



BESTCHROM

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BXK16/26/50 Lab scale chromatography column Instruction for use



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1. Introduction

BXP16/26/50 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 with domestic and chromatography systems (including AKTA). 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 16/26/50 lab column:

- User-friendly, easy operation
- Flared interface connector and highly elastic O-ring effectively prevent leakage
- Evenly distributed outflow, promoting column efficiency after packing
- Equipped with a thermostatic jacket for easy temperature control during the chromatography process

2. Technical parameters and materials

2.1 Technical parameters

Product	Inner diameter (mm)	Height (cm)	Volume (ml)	Column height (cm)	Operating pressure (bar)	Operating temperature (°C)	pH stability	Sieve pore size (μm)	Chemical stability
BXK 16/20	16	20	4-34	2-17	5 (Max)	2-60	1-14	10	Common aqueous solutions
BXK 16/40	16	40	44-76	22-37					
BXK 16/40 plus	16	40	16-54	8-32					
BXK 16/70	16	70	104-134	52-67					
BXK 16/70 plus	16	70	68-128	34-64					
BXK 16/100	16	100	164-194	82-97					
BXK 16/70 plus	16	100	128-188	64-94					
BXK 26/20	26	20	10-90	2-17	5 (Max)	2-60	1-14	10	
BXK 26/40	26	40	117-196	22-37					
BXK 26/70 plus	26	40	42-169	8-32					
BXK 26/70	26	70	276-355	52-67					
BXK 26/70 plus	26	70	180-339	34-64					
BXK 26/100	26	100	435-514	82-97					

Product	Inner diameter (mm)	Height (cm)	Volume (ml)	Column height (cm)	Operating pressure (bar)	Operating temperature (°C)	PH stability	Sieve pore size (µm)	Chemical stability
BXK 26/100 plus	26	100	339-498	64-94					
BXK 50/20	50	20	39-333	2-17	3 (Max)	2-60	1-14	10	Common aqueous solutions
BXK 50/30	50	30	235-529	12-26					
BXK 50/30 plus	50	30	0-471	0-24					
BXK 50/60	50	60	823-1117	42-57					
BXK 50/60 plus	50	60	470-1058	24-54					
BXK 50/100	50	100	1607-1901	82-97					
BXK 50/100 plus	50	100	1256-1845	64-94					

2.2 Materials

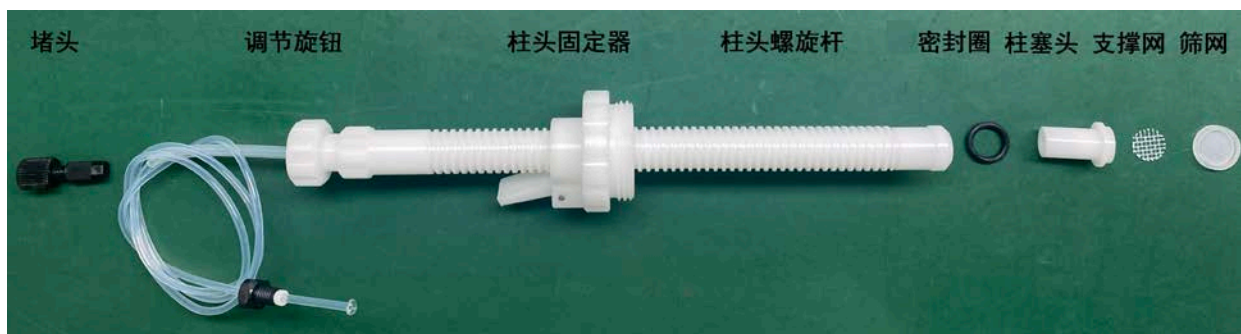
Upper/Lower thin hose	Upper/Lower adaptor	Tube	Seal ring	Upper/Lower support	Upper/Lower sieve
Fluorinated ethylene propylene copolymer (FEP)	polyformaldehyde (POM)	high borosilicate glass (inner tube) acrylic (outer tube)	EPDM rubber (EPDM)	polypropylene (PP)	10µm nylon (PA) 23µm polypropylene (PP)



3. Column structure

BXK tube consists of upper adaptor, tube and lower adaptor.

