



**BESTCHROM**

博 格 隆

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



# Contents

1.Introduction .....	1
2.Technical parameters and materials .....	2
3.Column structure .....	4
4.Column assembling .....	5
5.Column packing .....	8
6.Column efficiency testing .....	8
7.Cautions .....	10
8.Trouble shooting .....	11
9.Order information .....	12

## 1.Introduction

BXK16/26/50 lab-scale column is innovatively designed low pressure column. It is applicable in the processing of macro-biomolecules such as recombinant protein, antibody, vaccine and blood products, as well as R&D of micro-biomolecules such as antibiotics, peptide, synthetic drugs and other natural substances. The columns can be loaded by agarose-based, dextran-based and polymer-based resins including Bestdex, Bestarose, Chromdex. It can also be connected to chromatography systems (including AKTA). The product consists of high borosilicate glass inner tube, acrylic outer tube and POM plastic, which is suitable for most aqueous solutions via providing excellent bio-compatibility and chemical resistance.

Advantages of BXK 16/26/50 lab-scale column:

- User-friendly, easy operation.
- 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 (℃)	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)							
BXK 26/40	26	40	117-196	22-37								
BXK 16/70 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								
BXK 26/100 plus	26	100	339-498	64-94								

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 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	Gasket	Tube	Seal ring	Upper/Lower supporting mesh
Fluorinated ethylene propylene copolymer(FEP) Ethylene tetrafluoroethylene copolymer (ETFE)	polypropylene(PP)	polyformaldehyde(POM)	high borosilicate glass(inner tube) acrylic(outer tube)	EPDM rubber(EPDM)	polypropylene(PP)

1. The hose of BXK16/26 column is made of ETFE, the hose of BXK50 column is made of FEP.

### 3.Column structure

BXK tube consists of three parts: top adaptor, tube and bottom adaptor.

- **Tube body:** 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 the following parts(from left to right) :** plug<sup>1</sup>, hose, adjusting screw, adaptor fixator, adaptor screw rod, O-ring , column stopper , supporting mesh and sieving mesh.



**Note:** ①plug②adjusting screw③adaptor fixator④adaptor screw rod

⑤O-ring⑥column stopper⑦supporting mesh⑧sieving mesh

- **Lower adaptor consists of the following parts<sup>2</sup>:** plug, hose, bottom screw rod, adjusting screw, bottom fixator, O-ring, sieving mesh , supporting mesh and column stopper.



**Note:** ①plug②bottom screw rod③adjusting screw④bottom fixator⑤O-ring⑥column stopper⑦supporting mesh⑧sieving mesh

1. For the connection of BXK50 adaptor plugs,use M6 connector and M6 1/16 connector for connection.



2. Plus has no bottom adaptor, both adaptors are top adaptors.





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



#### 4.Column assembling

- **Assembling of lower adaptor**


Step	Application drawing
a.Rotate the bottom rod into the adjusting screw	
b.Assemble the screw rod and the bottom fixator	

<p>c.Insert the O-ring into the column stopper</p>	
<p>d.Connect the column stopper (with O-ring)to screw rod, tighten it(make sure gasket and hose are properly connected before assembling)</p>	
<p>e.Place the supporting mesh on the column stopper</p>	
<p>f. Buckle the sieving mesh on the column stopper</p>	


g. Rotate bottom fixator into the column bottom.



### ● Assembling the upper adaptor

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

### ● Column tube packing

Step	Application drawing
a. With the help of column unpacking tool, turn the lock ring clockwise, fasten it, fix adaptor connector  <b>Note: When disassembling, loosen lock rings in both ends, rotate adaptor connector to prevent damage to acrylic tube.</b>	

## 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 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<sup>1</sup>, 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 media and bed heights, please refer to user instructions or seek technical support from Bestchrom team.**

## 6.Column efficiency testing

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 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

