BESTCHROM
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Chromdex 30 PG
Chromdex 75 PG
Chromdex 200 PG
Gel Filtration Resin

Instruction for use

Contents

1. Introduction

Chromdex 30 PG (prep grade), Chromdex 75 PG and Chromdex 200 PG are based on high crosslinked agarose and filled with crosslinked dextran. Their average particle size is 34μm. They have both high selectivity of dextran and physical properties of agarose, high resolution and high hardness. The column bed is a good choice for fine purification stage due to its small variation with buffer concentration, stable chemical properties, low non-specific adsorption, high recovery and easy amplification.

2.Technical characteristics

Product		Chromdex 30 PG	Chromdex 75 PG	Chromdex 200 PG
Appearance		White slurry, can be layered		
Matrix		Highly cross-linked agarose and dextran		
Separation range	Linear molecule	0.4KD~7KD	0.5KD~30KD	1KD~100KD
	Globular molecule	0.3KD~10KD	3KD~70KD	10KD~600KD
Particle size+		24-44μm		
Average particle size		34μm		
Pressure flow velocity		30~50cm/h(0.1MPa BXK26/100,H=80cm, 20℃)		
Max. pressure		0.3MPa		
pH stability		3~12(working),1~14(CIP)		
Chemical stability		Stable in common aqueous buffers: 0.5M NaOH++, 1.0M acetic acid , 6M GuHCl,8M Urea, 50mM PB pH7.0、2%SDS 24% ethanol		
Storage+++		2~30℃, 20% ethanol or 2% benzyl alcohol		

+Particle size is normally distributed, and particles within this range account for more than 75% of the total.

++0.5M NaOH only be used for cleaning

+++2% benzyl alcohol is only used for international transport or special requirements from customer

3. Method of chromatographic

3.1 Column packing

Note：It is best to equilibrate the resin slurry to room temperature before column packing.

- According the column volume to calculate the amount of resin.

  Resin volume=column volume×1.15(Compression factor=1.15)

  According to the volume of the settlement resin required, the suspended slurry of the resin required is calculated by the follow:

  Required resin slurry[1] volume = Settlement resin volume ÷ Resin slurry [1] concentration. The original concentration of resin slurry [1] is shown in the follow table.

Pack size	Resin slurry [1] concentration (%)
100mL、500mL、1L、5L、10L	80
20L、40L	75

1: It refers to the original packaging resin slurry sold by Bestchrom.

Note: For non-original packaging, customer can calculate the required volume according to the actual concentration of resin slurry.

- Washing the resin:Thoroughly shake the resin and weigh certain volume of resin calculated by the method mentioned above. Pour it into a funnel, drain the liquid, and wash with about 3mL distilled water/mL resin for 3 times. Use a glass stick or stirrer to stir each time when adding distilled water, which helps to wash the shipping solvent away.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water to obtain a 45%~55% slurry, stir well and set aside for use.
- Take a cleaned BXK column (BXK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications).Take BXK16/70 for example, purge the bubbles trapped at the end-piece net by draining some distilled water through the column outlet. Leave about 1cm water at the bottom of the column and close the bottom outlet. Adjust the column so that it is perpendicular to the ground.
- Slowly pour the slurry into the column at one time (use a packing reservoir if necessary). Do not bring any air bubbles into the column.

  Packing reservoir: Empty glasstube with same diameter as the BXK column.
- Fill the remainder of the column with packing solution. Connect the packing reservoir to the chromatography system, open the flow velocity, drain the bubbles in the hose, close the flow velocity, and tighten the top cover of the packing reservoir.

✧ After pouring, stir well again with Stirrer, and then wash the resin particles on the inner wall of the column from top to bottom with the packing solution, and let the resin settle naturally until there is about 1cm of clarifying solution on the suspension. Mount the adapter and connect the adapter to the chromatography system or peristaltic pump. Lower the adapter to descend to

contact with the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. With the outlet of the top piece is opened, slowly move the adapter down until all bubbles are drained.

Note: This operation is only applicable to BXK 100 and above columns. Flushing the inner wall reduces the resin particles sticking between the seal ring and the column wall, avoiding the risk of leakage.

- When the bed height is 60cm, the flow velocity can be set to 30cm/h. Open the bottom plug, start the pump and run the setting flow velocity until the bed is stabilized, mark the bed height.
- Remove the packing reservoir (if any), Install the adaptor lower the adaptor to about 0.5cm above the resin surface, set the flow velocity (Chromdex 30 PG is 240cm/h, Chromdex 75 PG is 270cm/h, Chromdex 200 PG is 360cm/h), and continue to press the column using the above flow velocity until the bed is completely consolidated, mark the consolidated bed height.
- Stop the pump, open top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.3cm below the marked position, tighten the O-ring seal, close adaptor stop plug, and complete the column packing.

3.2 Evaluation of Packing

- The packing quality of chromatographic column can be confirmed by column efficiency measurement and evaluation. The tests are required after the column packing, during the column working life and when the separation and purification performance weakens. The method usually relies on the height equivalent to a theoretical plate(HETP) and the asymmetry factor(As).
- Acetone or NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	Acetone method	NaCl method
Sample	1.0%(v/v)acetone in water	0.8M NaCl in water
Sample volume	1.0%CV	1.0%CV
Mobile phase	Water	0.4M NaCl in water
Flow velocity	20cm/h	20cm/h
Monitor	UV280 nm	Conductivity

- Method for measuring HETP and As:

  Use UV curve or the conductivity curve to calculate the height equivalent of theoretical plate (HETP), number of theoretical plates(N) and the asymmetry (As):

