UniGel Series

Ion Exchange Chromatography Resins

Product Instruction Manual

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Ion Exchange Chromatography Resins

UniGel series of IEX resins include weak cation (CM), strong cation (SP), weak anion (DEAE), and strong anion (Q) four types of products . Their supports are highly crosslinked polymethacrylate beads of uniformed particle size and open pore structure. Through polyhydroxyl surface modification and tentacle surface extender chemistry, these UniGel ion exchange (IEX) resins are enabled with excellent binding capacity and minimal non-specific adsorption property. They are designed to meet the demands of downstream bioprocessing, from capture, intermediate purification to polishing, of biomolecules from small proteins to large biologics such as viral particles and vaccines. Their reproducibility, scalability, and reliability have been well proved.

Table 1. Classification and uses of ion exchange functional groups

Type	Chemical structure	Main features	PKa Value
CM	$-CH_2COO—$	Less acidc suitable for pH pH>4	4-6
SP	$-(CH_2)_3SO_3—$	Strongly acidic, suitable for pH 2-12	<2
DEAE	$-CH_2N^+H(CH_3)_2$	Less basic , suitable for pH<9	>9
Q	$-CH_2N^+(CH_3)_3$ (Q)	Strongly basic, suitable for pH2-12	>12

Note: 1. Mobile phase pH is between pI and pKa;

2. The isoelectric point difference between the pH of mobile phase and the sample to be separated is 1.0pH;

3. Strong positive exchange medium should be selected for pH<3.0, and strong negative exchange medium should be selected for pH>10.0.

Benefits of UniGel ion exchange chromatography resin：

1) Ultra-high dynamic binding capacity >105 mg lysozyme (cation exchange) and >90 mg BSA (anion exchange;

2) It can provide a variety of ion exchange resin with different particle sizes such as 30/50/65/80μm to meet the needs of resins to pure;

3) The packing factor and swelling coefficient of packed column are small (much lower than agarose and dextran gel), and the stability of column bed is high;

4) High strength matrix can use faster flow rate and higher column bed, improve purification efficiency;

5) Superior alkaline resistance, can effectively extend the service life, improve production efficiency, reduce production costs, improve enterprise efficiency.

Table 2. Basic attributes of UniGel

Project	UniGel-30CM	UniGel-30SP	UniGel-30DEAE	UniGel-30Q
Separation principle	CM	SP	DEAE	Q
Matrix	PMMA			
Particle size	~ 30 μm			
Ligand	$—CH_2COO^-$	$—(CH_2)_3SO_3-$	$—CH_2N^+H(CH_3)_2$	$—CH_2N^+(CH_3)_3$
Ligand density	~0.28 meq/mL	~0.11 meq/mL	~0.09 meq/mL	~0.09 meq/mL
Dynamic loading capacity	~105 mg/mL（Lys）	~115 mg/mL（Lys）	~80 mg/mL（BSA）	~80 mg/mL（BSA）
Maximum pressure resistance	1.0 MPa			
CIP	1 M NaOH	1 M NaOH	0.5 M NaOH	0.5 M NaOH
Recommended flow rate	50-300 cm/h			

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pH stability	2-12
Chemical stability	All commonly used buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 70% ethanol, 30% isopropanol, 30% acetonitrile, 1% SDS, 6M guanidine hydrochloride, 8M urea and other commonly used organic solvents; contact with strong oxidizers should be avoided.
Operating temperature	4-30 ℃
Storage conditions	20% ethanol，4-25 ℃

Project	UniGel-80CM	UniGel-80SP	UniGel-80DEAE	UniGel-80Q
Separation principle	CM	SP	DEAE	Q
Matrix	PMMA			
Particle size	~ 80 μm			
Ligand	$—CH_2COO^-$	$—(CH_2)_3SO_3$-	$—CH_2N^+H(CH_3)_2$	$—CH_2N^+(CH_3)_3$
Ligand density	~0.28 meq/mL	~0.11 meq/mL	~0.09 meq/mL	~0.09 meq/mL
Dynamic loading capacity	~105 mg/mL（Lys）	~115 mg/mL（Lys）	~80 mg/mL（BSA）	~80 mg/mL（BSA）
Maximum pressure resistance	0.5 MPa			
CIP	0.5 M NaOH	0.5 M NaOH	0.5 M NaOH	0.5 M NaOH
Recommended flow rate	150-750 cm/h			
pH stability	2-12			
Chemical stability	All commonly used buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 70% ethanol, 30% isopropanol, 30% acetonitrile, 1% SDS, 6M guanidine hydrochloride, 8M urea and other commonly used organic solvents; contact with strong oxidizers should be avoided.			
Operating temperature	4-30 ℃			
Storage conditions	20% ethanol，4-25 ℃			

