UniHybrid 10-300 C8

Reversed Phase Hybrid Silica Gel Chromatography Resin

PRODUCT INSTRUCTION MANUAL

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NanoMicro

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Reversed Phase Hybrid Silica Gel Chromatography Resin

UniHybrid series of chromatographic resin is a new addition of monodisperse organic hybrid silica gel chromatographic resin launched by NanoMicro Technology after success of UniSil silica gel resin. This resin is produced by unique surface hybrid modification and bonding and end-capping technology on UniSil silica gel. This series of products have the advantages of uniform particle size, high chemical stability, high mechanical strength, unique selectivity, good symmetry of preparation peak, good reproducibility, etc., compared with traditional high-purity silica gel chromatographic resin, UniHybrid series have longer lifetime and lower cost.

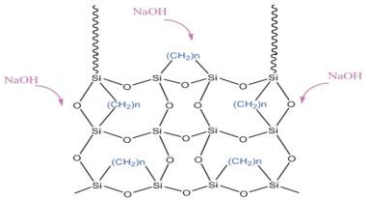

Figure 1. UniHybrid reverse phase chromatography resin based on surface hybridization

Ultra-pure silica gel matrix is a spatial network structure composed of Si(-O-)4 tetrahedrons. Due to the hydrolysis of Si-O-Si structure under acid or alkaline conditions, the bonded phase of the resin falls off and even the substrate dissolves. Then the limit of mobile phase pH range used by traditional high-purity silica gel resin is pH 2-8, or even not exceeds pH 7.5 for a long time. Therefore, in many separation and purification applications, mobile phase pH is severely limited. And in some cases where very high pH regeneration, such as in 0.1M NaOH solution, is required, the resin lifetime is extremely short.

Based on high purity monodisperse porous silica gel microsphere produced by NanoMicro, UniHybrid series of chromatographic resin introduce a layer of organic-inorganic hybrid coating containing methylene bridge structure by a dense surface hybridization on the surface of microsphere and pore structure, and then bond stationary phases, and densely end-cap. This unique surface hybrid technology combines the excellent properties derived from the silica and polymer chromatographic resins: the chemical stability of the polymer chromatographic resin, with acid and alkaline pH tolerance, and the mechanical strength of high-purity silica gel chromatographic resin. At the same time, due to the surface chemical treatment, the density of silicon hydroxyl group changes, giving the hybrid silica gel unique separation selectivity.

Compared with similar products in the market, UniHybrid 10-300 C8 has the following unique advantages:

- It has a very narrow particle size distribution with coefficient of variation (CV) of ⩽ 5%.
- It has precisely controlled pore size, pore size distribution, effective specific surface area, and pore volume, resulting in high sample loading capacity.
- It has unique end-capping technology that effectively controls the residual silanol groups on the particle surface to meet different application demands.
- It has good chemical stability suitable for separation and purification at pH 1-11.

Specific performance specifications for UniHybrid 10-300 C8 are listed in the table below:

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Table 1. Specifications of UniHybrid 10-300 C8

Separation mode	Reversed-phase chromatography
Matrix	Monodisperse silica gel
Particle size	10 μm
Pore size	300 Å
Particle size CV	⩽5%
Max. pressure	＜600 bar
Pore volume	~1.00 cm^3/g
Specific surface area	~100 m^2/g
Bulk density	~0.55 g/mL
Carbon content	⩾5 %
Range of pH	1~11

Operation Guidelines

Packing (dynamic axial compression method is recommended)

1) Calculate the chromatography column volume Vc and mass (m) of media required to fill the column volume according to the formula below:

Eq. 1 $Vc = h \times \pi r^2$

Eq. 2 $m = \rho \times Vc$

*Vc: chromatography column volume; h: chromatography column height; r: chromatography column radius; ρ: media density.

** In order to obtain a tight column bed, it is recommended to use slightly excess amount of media, for example, 1.05 to 1.10 times the mass of the media required from calculation.*

2) Assemble the chromatography column hardware. Wet the sieving plate at the bottom of the chromatography column with slurry solvent using wash bottle or by backflow, retain liquid with the height of 1~2 cm at the bottom of the column, and then close the column outlet valve.

3) Preparation of the media slurry: Prepare 2.5 to 3 mL of the slurry solvent, such as isopropanol, for 1 g of media. Pour the media into the prepared slurry solvent while stirring (at no more than 50 rpm/min) until evenly dispersed. If it is possible, mix them by ultrasound rather than magnetic stirring for 30 minutes.

