HyperTrap Geldex 200 PG Column

Product Description:

Geldex PG is a series of gel filtration chromatography media for cross-linked agarose scaffolds that take advantage of the molecular weight differences of different molecules to separate them. The series consists of three chromatography media for different separation ranges. This series of chromatography media can be well used for the separation and purification of a variety of biomolecules, such as: peptides, polysaccharides, recombinant proteins, nucleic acids, viruses, etc.

The Geldex PG series of chromatography media offers excellent scale-up throughput: (1) The improved Geldex base frame is more rigid, so it can achieve higher process flow rates and improve process efficiency at lower backpressures. (2) The modified Geldex substrate has better chemical resistance and is compatible with a wide range of buffer environments. (3) Fine particle size design to improve resolution.

Both the Geldex PG series are available in two prepacked column sizes, with 16 mm and 26 mm I.D. and 600 mm bed height, packed with 16/700 and 26/700 empty columns, respectively. Taking globulin as a reference, the separation range of this product is 10-600 kD, which has the characteristics of low non-specific adsorption and good chemical stability.

Product parameters

1. HyperTrap Geldex 200 PG Column prepacked column parameters

Column volume	120 mL	320 mL
Bed dimensions	16 x 600 mm	26 x 600 mm
Sample volume*	≤5 mL	≤13 mL
Pressure-resistant	0.5 MPa	0.3 MPa
Flow rate**	1 mL/min	1-3 mL/min
Operating temperature	4 - 30℃	4 - 30℃
Storage conditions	Store the components at 4℃ for 5 years.	

*It can be adjusted according to the actual situation

**This flow rate is the recommended common flow rate, the pressure resistance and flow rate of different types of chromatography resins in the prepacked column will be different, please refer to the product manual of the corresponding chromatography resin.

2. HyperTrap Geldex 200 PG Column chromatography media parameters

Name	Description
Chromatography media type	Gel filtration
Scaffolding	High degree of cross-linking agarose with dextran linker arm
Separation range	10-600 kD
Main particle size distribution*	22-44 μm
Flow rate is recommended	10-50 cm/h
Maximum flow rate	100 cm/h
Pressure-resistant	0.3 MPa
Operating temperature	4 - 30℃
pH stability**	2-14
Chemical resistance	Common aqueous solution, 30% isopropanol***, 75% ethanol***, 1 M NaOH, 1 M acetic acid, 6 M guanidine hydrochloride, 8 M urea

*Percentage of distribution in range≥80%.

**After 7 days of storage at 40°C, pH 2-14, the physicochemical properties and functions of the chromatography medium showed no significant change

***v/v, volume ratio.

Experimental manipulation

1. **Preparation of buffers**

Buffer selection: The stability of the sample in the buffer should be considered. For separation and purification, it is advisable to use a salt-containing buffer rather than purified water to avoid possible non-specific adsorption. If buffer exchange or desalting is performed, the buffer of interest is used directly.

2. **Chromatography conditions**

- Flow rate selection: According to the height of the column bed, the linear flow rate of 10-50 cm/h is generally selected.
- Sample preparation: To prevent the sample from clogging the column, the sample needs to be filtered with a 0.2/0.45 μm (after inclusion body breakage) microfiltration membrane prior to loading.

3. **Pre-packed column settings**

1) Open the package and remove the pre-packed column.

2) Connect the prepacked column: Unscrew the upper and lower plugs of the prepacked column and connect to the chromatography system (peristaltic pump or syringe, etc.), and rinse the prepacked column with pure water 2-3 times the column volume to drain the preservation solution (typically

20% ethanol). In order to prevent air bubbles from entering during the connection, the connector can be connected by dripping pure water into the inlet end of the prepacked column and filling it before connecting the system connector.

3) Wash and sterilize: For the first use, it is recommended to wash and disinfect the column and rinse 2 times the column volume with pure water or buffer. Cleaning and disinfection buffers are recommended, please refer to the subsequent cleaning and regeneration steps.

4) Sample preparation: To prevent the sample from clogging the column, it is recommended that the sample be filtered with a 0.45 μm microporous membrane prior to sample loading.