High chemical stability

It is generally designed according to the use environment of different products. The UniGel series high-load ion exchange resin is placed in 0.5 M NaOH solution for 15 days, and its dynamic binding capacity on proteins is tested at different time points. It can be seen that the change volatility of the value at different time points is observed. The UniGel series of high-load ion exchange resin has high alkaline resistance and good chemical stability.

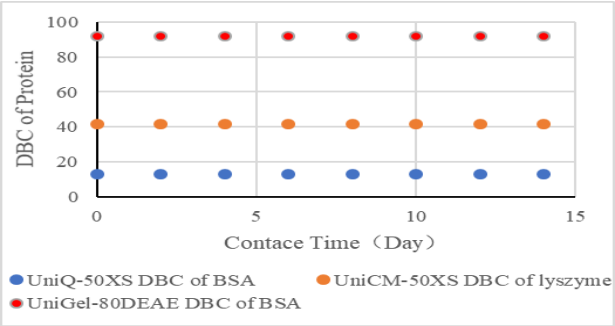

Figure 2. Chemical stability test results of UniGel ion exchange chromatography resin.

Efficient mass transfer for high dynamic load at high flow rates

UniGel series IEX resins introduce a specific surface funtionalization and surf ace extenders, leading to a high dynamic binding capacity. The following experiments were conducted with BSA (2 mg/mL) in 25 mM Tris HCl (pH 7.4) as the sample at a flow rate of 180 cm/h. The black line indicates the dynamic binding capacity of UniGel-80DEAE, and the red indicates the dynamic binding capacity of conventional ion exchange fillers, indicating that the capacity of UniGel-80DEAE is significantly higher than that of conventional fillers. The binding capacity of UniGel-80DEAE was higher than that of conventional ion exchange fillers under the same conditions.

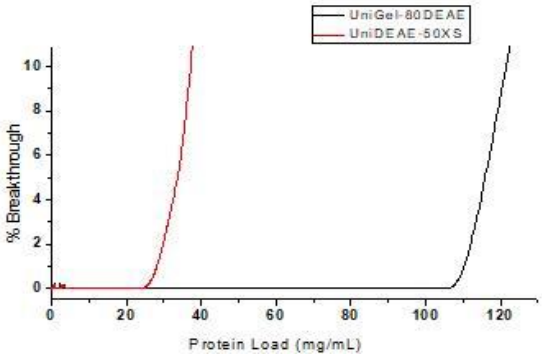

Figure 3. UniGel ion-exchange chromatography dynamic binding capacity test for resin.

Column Packing

UniGel series products are stored in 20% ethanol. 1.5 Kg of the product homogenate corresponds to 1L of medium volume. Recommended mobile phase for column-packing with the homogenate: 0.1 M NaCl or purified water. Recommended pack factor: 1.05.

UniGel product series are made of a "hard agarose gel" of high mechanical strength. They can be packed into either low-pressure columns or mid-/high-pressure columns. Recommended pore size of column sieving plate: is10-23 μm. Column-packing procedures:

1) Calculation of the required mass of the medium homogenate

Required mass of medium homogenate (Kg) = Target column volume (L) ×1.5 Kg/L ×1.05.

Example: The mass of homogenate required for packing a 25L column (40 cm I.D×20 cm) is: 25 L×1.5 Kg/L ×1.05=39.375 Kg. For guaranteed measurement accuracy of the medium volume, the concentration homogeneity of the homogenate must be verified before weighing (the homogenate can be stirred slowly with a plastic spoon or a mechanical agitator at <50rpm to achieve the desired homogeneity and then allowed to stand for NMT 10 minutes).

2) Preparation of the homogenate for column-packing

Prior to the start of the packing, prepare sufficient amount of 0.1 M NaCl (or purified water) for use as loading solution for column-packing. The loading solution referred to hereinafter is 0.1 M NaCl (or purified water).