- **Tube body is double-layered.** Inner tube is made of high borosilicate glass while its outside is made of acrylic. The design can not only provide better protection, but also enable the thermal insulation by introducing water in the gap between two layers. Column length varies from 20cm,30cm,40cm,60cm,70cm to 100cm and diameter ranges from 16mm, 26mm to 50mm.
- **Upper adaptor consists of plug, soft pipe, adjusting knob, adaptor fastener, adaptor rod, sieve , supporting sieve and column stopper.**



- **Lower adaptor consists of plug, soft pipe, bottom rod, adjusting knob, adaptor fastener, O-ring, sieve , supporting sieve and column stopper.**

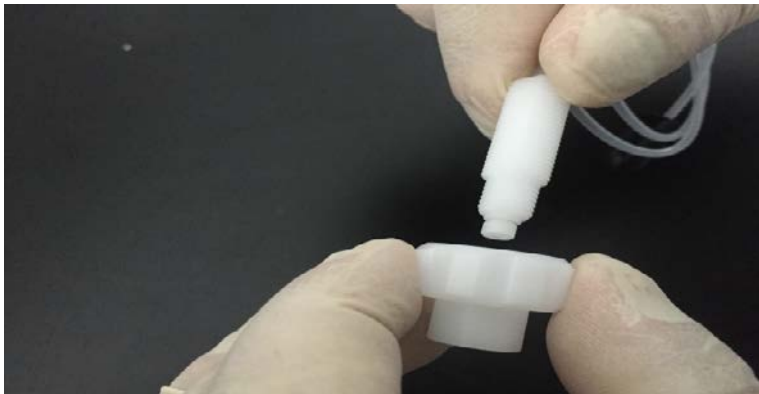

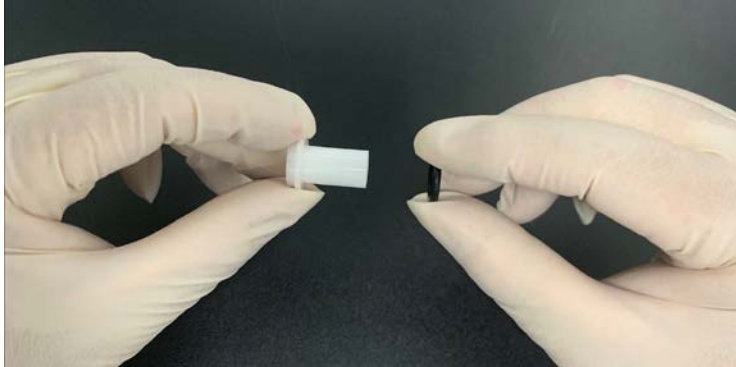






***No lower adaptor is available. Both adaptors are upper ones.**

- Packing reservoir: an accessory for packing column with high column bed. The reservoir consist of upper lid, acrylic pipe and connector.

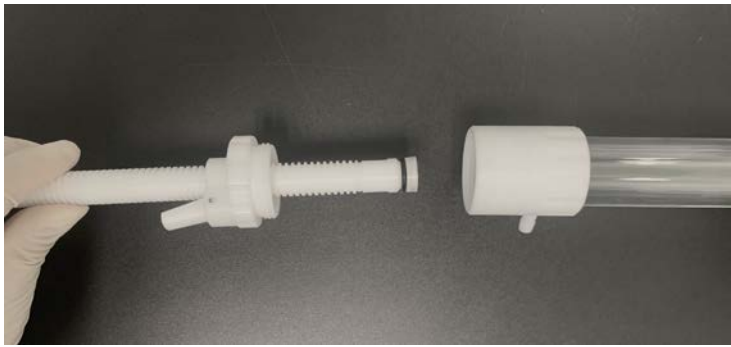
4. Packing column

- Assembling of lower adaptor

Step	Application drawing
a. screw the bottom rod into the adjusting knob	
b. Penetrate the rod through the bottom fastener	
c. Insert the O-ring into the column stopper	

<p>d.</p> <p>Connect the column stopper with O-ring to screw rod, tighten it.</p>	
Step	Application drawing
<p>e.</p> <p>place the supporting sieve to the column stopper</p>	
<p>f. Fasten the sieve on the column stopper</p>	
<p>g.</p> <p>screw bottom fastener to the column bottom</p>	

● **Assembling the upper adaptor**

Step	Application drawing
a. adaptor assembling is similar to lower adaptor	/
b. Connect the assembled upper adaptor to column tube, tighten the adaptor fastener. Adaptor assembling is completed	

5. Column packing

10~15cm loading bed is recommended for adsorption chromatography; For molecule sieve, column bed should be 60~90cm.

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

6. Column efficiency testing and assessment

Efficiency of packed column can be assessed.

- 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 to 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

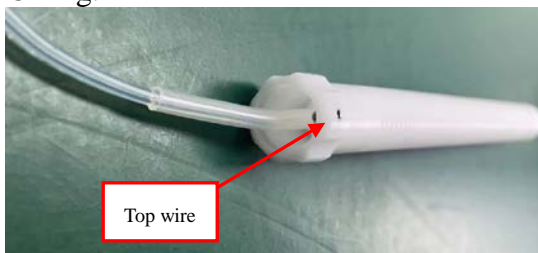
As a guideline, if the value of HETP is less than 3 times the average particle size (d50) of the resin and the As is between 0.8~1.5, column is very efficient. The unsatisfactory result need to be analyzed and re-packing is necessary.

