
UniGel-65Heparin

Heparin affinity chromatography resin

Instruction

UniGel-65Heparin

Affinity resin

UniGel® is a surface hydrophilically modified monodisperse spherical porous cross-linked polyacrylate "hard gel" chromatographic medium, featuring excellent pressure resistance, hydrophilicity and biocompatibility. The Protein A affinity fillers and ion exchange fillers produced based on UniGel® have been widely applied in the purification of biopharmaceuticals. UniGel®-65Heparin is a heparin affinity filler developed based on UniGel®. It uses a special extended arm and bonding technology to couple high-purity sodium heparin ligands, offering good loading capacity and selectivity, with low ligand shedding and minimal non-specific adsorption. It is mainly used for the affinity chromatographic separation and purification of thrombin III, coagulation factors, lipoproteins, esterases, hormones and interferons, etc.

Product advantages:

- (a) Monodisperse high-strength porous cross-linked polyacrylate microspheres, allowing for higher operating pressure and high flow rate;
- (b) Utilizes a unique immobilization bonding technology, resulting in lower ligand shedding;
- (c) Higher dynamic loading capacity throughout the process and less non-specific adsorption, improving production efficiency;
- (d) Longer service life, reducing production costs.

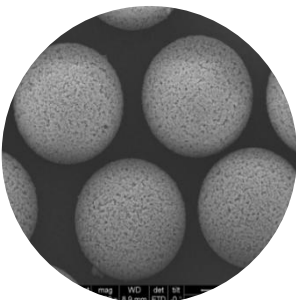


Figure 1. Electron micrograph of UniGel-65Heparin affinity chromatography medium.

Table 1. Characteristics of UniGel-65Heparin.

Product name	UniGel-65Heparin
Chromatography technique	Heparin affinity
Matrix	PMMA
Particle size	65µm
DBC	~5 mg·mL ⁻¹ (ATIII)
Maximum pressure	0.8MPa
CIP	2 M NaCl
Recommended flow velocity	100-500 cm/h
pH stability	4-12
Chemical stability	It remains stable in common water-soluble buffer solutions (0.05 M sodium acetate, 8 M urea, 6 M guanidine hydrochloride, non-ionic detergent, 10% glycerol).
Storage	20% Ethanol, 4-25 °C

Column packing instruction

Determination of slurry

UniGel-65Heparin resin is supplied in 20% ethanol in original package as a ~65% slurry (v/v). Replacement of storage solution with 5 column volumes of 0.5 M NaCl solution prior to column packing is recommended.

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

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

It is recommended to prepare 50~70% resin slurry in 0.5M NaCl solution for packing a UniGel-65Heparin column.

Packing preparation

Calculate the column volume (Vc):

$$\text{Eq. 2 } Vc = h \times \pi r^2$$

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

Determination of slurry volume (Vs) needed for packing a column of desired bed height: the recommended compression factor (CF) for packing UniGel-65Heparin resin is 1.15 -1.2. The required slurry volume can be calculated according to Eq. 3.

$$\text{Eq. 3 } Vs = 100 \times (Vc \times CF)/Cs$$

Column packing method (as NmXK 16/20 column)

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

- 1) Wet the column bottom frit with packing buffer and remove air; leave 1~2 cm of packing buffer in the column and close the bottom outlet.
- 2) Flush column top adaptor with packing buffer to remove trapped 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 as necessary. Rinse the column inner wall with packing buffer. When a clear supernatant of 2~3 cm has developed 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.

Column qualification

A packed column should be equilibrated with 2 CV of 0.5 M NaCl, before the sample is injected. Refer to Table 3 for detailed test conditions.

Table 2. Column qualification test condition

Sample	5% (v/v) Acetone in pure water or 2 M NaCl solution
Injection volume	1 ~ 5 % Column volume
Mobile phase	Pure water or 0.5 M NaCl solution
Linear flow rate	50 ~ 200 cm/h
Detector	5 % Acetone: UV@280 nm 2 M NaCl: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >2500

How to use

Adsorption binding

UniGel-65Heparin is mainly used as a affinity chromatography medium for purifying coagulation factors or nucleic acid-binding proteins. The affinity of different biomolecules to UniGel-65Heparin may vary. Their binding capacity will also depend on the chromatography conditions,

such as buffer composition, pH, flow rate, and temperature. Additionally, due to the presence of negatively charged sulfate and carboxyl groups in the heparin ligand, UniGel-65 Heparin may also bind proteins through cation exchange. When used in plasma protein purification applications, the binding buffer for UniGel-65Heparin is typically 10-20 mM sodium citrate buffer with a pH of 7.4. The binding is an affinity interaction, so it is recommended to add a low concentration of NaCl in the binding buffer to eliminate non-specific ion interactions. In other applications, pH 7.0 10 mM phosphate sodium, pH 7.0 or 20 mM Tris-HCl, pH 8.0 can be used as the binding buffer.

Adsorption elution

Elution is usually carried out by increasing the ionic strength of the buffer, using continuous linear gradient elution or stepwise gradient elution. The most commonly used elution buffer is NaCl, KCl or ammonium sulfate, with a concentration up to 1.5–2 M.

Cleaning and regeneration

Regular in-situ CIP cleaning can prevent contaminants from accumulating in the medium bed and help maintain the loading capacity, flow rate and general performance of UniGel-65Heparin for heparin affinity. UniGel-65Heparin can be cleaned with 2 M NaCl as the CIP cleaning solution. It is not recommended to use a high concentration of NaOH for cleaning as it will lead to a decrease in the medium's function and lower loading capacity. Among them, 8 M urea and 6 M guanidine hydrochloride can maintain the stability of the

medium as CIP cleaning solutions.

Recommended CIP cleaning procedures are as follows:

Ion-binding proteins:

1 CV 2 M NaCl, 10-15 min;

Precipitated or denatured proteins:

2 – 4 CV 0.1M sodium hydroxide, 6 M guanidine hydrochloride or 8 M urea, 1 h;

Hydrophobic-binding proteins:

4 CV 0.1-0.5% non-ionic surfactant, 1 – 2 h;

Choose different CIP conditions based on different pollutant types. Due to the differences in the feed liquid, the frequency of CIP is also different, but generally, the CIP cycle is recommended to be carried out once every 5 separation cycles. Afterward, it is necessary to thoroughly clean the chromatography column using the equilibrium buffer.

Storage

Rinse column/resin fully with pure water to remove residual buffer, then equilibrate the column/resin with 20% ethanol. Store column/resin at 4 ~ 25 °C. Replacement of storage solution every three months is recommended to prevent column/resin from drying out or growth of microorganisms.

Attention: Sample, buffers and solutions should be filtered with a 0.45 µm membrane prior to chromatography operation.

Ordering information

Product name	Package	Cat. No.
UniGel-65Heparin	30 mL	17012-065100-2030
	50 mL	17012-065100-2050
	100 mL	17012-065100-2100
	300 mL	17012-065100-2300
	500 mL	17012-065100-2500
	1 L	17012-065100-1001
	5 L	17012-065100-1005
	10 L	17012-065100-1010
	50 L	17012-065100-1050
	100 L	17012-065100-1100

Note: Prepacked columns of 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm are available. Please contact us for further details.

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