Separation principle	Strong cation exchange chromatography
Matrix	Polymethacrylate (PMMA)
Particle size	55 μm
Pore size	300 Å
Ligand	$—(CH_2)_3SO_3^-$
Ligand density	~0.25 meq·mL^{-1}
Dynamic binding capacity	~55 mg·mL^{-1} (Lysozyme)
Maximum pressure	0.8 MPa
Cleaning-in-place	1 M NaOH
Recommended flow velocity	100-500 cm/h
pH stability	2~12
Chemical stability	Stable in commonly used buffers, 1 M HAc, 1 M NaOH, 1 M HCl, 70% ethanol, 30% isopropyl alcohol, 30% acetonitrile,1% SDS, 6 M guanidine hydrochloride, 8 M urea, etc. Avoid long-term exposure to strong oxidant.
Operational temperature	4-30 °C
Storage	20% Ethanol, 4-25°C

Column Packing Instruction

Uni®SP-50XS strong cation exchange chromatography resin is supplied in 20 % ethanol as ~65 % slurry (v/v). Replacement of storage solution with 5 column volumes of 0.5 M NaCl solution prior to column packing is recommended.

Determination of Slurry Percentage and Resin Volume for Column Packing

Resin slurry percentage (Cs) refers to the ratio of settled resin volume (Vr) to the total volume of the slurry. To determine Cs of a resin slurry, gently resuspend rein in an appropriate vessel, then pour 10 ml of the homogenous slurry into a calibrated cylinder and allow to settle overnight (>12 hrs). Record the volume of the settled resin bed (Vr) and calculate the slurry percentage (Cs) using *Eq. 1.*

Eq. 1 $Cs\ (\%) = 100 \times (Vr/10) = 10\ Vr$

For best results, 50-70 % resin slurry in 0.5 M NaCl solution is recommended for packing a Uni®SP-50XS column.

Packing Preparation

1) Calculate the column volume (Vc):

Eg. 2 $Vc = h \times \pi r^2$

h: Bed height of column; r: Radius of column

2) Determination of slurry volume needed for packing a column of desired bed height (Vs): The recommended compression factor (CF) for packing Uni®SP-50XS resin is 1.15 -1.2. The required slurry volume can be calculated according to Eq. 3.

Eg. 3 $Vs = 100 \times (Vc \times CF) / Cs$

Packing Uni®SP-50XS in an Nmxk 16/20 Column

Please carefully review manufacturers' instruction manuals for column and chromatography system before performing the following procedure.

1) Wet the column bottom frit with packing solution and remove air; leave 1-2 cm of solution in the column and close the bottom outlet.

2) Flush column top adaptor with packing solution to remove strapped air.

3) Transfer an appropriate amount of fully resuspended resin slurry (Vs) into the column in a single operation; use an extension tube or a packing reservoir if necessary. Rinse the column inner wall with packing solution. When a clear supernatant of 2-3 cm has formed above the column bed, insert the top adaptor into the column and tighten the seal. Avoid trapping air bubbles during this process.

4) Connect the top adaptor to a chromatography system, open the bottom outlet, and turn on the system pump to consolidate the resin using constant flow rate or pressure. When a stable consolidated column bed is formed, stop the system pump and close the column bottom outlet.

5) Loosen the column top adaptor seal slightly and push the adaptor 2-3 mm down slowly, then tighten the seal.

Performing Column Qualification Test

A packed column should be equilibrated with 2 CV of eluent at test velocity, such as 100 cm/hr, before the probe solution is injected. Refer to Table 2 for detailed test conditions.

Table 2. Recommended test conditions for Uni®SP-50XS column qualification

Probe	5 % (v/v) Acetone in water or 2 M NaCl solution
Probe volume	1% - 5% column volume
Eluent (Mobile phase)	water or 0.5 M NaCl solution
Linear velocity	50 - 200 cm/h
Detector	5 % Acetone as probe: UV @ 280 nm 2 M NaCl as probe: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >2500

How to Use

1) Equilibration: equilibrate the column with 5 BV or more of equilibration buffer (e.g. Buffer A, 20 mM PBS, pH=7.0), until the conductivity and pH of the effluent remain constant (consistent with the equilibration buffer). The specific buffer system should be screened and optimized according to the stability and isoelectric point of the target protein and the type of ion exchange resin.

2) Loading: dissolve the solid sample in the equilibration buffer; Low concentration sample solution can be concentrated in advance; and high concentration sample solution can be diluted with equilibrium buffer. Carry out the centrifugation or membrane filtration for the sample to prevent clogging of the column. Calculate the loading volume based on the resin loading capacity and the target protein content in the sample and ensure that the loading buffer is consistent with the equilibration buffer before loading.

