
Uni®HR Series

Hydrophobic chromatography medium

Product instruction manual

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Hydrophobic chromatography medium

Hydrophobic chromatography is a technique to separate biological macromolecules by utilizing their hydrophilicity differences. Under the condition of high salt, the biological macromolecules adsorbed on the hydrophobic groups of the hydrophobic chromatography medium, and by reducing the salt concentration, different proteins were eluted from weak to strong in order to achieve the purpose of separation and purification. Hydrophobic chromatography is one of the most commonly used methods for separation and purification of biomacromolecules with mild operating conditions and high resolution.

Nano hydrophobic chromatography medium Uni®HR uses monodisperse highly crosslinked PMMA (polymethyl methacrylate) hydrophilic modified microspheres as a matrix. By advanced bonding technology, phenyl and butyl hydrophobic groups are fixed on the surface of the matrix, and biomolecules are adsorbed under high salt conditions and elution under low salt conditions. It has the advantages of high adsorption capacity and low non-specific adsorption, and is suitable for further separation and purification after ion exchange chromatography.

Product advantages:

- a) Using monodisperse microsphere packing, better batch to batch stability
- b) Patented surface bonding technology for higher dynamic load
- c) The compression coefficient of packing column is much lower than that of agarose and dextran gel
- d) More complete packing specifications, to provide customers with professional customized services

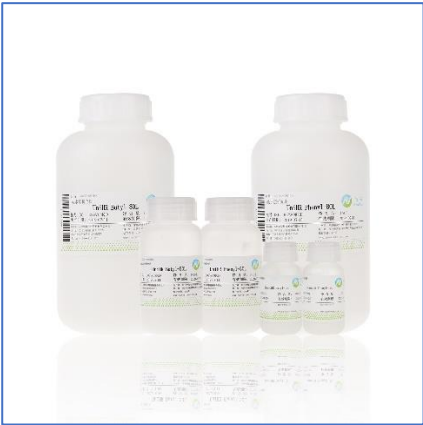


Figure 1. Uni®HR hydrophobic chromatography product diagram

Table 1. List of properties of hydrophobic chromatography media

	Argument
Matrix	PMMA
Ligand	Phenyl or Butyl
Dynamic loading capacity	10~30 mg/ml (Lys)
Particle size (μm)	30/60/80
Aperture (Å)	500/1000
Purification	Capture and moderate purification
Main feature	The skeleton has good hydrophilicity, good biocompatibility, alkaline resistance and high sample recovery
Typical application	Suitable for the capture and purification of antibodies, proteins, peptides, nucleic acids, antibiotics and other biological macromolecules

Table 2. Characteristics of Uni®HR

	Uni®HR Butyl-30S	Uni®HR Phenyl-30S	Uni®HR Butyl-30L	Uni®HR Phenyl-30L
Separation principle	Hydrophobic action			
Matrix	PMMA			
Particle size	35 µm	35 µm	32 µm	32 µm
Ligand	Butyl	Phenyl	Butyl	Phenyl
Dynamic loading capacity	~23 mg·mL ⁻¹ (Lys)	~30 mg·mL ⁻¹ (Lys)	~15 mg·mL ⁻¹ (Lys)	~15 mg·mL ⁻¹ (Lys)
Maximum pressure resistance	1.0 MPa			
CIP	1 M NaOH / 70% Ethanol			
Recommended flow rate	50-300 cm/h			
pH stability	2-12			
Chemical stability	All common buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 70% ethanol, 30% isopropyl alcohol, 30% acetonitrile, 1%SDS, 6M guanidine hydrochloride, 8 M urea and other common organic solvents; Avoid exposure to strong oxidants.			
Service temperature	15-30 °C			
Storage	20% Ethanol, 4-25 °C			

	Uni®HR Butyl-60S	Uni®HR Phenyl-60S	Uni®HR Butyl-80L	Uni®HR Phenyl-80L
Separation principle	Hydrophobic action			
Matrix	PMMA			
Particle size	60 µm	60 µm	80 µm	80 µm
Ligand	Butyl	Phenyl	Butyl	Phenyl
Dynamic loading capacity	~20 mg·mL ⁻¹ (Lys)	~20 mg·mL ⁻¹ (Lys)	~10 mg·mL ⁻¹ (Lys)	~10 mg·mL ⁻¹ (Lys)
Maximum pressure resistance	0.8 MPa	0.8 MPa	0.5 MPa	0.5 MPa
CIP	1 M NaOH / 70% Ethanol			
Recommended flow rate	100-500 cm/h	100-500 cm/h	150-750 cm/h	150-750 cm/h
pH stability	2-12			

Chemical stability	All common buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 70% ethanol, 30% isopropyl alcohol, 30% acetonitrile, 1%SDS, 6M guanidine hydrochloride, 8 M urea and other common organic solvents; Avoid exposure to strong oxidants.
Service temperature	15-30 °C
Storage	20% Ethanol, 4-25 °C

Column Packing

UniHR series product is 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: 1.1.

UniHR series product is 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: is 10-23 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 × 1.1.

Example: The mass of homogenate required for packing a 25L column (40 cm I.D.×20 cm) is: 25 L×1.5 Kg/L ×1.1=41.25 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 1.1.