4. Column efficiency measurement (optional)

Select one of the two test methods shown in the table below for column efficiency testing. The mobile phase equilibration column was used to stabilize the baseline, the sample was loaded into the column, the mobile phase was continued to be rinsed, and the chromatographic peaks were returned to the baseline after the operation was completed, and the chromatographic peaks were integrated to evaluate the loading effect.

Table2 Statistical table of the two column efficiency determination methods

	Acetone method	NaCl method
Sample	1% (v/v) acetone in water	2 M NaCl in water
Sample volume	1% column volume	1% column volume
Mobile phase	water	0.2 M NaCl in water
Velocity of flow	30 cm/h	30 cm/h
Detectors	UV 280 nm	electrical conductivity

The main evaluation criteria for column loading effect are N/m (number of plates per meter) and As (symmetry factor), which are calculated as follows:

$$\frac{N}{m} = 5.54x(\frac{V_R}{W_h})^2 \times \frac{1}{L}$$

$$As = {}^{b}/_{a}$$

Column efficiency qualification standard: N/m > 5000, 0.8 < As < 1.5

*Parameter Notes:

L = column height, VR = reserved volume, Wh = peak width at half height, a = width at 10% peak height on the left half, b = width at right half height at 10% peak height

5. Chromatography steps

(1) Equilibration: Equilibrate the column well with buffer until the pH and conductivity are stable and largely consistent with the equilibration buffer, a step that typically requires 1-2 times the column volume.

(2) Loading: Usually, the amount of sample loaded for separation and purification is not more than 5% of the column volume, and the sample concentration is not easy to be too high, so as not to overpressure or affect the resolution.

(3) Elution: Use buffer to elution and collect components from different positions, usually requiring 1~1.5 times the column volume.

(4) Regeneration: Rinse the column with a buffer containing high salt (e.g., 1 M NaCl).

(5) Re-equilibration: Equilibrate the column again with buffer.

6. Cleaning and regeneration

As the chromatography media is used, contaminants (e.g., lipids, endotoxins, proteins, etc.) accumulate on the column. Regular, cleaning-in-place is essential to keep the column in stable working order. The frequency of CIP is determined based on the degree of contamination of the chromatography media (if the contamination is severe, it is recommended that CIP should be performed after each use to ensure reproducibility of results and extend the working life of the chromatography media).

For different types of impurities and contaminants, cleaning can be carried out under the following conditions:

- Removal of highly binding proteins: Wash with 1 column volume of 2 M NaCl solution, or use a high-salt buffer not lower than pH 2, such as 1 M NaAc.
- Removal of strongly hydrophobic and precipitated proteins: Wash with 1 column volume of 0.5 M NaOH and then wash the lye with 2-3 column volumes of purified water.
- Lipoprotein and lipid removal: Wash with 1 column volume of 70% ethanol or 30% isopropanol, then rinse with 2-3 column volumes of purified water.

  Note: 70% ethanol or 30% isopropanol should be degassed before use, the flow rate can be selected from 10-30 cm/h during cleaning-in-place, and reverse cleaning can be used when blockage is severe.

7. Sterilization

In order to reduce the microbial load, it is recommended to use 0.5 M NaOH solution to treat the chromatography medium with a treatment time of 30~60 min.

8. Destruction & Recycling

- Since Geldex PG chromatography media is difficult to degrade in nature, it is recommended to incinerate the waste chromatography media in order to protect the environment.
- For chromatography media that have come into contact with biologically active samples such as viruses and blood, please follow local biosafety requirements before destroying or disposing of

them.

9. **Stockpile**

Store unopened chromatography media in its original container, soak the packed column in 20% ethanol and close the upper and lower column heads. The storage environment is 4~30°C.

Precautions

1. It is recommended that the buffers and protein solutions used for purification be filtered through a 0.22 μm or 0.45 μm filter before being used on the column.
2. The outer tube of the column is made of glass, and the inner tube is made of glass.
3. This product is for scientific research purposes only.

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