Lab-scale small column (I.D.≤50 mm):

Subject the calculated amount of medium suspension in 20 % ethanol to suction filtration, add the loading solution (in 3 times the volume of the suspension) into the filter funnel, gently stir with a plastic spoon and then perform suction filtration again. Repeat the above-mentioned procedure twice so that the 20% ethanol in the medium suspension is completely replaced by the loading solution. Finally, add loading solution to make up the total volume of the homogenate to 1.67~2 times the target column volume (Homogenate concentration: 50%~60%).

Larger scale column (I.D.>50 mm):

Measure and transfer the calculated amount of medium suspension in 20 % ethanol into a homogenate tank and allow the suspension to settle for more than 2 hours, then remove the supernatant (which might be a bit turbid). Replenish loading solution in the same volume of the removed supernatant. Gently stir the homogenate and then allow it to settle for more than 2 hours, then remove the supernatant. Repeat the above-mentioned procedure twice or thrice so that the ethanol is completely replaced by the loading solution. Finally, add loading solution to make up the total volume of the homogenate to 1.67~2 times the target column volume (Homogenate concentration: 50%~60%).

Note: During the preparation of the homogenate for column-packing, avoid grinding or rubbing the Resin; do not use magnetic stirrer or silicone tube peristaltic pump; if mechanical agitator is used, the paddle should not be too close to the column wall. Furthermore, when it is necessary to re-pack the column, homogenate should be prepared according to the above-mentioned filtration or settlement procedure.

3) Packing

- **Flow packing of column:**

  3-1) Thoroughly homogenize the above-mentioned medium suspension

and transfer it into the column tube.

3-2) Start the column-packing with the loading solution as mobile phase (the effluent may be a bit turbid at the beginning of the column-packing but will become clear after the flow of 1-2 CV of loading solution).

Note: The starting flow rate for column packing should not be too high.

3-3) When the column bed height stabilizes, increase the flow rate to maximum target value (Recommended value: 2 times the operation flow rate) or increase the column-packing pressure to maximum target value (Recommended value: NMT 1.0 MPa). Once the pressure becomes constant, allow the column-packing to stabilize for another 20 minutes.

3-4) Simply adjust the piston to 3mm below the gel surface.

- **Axial compression packing of column (I.D.>300 mm):**

3-1) Clean and degas the column.

3-2) Connect the homogenate tank to the column, remove the surplus solution in the column, lower the piston to 5cm away from the lower frit.

3-3) Open the feeding valve, lift the piston for draw-off. Draw-off rate: 200~300 cm/h. Determine the draw-off amount according to the homogenate concentration, and calculate the piston travel distance. Close the feeding valve, move the piston downwards for column compression at a compression rate of 60~100 cm/h.

Keep compressing until the complete settlement of filler; stop the piston when the column bed height no longer increases, then record the column bed height. Calculate the final position of the piston with a compression ratio of 1.05.

Note: Generally the mass of filler is calculated using a percentage >120% since the ingression of air during the homogenate draw-off is inevitable.

Packing Efficiency Assessment

After the chromatographic column is packed, equilibrate the column with 2 CV 0.5 M NaCl solution and conduct column efficiency test at a flow rate of 100 cm/h. For the specific test parameters, refer to Table 3:

Table 3. Packing efficiency test method for columns packed with UniGel series ion exchange chromatography Resin

Sample	2 M NaCl
Sample injection	1~5 % column volume
Eluent	0.5 M NaCl
Linear flow rate	100 cm/h
Detection	conductivity curve if 2M NaCl is used as sample
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >3000

How to use

1) **Equilibration** Equilibrate the column with more than 5 CV of equilibration buffer (e.g. Buffer A, 20 mM PBS, pH7.0), 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 medium's loading capacity and the target protein content in the sample and make sure 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 chromatographic medium sequentially by changing the salt concentration or the pH of the mobile phase.

4) **Clean in Place (CIP)** Regular CIP prevents the accumulation of precipitated protein contaminants in the medium 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

The used chromatography Resin or prepacked column should be sealed and stored in 20% ethanol or 10 mM NaOH after completely generation. The recommended storage temperature is 4 ~ 25 ℃.

For unused chromatography Resin or prepacked column, it is recommended to replace the 20% ethanol once every 3 months to prevent ethanol volatilization and growth of microorganisms.