4) Slowly pour the slurry into the column, and then wash the inner wall of the column with a wash bottle containing slurry solvent to prevent mixing with gas.

5) Open the outlet at the bottom of the column while packing the column using dynamic axial compression. For silica gel media with particle sizes of 8 μm and 10 μm, a pressure of 80 to 100 bar is recommended; For silica gel media with particle sizes of 20 to 40 μm, a pressure of 40 to 70 bar is recommended.

Performing Column Qualification Test

Generally, the chromatography column performance should be tested before use. The test results and test parameters should be saved so that the performance of the column can be evaluated in the future. The recommended mobile phase for column efficiency test is methanol, and the test sample is toluene or naphthalene. Prior to test, 2~3 column volumes of mobile phase should be used to equilibrate the column. Refer to Table 2 for detailed test conditions.

Table 2. Recommended test conditions for UniHybrid 10-300 C8 column qualification

Probe	2% (v/v) toluene in methanol solution
Probe volume	0.1% column volume
Mobile phase	Methanol
Linear velocity	100～250 cm/h
Detector	UV@254 nm
Recommended qualification specifications	As: 0.8-1.5; Plates（N/m): >30,000

Recommended Mobile Phase

When using UniHybrid 10-300 C8 resin, the organic solvents such as isopropanol, methanol, ethanol, acetonitrile, and tetrahydrofuran

can be used as mobile phases, or ion-pairing reagents such as TFA for improving separation. This media is not suitable for running in 100% aqueous phase or mobile phase with organic phase ratio lower than 10% for long-term.

Cleaning

If chromatography media needs to be cleaned, it is recommended to be repeatedly washed with a mobile phase of lower polarity, such as pure acetonitrile, methanol, or a 95%:5% mixture of dichloromethane and methanol. If the above cleaning method is not effective, the extreme method, i.e., dimethyl sulfoxide or dimethylformamide at low flow rates can be used.

Note: When washing with solvents of different polarities, the liquid in the media should be replaced by washing with a solvent miscible with the previous washing solvent, such as isopropanol. Washing with mobile phase with organic/aqueous phase ratio greater than 50% is necessary for cleaning and storage after use.

Regeneration

After long-term use of chromatographic column, the column efficiency (number of theoretical plates) tends to decrease. In this case, the column can be regenerated by a washing procedure. Generally, the washing procedure is defined as more than 2 column volumes (the linear flow rate should be controlled at 150 ~ 200 cm/h). The column may also be washed with a series of solvents with increasing non-polarity, such as acetonitrile (methanol) → isopropanol → dichloromethane, and then changed back to the original mobile phase in reverse order.

Storage

Used media should be washed clean from salts and then sealed with a high organic phase (pure ethanol or pure isopropanol) in a cool and dry place. The unopened media can be directly stored in a cool and dry place. Recommended storage temperature is 4 ~ 35°C.

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

*Note: In the process of use, the used sample and mobile phase must be filtered by a filter membrane with pore size of 0.45 μm.

Trouble Shooting Guide

Problems that occasionally occur during If you have any problems when using UniHybrid 10-300 C8 reversed phase 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	On-line filter of instrument clogged	Remove and wash filter or replace the filter if possible; Filter sample and eluent prior to use.
	Column contamination after long-term use	Replace the chromatography column or replace media.
Most of the sample passing through the column after loading	Too low ion strength or no salt in ionizable sample solution	Appropriately increase ionic strength by adding NaCl or other measures.
	Inappropriate pH of sample	Adjust pH to increase binding strength.
	The column is contaminated	Wash the column or remove strong contaminants remaining in the sample.
	Too high organic solvent ratio in sample solution	Decrease the organic phase ratio of sample solution close to that of the mobile phase.
Failure to elute sample during the elution process	Too weak elution strength of the eluent	Increase the organic solvent ratio in sample solution or replace the eluent with another eluent with stronger elution strength (less polarity)
		Adjust pH of the eluent
Baseline drift	Bubbles after mixing of mobile phase	Try to elute as early as possible after mixing the mobile phase
	Chromatography column not well-equilibrated	Increase the equilibration time.
	Different absorption coefficients for eluent A and B at the same UV wavelength.	Detect at different wavelengths or run a blank gradient.
	Contaminated eluent	Use a new solvent or a high purity solvent.

Order Information

Product	Package Size	Catalog #
UniHybrid 10-300 C8	30g, 50g, 100g, 300g, 500g, 1kg, 5kg, 10kg, 50kg, 100kg	19502-100030

Note: We can also provide you with preparation columns 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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