3) Elution: after sample loading, continue to wash the sample with equilibrium buffer until the baseline is stable. Elute the sample adsorbed on the chromatography resin sequentially by changing the salt concentration or the pH of the mobile phase.

4) Cleaning in place (CIP): regular CIP prevents the accumulation of precipitated protein contaminants in the column bed and helps to maintain the loading capacity, separation efficiency, flow characteristics, etc. of the chromatography resin. Prepare a specific CIP method for each process based on the type of contaminant. The frequency of CIP depends on the nature of the starting feed liquid and process conditions. The recommended CIP method is as follows.

Wash sequentially with 5 CV of 1-2 M NaCl, 5 CV of 0.5-1 M NaOH, 3 CV of water and 5 CV of 1 M acetic acid.

Storage

Seal and store the used chromatography resin or prepacked column in 20% ethanol or 10 mM of NaOH at the recommended temperature of 4-25 °C.

It is recommended to replace the 20% ethanol once every 3 months for unused chromatography resin or prepacked column to prevent ethanol volatilization and microbial growth.

Note: The shelf life of unopened resin is 5 years.

Trouble Shooting Guide

Problems that occasionally occur during Uni®SP-50XS chromatography and suggested solutions are listed below. We also have an experienced and dedicated application team to provide full technical support, from method development, scale-up design, to commercial production.

Problem	Possible Cause	Recommended Solution
Increase of column pressure	Too high flow rate	Reduce the flow rate
	Valve between pump and collector not opened	Open the valve
	On-line filter clogged of instrument	Remove impurities and wash or replace the filter, and filter the sample and buffer through 0.45 μm or 0.2 μm membrane prior to use
	Sample precipitation on the column	Perform CIP (refer to regeneration conditions)
	Dirty sample or substances with a strong adsorption remaining on the resin	Perform CIP (refer to regeneration conditions)
	Column bed compressed	Repack the column
	Long service time of the column	Replace the column or the chromatography resin
Insufficient sample adsorption	Too high ion strength in the sample solution	Reduce the ion strength in the sample solution by dilution or desalination, etc.
	Inappropriate pH of sample	Adjust pH to increase binding strength.
Failure to elute sample during the elution process	Too weak elution strength of the eluent	Replace the eluent with another one with stronger elution strength
	Inappropriate pH of eluent	Adjust pH of the eluent
	Residual highly hydrophobic impurities in the column	Perform CIP (refer to regeneration conditions)
Reduced resolution	Inappropriate elution conditions, e.g. too steep	Change the elution conditions to shallower gradient

	gradient or too high flow rate	or isocratic elution and to reduced flow rate
	Column packed improperly	Repack the column
	Dead volume left at the top or behind the column	Pack more resin or reduce the void behind the column
	Overload	Clean and re-equilibrate the column to reduce the loading volume
Decreased binding of the sample after repeated injections	Impurities in the sample bound to the resin, which interfere with the normal binding	Perform CIP (refer to regeneration conditions)
Cracks in the column bed in use	Insufficient elimination of swelling	Mix well and fully equilibrate with 0.5 M NaCl
	Bubbles in the solution	Depressurize and filter to remove the air
	Outside air entered the system	Add more buffer to remove the air completely
	The column was not properly packed	Repack
Baseline drift	Chromatography column not well-equilibrated	Increase the equilibration time.
	Different absorption coefficients for eluents A and B at the same UV wavelength	Detect at different wavelengths or run a blank gradient.
Presence of unknown impurity peaks	Incomplete elution of the previous sample	Regenerate the column
	Impure eluent	Run a blank control gradient or use a high-purity chromatography grade reagent
	Trace ionic impurities bound to the column and concentrated during equilibration and loading, with peaks appearing during elution	Clean the column
Too long equilibration time	Direct re-equilibration after CIP	Equilibrate with 2 CV of buffer B (containing 1 M NaCl) and with buffer A

Order Information

Product	Size	Catalog #
Uni®SP-50XS	30 mL	04022-050030-2030
	50 mL	04022-050030-2050
	100 mL	04022-050030-2100
	300 mL	04022-050030-2300
	500 mL	04022-050030-2500
	1 L	04022-050030-1001
	5 L	04022-050030-1005
	10 L	04022-050030-1010
	50 L	04022-050030-1050
	100 L	04022-050030-1100

Note: We can also provide you with a preparation column with an inner diameter of 10/21.2/30/50 mm and a length of 100/150/250 mm. Please contact us for further details.

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