HETP=L/N

$N=5.54(V_R/W_h)^2$

Note: V_R = retention volume

W_h = half-peak width

L = column height

N = the number of theoretical plates

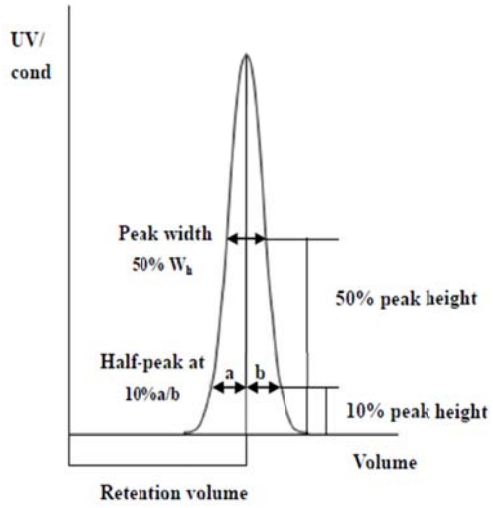
(The units of V_R and W_h should be the same)

As=b/a

Note:

a= 1st half peak width at 10% of peak height

b= 2nd half peak width at 10% of peak height



- Evaluation the column packing

  As a guideline, if the value of HETP is less than 3 times the average particle size(d_{50}) of the resin and the As is between 0.7~1.3, the column is very efficient. (For Chromdex resin, the number of trays per meter should be greater than 10,000) .The unsatisfactory results should be analyzed and the column should be repacked.

3.3 Chromatographic method

- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading, when the viscosity is too high, it can be diluted appropriately, and the protein concentration generally does not exceed 70mg/ mL.
- Equilibration: Use the recommended flow velocity to flush the column with equilibration buffer. The choice of equilibration buffer depends on the stability of the sample. The type and pH of buffer have little influence on the effect of gel filtration, but agarose contains a small amount of sulfate and carboxyl groups, in order to reduce the non-specific adsorption of basic protein samples, it is recommended to add at least 0.15M NaCl into the equilibration buffer. The pH and conductivity of the buffer to be exported and the buffer before entering the column indicate that the column is well balanced, generally 2~5CV are required.
- Sampling: The sample is loaded onto the column through the sample loop of the chromatography system. The volume of the sample will be different according to the difference between the target and the impurity. Generally, the sample volume of 0.5 to 5% of the column volume is loaded, and the loading volume can be adjusted appropriately according to the separation effect.
- Separation: Continue to rinse the column with equilibration buffer to collect the different components that flow out, until no more biomolecules flow out, generally 1~1.5CV are required.
- Regeneration: Rinse 2~3CV with balanced buffer solution.
- Rebalancing: After rinsing with equilibration buffer, the second sample can be loaded and repeated.

4. Cleaning-in-place(CIP)

Chromdex 30 PG、Chromdex 75 PG、Chromdex 200 PG in some processes, there are denaturated proteins, lipids, strong hydrophobic proteins, etc., in the regeneration process is not easy to elute, Or after using for a period of time, the column efficiency may decrease, the back pressure may increase, the separation effect will become worse, the color of the chromatographic resin will change, etc., the following process can be used for in-place cleaning (it is generally recommended to clean once every 5 cycles).

- First rinse 1CV with buffer containing 1M NaCl
- Removal of denatured protein
  - 2CV were backwashed with 0.5M NaOH at a flow velocity of 20 cm/h.

    Note：Denatured protein can also be removed with protease, using 1mg/mL gastric enzyme dissolved in 0.1 M acetic acid solution containing 0.5 M NaCl.
- Remove lipids or lipoproteins
  - Use 70% ethanol or 30% isopropanol to flush 4CV at a flow velocity of 40cm/h (in order to prevent air bubbles, a gradient method can be used to gradually increase the ratio of organic solvents).
  - Or use 1% non-ionic detergent
- Inorganic pollutants
  - Flush 2CV with 0.5 M acetic acid
- Rinse 4CV with distilled water

5. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation,it is recommended that Chromdex 30 PG、Chromdex 75 PG and Chromdex 200 PG can be treated with 0.5~1M NaOH before and during use.

6. Storage

Chromdex 30 PG、Chromdex 75 PG and Chromdex 200 PG are supplied in 20% ethanol or 2% benzyl alcohol. They should be stored in 20% ethanol and sealed at 2-30℃ after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months.

7. Disposal and Recycling

Chromdex 30 PG、Chromdex 75 PG and Chromdex 200 PG are very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
Chromdex 30 PG	AG317107	100mL
	AG317111	500mL
	AG0213	1L
	AG0214	5L
	AG0215	10L
	AG317115	20L
	AG317116	40L
Chromdex 75 PG	AG318107	100mL
	AG318111	500mL
	AG0073	1L
	AG0074	5L
	AG0075	10L
	AG318115	20L
	AG318116	40L
Chromdex 200 PG	AG319107	100mL
	AG319111	500mL
	AG0083	1L
	AG0084	5L
	AG0085	10L
	AG319115	20L
	AG319116	40L

Prepacked columns	Code No.	Pack size
EzLoad 16/60 Chromdex 75 PG	EG004	1 pcs
EzLoad 16/90 Chromdex 75 PG	EG020	1 pcs
EzLoad 26/60 Chromdex 75 PG	EG005	1 pcs
EzLoad 26/90 Chromdex 75 PG	EG00713	1 pcs
EzLoad 16/60 Chromdex 200 PG	EG006	1 pcs
EzLoad 16/90 Chromdex 200 PG	EG021	1 pcs
EzLoad 26/60 Chromdex 200 PG	EG007	1 pcs
EzLoad 26/90 Chromdex 200 PG	EG00813	1 pcs