Column efficiency and As for common resins:

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

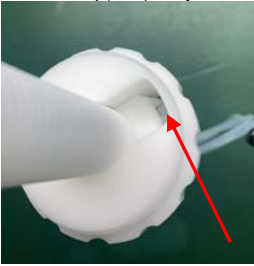
7. Cautions

- The outer tube of the chromatography column is made of acrylic material, which will not tolerate organic solvents with concentrations greater than 40% (ethanol, acetonitrile, acetone, etc.) to avoid cracks.
- 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 loosening 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.

8. Trouble shooting

Trouble	Cause and solution
Leakage from seal ring(O-ring)	<ol style="list-style-type: none"> 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)	<p>Adaptor spring is damaged, replace with a new one</p> 
Back pressure is unusually high	<ol style="list-style-type: none"> 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	<ol style="list-style-type: none"> 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	<ol style="list-style-type: none"> 1. Make sure the lower adaptor is correctly mounted 2. Make sure the sieve specification matches the resin particle size

9. Order information

Product	Item code	Pack/pcs
BXK 16/20	B-1620	1
BXK 16/40	B-1640	1
BXK 16/40 plus	BC226221	1
BXK 16/70	B-1670	1
BXK 16/70 plus	BC228221	1
BXK 16/100	B-16100	1
BXK 16/100 plus	BC229221	1
BXK 26/20	B-2620	1
BXK 26/40	B-2640	1
BXK 26/40 plus	BC326221	1
BXK 26/70	B-2670	1
BXK 26/70 plus	BC328221	1
BXK 26/100	B-26100	1
BXK 26/100 plus	BC329221	1
BXK 50/20	B-5020	1
BXK 50/30	B-5030	1



BXK 50/30 plus	BC425221	1
BXK 50/70	B-5070	1
BXK 50/70 plus	BC427221	1
BXK 50/100	B-50100	1
BXK 50/100 plus	BC329221	1

Accessories	Item code	Pack/pcs
adaptor (16column)	B-16A	1
adaptor O-ring (16column)	BS230015	5
10um sieve (16column)	BS220045	5
23um sieve (16column)	BS220055	5
supporting sieve (16column)	BS220035	5
glass tube (16/20column)	BS214001	1
glass tube (16/40column)	BS216001	1
glass tube (16/70column)	BS218001	1
glass tube (16/100column)	BS219001	1
plastic outer tube (16/20column)	BS214011	1
plastic outer tube (16/40column)	BS224011	1
plastic outer tube (16/70column)	BS218011	1
plastic outer tube (16/100column)	BS219011	1
16 reservoir	B-16R	1
16 reservoir bottom components (with seal ring)	BS-16R	1
Column stopper (16column)	BS200001	1
16/26reservoir plastic encloser	BS214001	1



BXK16 column tube joint	BA400031	1
adaptor (26column)	B-26A	1
adaptor O-ring (26column)	BS330005	5
10um sieve (26column)	BS320015	5
23um sieve (26column)	BS320025	5
supporting sieve (26column)	BS320035	5
glass tube (26/20column)	BS314001	1
glass tube (26/40column)	BS316001	1
glass tube (26/70column)	BS318001	1
glass tube (26/100column)	BS319001	1
Accessories	Item code	Pack/pcs
plastic outer tube (26/20column)	BS314011	1
plastic outer tube (26/40column)	BS316011	1
plastic outer tube (26/70column)	BS318011	1
plastic outer tube (26/100column)	BS319011	1
26reservoir	B-26R	1
26reservoir bottom components (with seal ring)	BS-26R	1
BXK26column joint	BA400041	1
Column stopper (26column)	BS300001	1
26column stand	BA5312001	1
50 adaptor (complete)	B-50A	1
adaptor O-ring (50column)	BS430005	5
10umsieve (50column)	BS420015	5
23umsieve (50column)	BS420025	5
supporting sieve (50column)	BS420035	5



glass tube (50/20column)	BS414001	1
glass tube (50/30column)	BS415001	1
glass tube (50/60column)	BS417001	1
glass tube (50/100column)	BS419001	1
plastic outer tube (50/20column)	BS414011	1
plastic outer tube (50/30column)	BS415011	1
plastic outer tube (50/60column)	BS417011	1
plastic outer tube (50/100column)	BS419011	1
Column stopper (50column)	BS400001	1
50reservoir plastic encloser	BS416001	1
50 bottom adaptor(complete)	BS450011	1
50reservoir	B-50R	1
50column stand	BA5412001	1