BESTCHROM
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BXP16
Lab scale high precision chromatography column
Instruction for use

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1.Introduction

BXP16 lab column is innovatively designed low/medium pressure column. It is applicable in the processing of macromolecules 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. This product is composed of accessories such as a high borosilicate glass inner tube, a acrylic outer tube and a POM plastic column head, which provides bio-compatibility, chemical resistance and compatibility with most aqueous and organic solutions.

Advantages of BXP16 lab column:

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

2.Technical parameters

Product	BXP16/30	BXP16/40
Inner diameter(mm)	16	16
Height(cm)	30	40
Volume(mL)	0-46	20-64
Column height(cm)	0-23	10-32
Operating pressure（bar）	20(Max)	
Operating temperature/℃	2-60	
pH stability	1-14	
Sieve aperture*（μm）	10	
Chemical stability	Common aqueous solutions	

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

3.Column structure

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



Note：①adaptors ②glass tube ③column joint ④adaptors

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

4.Column loading

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[1]), 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[2], 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

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 BXP16, dead volume can greatly affect column efficiency. It is recommended to choose fine column(column with 0.75mm 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

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

Note：

V_R = retention volume

W_h = half-peak width

L = column height

N = the number of theoretical plates

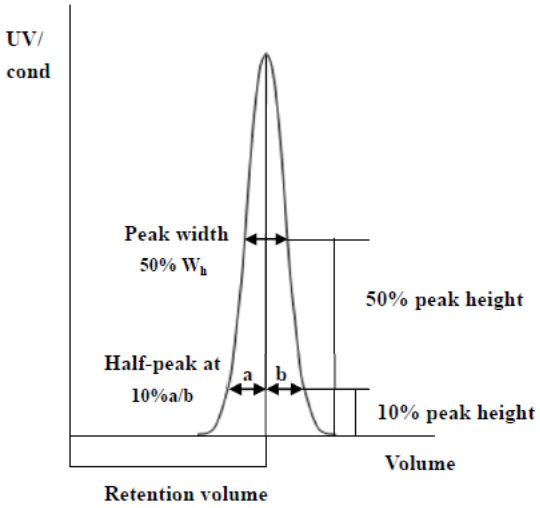
(The units of V_R and W_h should be the same)

As=b/a

Note：

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

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



- Evaluation the column packing

Generally, if the HETP value is less than three times the average particle size of the medium and the asymmetry factor falls within the range of 0.8 to 1.5, it indicates that the column efficiency is in good condition. For unsatisfactory column efficiency results, it is necessary to analyze the root causes and repack the column.

For packing media with different particle sizes, the column is considered to have a good packing performance when the measured column efficiency and asymmetry factor (As) meet the corresponding values specified in the table below.

Column efficiency and As for common resins

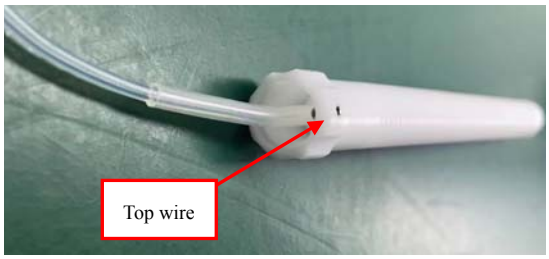
Particle size（μm）	Product	Column efficiency（N/m）	As
34	Bestarose HP	＞6000	0.8-1.5
34	Chromdex	＞10000	0.7-1.3
90	Bestarose FF	＞2500	0.8-1.5
90	Bestarose XL	＞2500	0.8-1.5
60~90	Diamond	＞3000	0.8-1.5
200	Bestarose BB	＞1000	0.8-1.5

Note: Column efficiency is related to the diameter and height of the chromatography column;

the above column efficiency is for reference only and can be adjusted upwards based on actual conditions.

6.Cautions

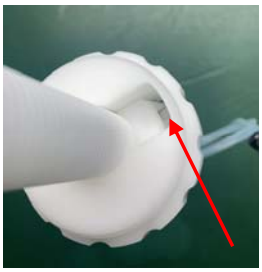
- Make sure the plunger heads at both upper and lower column end fittings are fully tightened, to prevent liquid leakage during the packing process.
- Press the column head vertically only. Rotational pressing is strictly prohibited to prevent plunger loosening, seal deformation and subsequent leakage.
- 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	1. Seal ring is worn, 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.

Trouble	Cause and solution
Leakage from the joint of adaptor and soft pipe when using	1.The plunger head and screw rod are not tightened. 2.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.Excessive column packing flow rate causes overpressure of the packing material inside the column.. 2.Gel is overly cracked. 3.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. 4.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 aperture matches the resin particle size.

8.Order information

Product	Item Code	Pack/pcs
BXP16/30	BC255221	1
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Glass tube（BXP16/30）	BS215011	1
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BXP16 column joint	BA410001	1
Complete Adaptor（16 column）	BS250031	1
Adaptor O-ring（16 column）	BS230015	5
10μm sieve（16 column）	BS220045	5
23μm sieve（16 column）	BS220055	5
Supporting sieve（16 column）	BS220035	5