BESTCHROM
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BXP16 Lab scale chromatography column

Instruction for use

Contents

1. Introduction

BXP16 lab column is innovatively designed low/medium pressure column. It is applicable in the processing of micro-molecules such as recombinant protein, antibody, vaccine and blood products, as well as R&D of bio-micromolecules such as antibiotics, peptide, synthetic drugs and other natural substances. The columns can be loaded by agarose-based, dextran-based and polymer-based resins such as Bestdex, Bestarose, Chromdex. They can also be connected to domestic and imported chromatography systems. The product is made of high borosilicate glass inner tube, acrylic outer tube and POM plastic, which provides bio-compatibility, chemical resistance and compatibility with most aqueous and organic solutions.

Advantages of BXP 16 lab column:

- User-friendly, easy operation
- Equipped with double adaptors, enabling higher flexibility during packing
- Flared interface connector and highly elastic O-ring effectively prevent leakage
- Higher tolerance towards organic solution
- Evenly distributed outflow, promoting column efficiency after packing

2. Technical parameters

Product	BXP 16/30	BXP 16/40 column	BXP 16/60 column
Inner diameter(mm)	16	16	16
Height(cm)	30	40	60
Volume(ml)	0-46	20-64	60-100
Column height(cm)	0-23	10-32	30-50
Operating pressure(bar)	20(Max		
Operating temperature/℃	2-60		
pH stability	1-14		
Sieve pore size*(μm)	10		
Chemical stability	Common aqueous solutions		

***10um sieve is standard, 23um sieve is also available**

3. Column structure

BXP16 column mainly consists of two adaptors, glass tube and column joint.

- **Tube：** Tube is made of high precision glass, Tube height ranges from 20cm、30cm、40cm and 60cm.
- **Adaptor components：** soft pipeline、M6 joint、adjusting knob、screw rod fastener、screw rod、sealing ring、adaptor inner tube、plug.

4. Column packing

10~15cm loading bed is recommended for adsorption chromatography; For molecule sieve, column bed should be as high as possible under the recommended flow rate.

1) Pack the column (connect to adaptor if necessary), wash column with purified water or 20% ethanol.

2) Remove the bottom adaptor and wash with buffer, drain the bubble under the sieve net, mount the adaptor to the column bottom, tighten the lower plug. Keep 1cm height of liquid in the column bottom, adjust column and keep it vertical to ground.

3) Add buffer to the media, prepare the slurry according to the user instruction

4) Stir slurry well and pour it slowly to the column at one time, make sure do not take any bubble in.

5)If a reservoir is available, slowly pour moderate amount of buffer to the reservoir. Connect upper adaptor to the chromatography system, drain the bubbles in the adaptor. Mount adaptor on the column, press adaptor under the gel surface, tighten the knob.

6) Set the flow rate, open bottom adaptor and bottom plug. Open the pump and press the gel.

7) When the column bed surface is stable for more than 15 min, shut pump and tighten the bottom plug.

8) Wash the upper adaptor with buffer solution, drain the bubble trapped in the sieve net, remove reservoir(if available), connect the upper adaptor to column.

9) Adjust adaptor to about 0.5~1cm above the gel surface, make sure adaptor is filled with liquid.

10) Open bottom plug, connect to pump, keep flow rate unchanged(make sure pressure is under max limit and 2MPa). Keep pressing gel till gel surface is stabled, mark the gel height.

11)Stop the pump, open the outlet of the top piece, close the outlet of the bottom piece,

loosen the seal ring slightly, press the adapter to about 3~5mm below the gel bed, tighten the seal ring, close the outlet, and complete the column packing.

1: Column tube + Column joint

2：The required flow rate varies from media and bed heights, please refer to user instructions or seek technical support from Bestchrom team.

5. Column efficiency testing and assessment

Efficiency of packed column can be assessed. The results can be affected by factors such as flow rate, loading sample and dead column volume. Due to the low volume of BXP10, dead volume can greatly affect column efficiency. It is recommended to choose fine column (column with 0.5mm inner diameter) and keep the tube connecting sample ring with column outlet as short as possible.

- Acetone or NaCl can be used as sample for the testing. Sample solution and eluent buffer 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
Loading	1.0% CV	1.0% CV
Buffer	Water	0.4M NaCl in water
Flow rate	30 cm/h	30cm/h
Monitor	UV 280 nm	Conductivity

- Method for measuring HETP and As
  According the UV curve or the conductivity curve to calculate the column efficiency (HETP), and the asymmetry (As):