Trouble Shooting Guide

If you have any problems with the UniGel series of ion exchange chromatography products, refer to the table below for solution or contact us.

Problem	Possible cause	Recommended solution
Increase in 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
	Sample precipitation on the column	Perform CIP (refer to regeneration conditions)
	Dirty sample or substances with a strong adsorption	Perform CIP (refer to regeneration conditions)
	Column bed compressed	Repack the column.
	Long service time of the column	Replace the column or the chromatography filler
Insufficient sample adsorption	Too high ion strength in the sample solution	Reduce the ion strength in the sample solution by dilution or
	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
	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 gradient	Change the elution conditions to a slower gradient or
	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 load
Decreased binding of the sample after	Impurities in the sample bound to the Resin	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	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
	Trace ionic impurities bound to the column and	Clean the column
Too long equilibration time	Direct re-equilibration after CIP	Equilibrate with 2 CV of buffer B (containing 1 M NaCl) and

Ordering Information

Format	Size	Catalog
UniGel-30Q	30 mL	04084-030100-2030
	50 mL	04084-030100-2050
	100 mL	04084-030100-2100
	300 mL	04084-030100-2300
	500 mL	04084-030100-2500
	1 L	04084-030100-1001
	5 L	04084-030100-1005
	10 L	04084-030100-1010
	50 L	04084-030100-1050
	100 L	04084-030100-1100
UniGel-80Q	30 mL	04084-080100-2030
	50 mL	04084-080100-2050
	100 mL	04084-080100-2100
	300 mL	04084-080100-2300
	500 mL	04084-080100-2500
	1 L	04084-080100-1001
	5 L	04084-080100-1005
	10 L	04084-080100-1010
	50 L	04084-080100-1050
	100 L	04084-080100-1100
UniGel-30SP	30 mL	04082-030100-2030
	50 mL	04082-030100-2050
	100 mL	04082-030100-2100
	300 mL	04082-030100-2300
	500 mL	04082-030100-2500
	1 L	04082-030100-1001
	5 L	04082-030100-1005
	10 L	04082-030100-1010
	50 L	04082-030100-1050
	100 L	04082-030100-1100
UniGel-80SP	30 mL	04082-080100-2030
	50 mL	04082-080100-2050

	100 mL	04082-080100-2100
	300 mL	04082-080100-2300
	500 mL	04082-080100-2500
	1 L	04082-080100-1001
	5 L	04082-080100-1005
	10 L	04082-080100-1010
	50 L	04082-080100-1050
	100 L	04082-080100-1100
UniGel-30CM	30 mL	04081-030100-2030
	50 mL	04081-030100-2050
	100 mL	04081-030100-2100
	300 mL	04081-030100-2300
	500 mL	04081-030100-2500
	1 L	04081-030100-1001
	5 L	04081-030100-1005
	10 L	04081-030100-1010
	50 L	04081-030100-1050
	100 L	04081-030100-1100
UniGel-80CM	30 mL	04081-080100-2030
	50 mL	04081-080100-2050
	100 mL	04081-080100-2100
	300 mL	04081-080100-2300
	500 mL	04081-080100-2500
	1 L	04081-080100-1001
	5 L	04081-080100-1005
	10 L	04081-080100-1010
	50 L	04081-080100-1050
	100 L	04081-080100-1100
UniGel-30DEAE	30 mL	04083-030100-2030
	50 mL	04083-030100-2050
	100 mL	04083-030100-2100
	300 mL	04083-030100-2300
	500 mL	04083-030100-2500
	1 L	04083-030100-1001
	5 L	04083-030100-1005

	10 L	04083-030100-1010
	50 L	04083-030100-1050
	100 L	04083-030100-1100
UniGel-80DEAE	30 mL	04083-080100-2030
	50 mL	04083-080100-2050
	100 mL	04083-080100-2100
	300 mL	04083-080100-2300
	500 mL	04083-080100-2500
	1 L	04083-080100-1001
	5 L	04083-080100-1005
	10 L	04083-080100-1010
	50 L	04083-080100-1050
	100 L	04083-080100-1100

We also offer 7.7 mm × 22 mm、16 mm × 25 mm、7.7 mm × 100 mm prepacked columns. More requirements about products or customized needs, please

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