Note: Generally the mass of filler is calculated using a percentage >120% since the ingress 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 60 cm/h. For the specific test parameters, refer to Table 3:

Table 3. Acetone or NaCl can be used as the indicator, and the indicator solution and mobile phase are prepared according to the following table:

Sample	1.0% (v/v) Acetone	2 M NaCl
Sample injection	1.0% column volume	1.0% column volume
Buffer	water	0.5 M NaCl
Linear flow rate	50~200 cm/h	50~200 cm/h
Detection	UV 280 nm	Electric conductivity

Recommended qualification	As: 0.8-1.5; Plates (N/m) : >2500
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How to use

1. The separation principle of hydrophobic chromatography media is separated according to the hydrophobic difference of biomolecules.
2. When using the hydrophobic chromatography medium, the (NH₄)₂SO₄ buffer is generally selected as the balance buffer, and the concentration of (NH₄)₂SO₄ is selected according to different samples.
3. The sample should also be dissolved in (NH₄)₂SO₄ buffer.
4. Select more suitable hydrophobic chromatography media through the screening of chromatographic media.

The use and optimization of salt in hydrophobic chromatography:

- 1) The commonly used salt is ammonium sulfate, but other salts can also be used.

Na₂SO₄>K₂SO₄>(NH₄)₂SO₄>Na₂HPO₄>NaCl

- 2) Temperature has a great effect on the solubility of salt, and it is generally recommended to operate at 20-25 °C. If you need to operate at low temperatures, you must consider the saturation and dissolution of the salt.

- 3) Hydrophobic chromatography is generally carried out around pH neutral.

Clean in Place (CIP)

With the increase of the use of chromatographic media, the accumulation of pollutants on the chromatographic column is also increasing, and regular in-place cleaning can prevent the accumulation of pollutants and maintain a stable working state. Determine the frequency of in-place cleaning according to the degree of pollution of the medium (if the pollution is serious, it is recommended that in-place cleaning should be carried out after each use to ensure repeatability of the results). Recommended cleaning conditions for different types of impurities and pollutants are as follows:

1. Rinse the strongly bound proteins with 3-5 CV of pure water first.
2. For the removal of strong hydrophobic proteins and precipitated proteins: clean with 3 CV 1 M NaOH first, then immediately rinse with 5 CV pure water, and finally rinse with balanced solution to the baseline.

Removal of lipoproteins and lipids: Wash with 70% ethanol or 30% isopropyl alcohol at 5-10 CV, then rinse with 5-10 CV pure water.

Storage

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

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.

Ordering Information

Format	Size	Catalog
Uni®HR Butyl-30S	30 mL	06132-030050-2030
	50 mL	06132-030050-2050
	100 mL	06132-030050-2100
	300 mL	06132-030050-2300
	500 mL	06132-030050-2500
	1 L	06132-030050-1001
	5 L	06132-030050-1005
	10 L	06132-030050-1010
	50 L	06132-030050-1050
	100 L	06132-030050-1100
Uni®HR Phenyl-30S	30 mL	06131-030050-2030
	50 mL	06131-030050-2050
	100 mL	06131-030050-2100
	300 mL	06131-030050-2300
	500 mL	06131-030050-2500
	1 L	06131-030050-1001
	5 L	06131-030050-1005
	10 L	06131-030050-1010
	50 L	06131-030050-1050
	100 L	06131-030050-1100
Uni®HR Butyl-30L	30 mL	06132-030100-2030
	50 mL	06132-030100-2050
	100 mL	06132-030100-2100
	300 mL	06132-030100-2300
	500 mL	06132-030100-2500
	1 L	06132-030100-1001
	5 L	06132-030100-1005
	10 L	06132-030100-1010
	50 L	06132-030100-1050
	100 L	06132-030100-1100
Uni®HR Phenyl-30L	30 mL	06131-030100-2030
	50 mL	06131-030100-2050
	100 mL	06131-030100-2100

	300 mL	06131-030100-2300
	500 mL	06131-030100-2500
	1 L	06131-030100-1001
	5 L	06131-030100-1005
	10 L	06131-030100-1010
	50 L	06131-030100-1050
	100 L	06131-030100-1100
Uni®HR Butyl-60S	30 mL	06132-060050-2030
	50 mL	06132-060050-2050
	100 mL	06132-060050-2100
	300 mL	06132-060050-2300
	500 mL	06132-060050-2500
	1 L	06132-060050-1001
	5 L	06132-060050-1005
	10 L	06132-060050-1010
	50 L	06132-060050-1050
	100 L	06132-060050-1100
Uni®HR Phenyl-60S	30 mL	06131-060050-2030
	50 mL	06131-060050-2050
	100 mL	06131-060050-2100
	300 mL	06131-060050-2300
	500 mL	06131-060050-2500
	1 L	06131-060050-1001
	5 L	06131-060050-1005
	10 L	06131-060050-1010
	50 L	06131-060050-1050
	100 L	06131-060050-1100
Uni®HR Butyl-80L	30 mL	06132-080100-2030
	50 mL	06132-080100-2050
	100 mL	06132-080100-2100
	300 mL	06132-080100-2300
	500 mL	06132-080100-2500
	1 L	06132-080100-1001
	5 L	06132-080100-1005
	10 L	06132-080100-1010

	50 L	06132-080100-1050
	100 L	06132-080100-1100
Uni®HR Phenyl-80L	30 mL	06131-080100-2030
	50 mL	06131-080100-2050
	100 mL	06131-080100-2100
	300 mL	06131-080100-2300
	500 mL	06131-080100-2500
	1 L	06131-080100-1001
	5 L	06131-080100-1005
	10 L	06131-080100-1010
	50 L	06131-080100-1050
	100 L	06131-080100-1100

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

NanoMicro Technologies, Inc.

National hotline: 400-828-1622	Email: info@nanomicro.com	(The company's products are only for scientific research or industrial use)	
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