  HETP=L/N

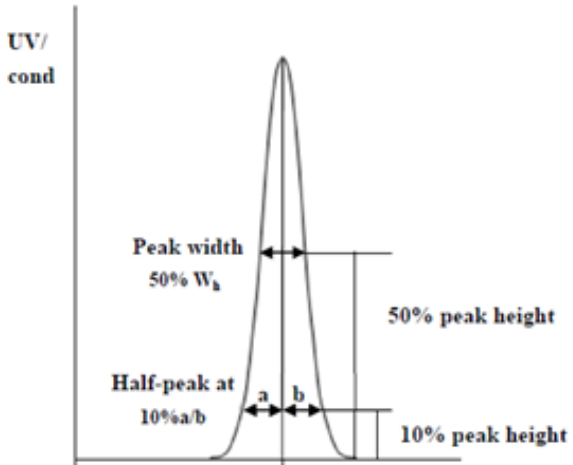
  N=5.54 (VR/Wh)2

  Note：

  VR = retention volume

  Wh = half-peak width

  L = column height



N = the number of theoretical plates

(The units of VR and Wh should be the same)

As=b/a

Note：

a= First half-peak width at 10% peak height

b = second half-peak width at 10% peak height

- Evaluation the column packing

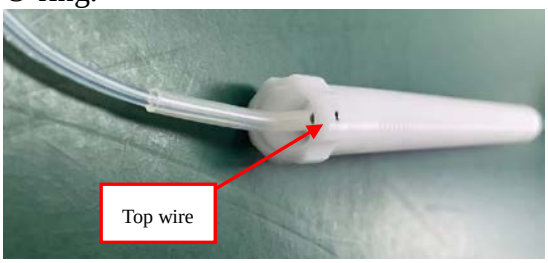
  As a guideline, if the value of HETP is less than 3 times the average particle size(d50) of the resin and the As is between 0.8~1.5,column is very efficient. The unsatisfactory result need to be analyzed and re-packing is necessary.

Particle size(μm)	Resin	N/m	As
34	Bestarose HP	＞8000	0.8-1.5
34	Chromdex	＞10000	0.7-1.3
90	Bestarose FF	＞3000	0.8-1.5
90	Bestarose XL	＞3000	0.8-1.5
75~90	Diamond	＞3500	0.8-1.5
200	Bestarose BB	＞2000	0.8-1.5

Column efficiency and As for common resins：

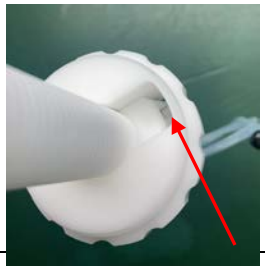
6. Cautions

- Make sure the column stopper and screw rod are tightened to avoid leakage
- For seriously blocked column after loading, reverse cleaning method can be used. Make sure lower the flow rate by 50% during washing.
- Keep the protective soft tube in the adaptor when using column, Do not fold soft tube to prevent breakage or effect on flow rate
- Keep top wires in adaptor tight for any loosing may cause damage to rod, which will be unable to tighten the O-ring.



- When mounting/removing adaptor, the O-ring should be loosen. If O-ring blocks, gently switch the adaptor. Never push/pull violently or shake adaptor to avoid breakage in glass tube.

7. Trouble shooting

Trouble	Cause and solution
Leakage from seal ring(O-ring)	1. Seal ring is damaged, replace with a new one; 2. Hard object is stuck between seal ring and glass tube. Wash the seal ring and tube; Mount the adaptor on after supernatant appearing on the gel surface.
Leakage from the joint of adaptor and soft pipe when using	M6 joint is not tightened when connecting to soft pipe.
Upper adaptor slides with seal-ring tightened when using(pressure is lower than 0.5MPa)	Adaptor spring is damaged, replace with a new one 
Back pressure is unusually high	1. Flow rate is higher than the max flow rate of resin during column packing. 1. Gel is overly cracked 2.Sample is not appropriately treated. Adaptor sieve is blocked by protein precipitation. Wash the sieve in absolute ethanol or 1M NaOH for 30min in ultrasonic cleaner. Replace a sieve if necessary. 3.Soft pipe is folded or blocked by alien objects
Flow rate is lower than setting rate	1. Check for the existence of air in pipes and tubes 2. Check for leakage

	3. Check for the normal operation of device
Resin leakage from the column lower outlet	1. Make sure the lower adaptor is correctly mounted 2. Make sure the sieve specification matches the resin particle size

8. Order information

Product	Item Code	Pack/pcs
BXP16/30 column	BC255221	1
BXP16/40 column	BC256221	1
BXP16/60 column	BC257221	1
Glass tube(BXP16/30 column)	BS215011	1
Glass tube(BXP16/40column)	BS216011	1
Glass tube(BXP16/60column)	BS217011	1
BXP16 column joint	BA410001	1
Complete Adaptor(16 column)	B-16A	1
Adaptor O-ring(16 column)	BS230015	5
10um sieve(16 column)	BS220045	5
23um sieve(16 column)	BS220055	5
Supporting sieve(16 column)	BS220035	5