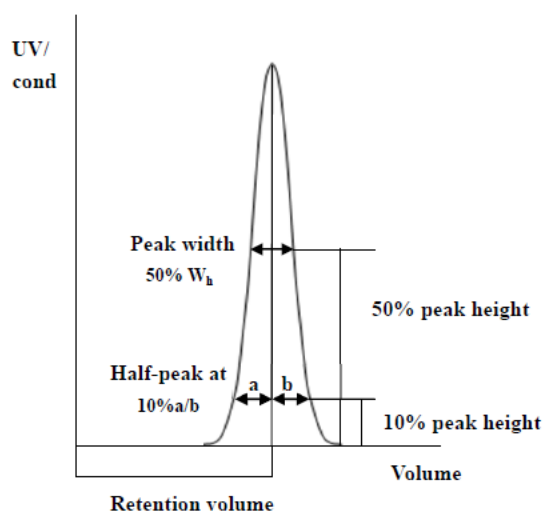
- Evaluation the column packing

The peak shape should be symmetric, and the asymmetry factor should be as close as possible to 1 (0.7~1.5 is generally acceptable). The change of peak shape is often the first sign of column bed deterioration.

For fillers with different particle sizes, the column efficiency can be considered as good if the following values are reached:

Column efficiency and As for common resins:

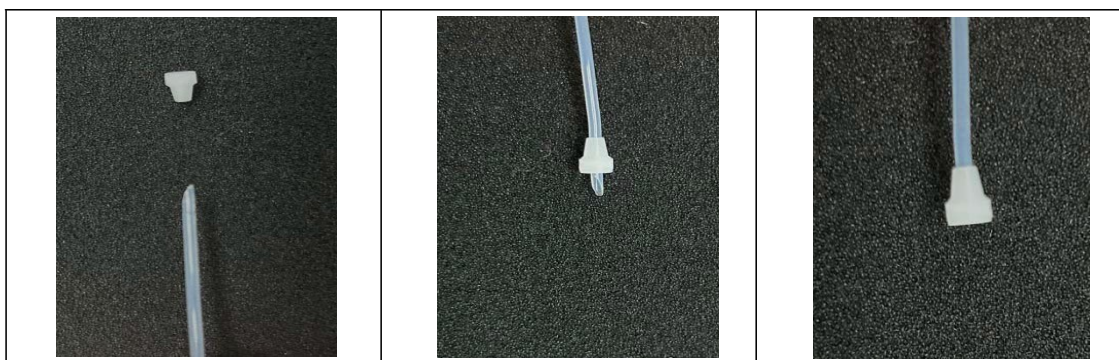
Particle size ( $\mu m$ )	Resin	N/m	As
34	Bestarose HP	>8000	0.8-1.5




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.) . Otherwise, it may cause cracks on tube.
- Make sure the column stopper and screw rod are tightened to avoid leakage.
- Keep the protective hose in the adaptor when using column, Do not fold connecting hose to prevent breakage or impair flow rate.
- When mounting/removing adaptor, the O-ring should be loosen, lower/raise adaptor vertically. If O-ring blocks, gently rotate the adaptor in left and right direction. Never push/pull violently or shake adaptor to avoid breakage in glass tube.
- Since fractional force between O-ring and glass tube is small, slightly rotate adaptor is possible even O-ring is completely open. Never rotate adaptor in counter-clockwise direction to prevent the loose of column stopper and screw rod, which will cause leakage in adaptor.
- Assembly of hose and gasket: first sharpen the hose, let the hose go through gasket, cut the surplus hose and make sure no hose is exposed from the gasket (See the following picture).



## 8. Trouble shooting

Trouble	Cause and solution
Leakage from O-ring	<ol style="list-style-type: none"> <li>1. O-ring is damaged, replace with a new one.</li> <li>2. Hard object is stuck between O-ring and glass tube.</li> </ol> <p>Wash the O-ring and tube. Mount the adaptor after supernatant appearing on the resin bed surface.</p>
Leakage from the connector of adaptor and hose when using	<ol style="list-style-type: none"> <li>1. Loose connection between column stopper and screw rod.</li> <li>2. M6 connector is not tightened when connecting to hose.</li> </ol>
Top adaptor slides when O-ring is tightened (pressure is lower than 0.5MPa)	<p>Adaptor spring is damaged, replace a new one</p> 
Back pressure is unusually high	<ol style="list-style-type: none"> <li>1. Flow rate is higher than the max flow rate of resin during column packing.</li> <li>2. Too many broken/shattered beads in resin.</li> <li>3. Sample is not appropriately treated. Adaptor sieving mesh is blocked by protein precipitation. Wash the sieving mesh in absolute ethanol or 1M NaOH for 30min in ultrasonic cleaner. Replace a sieving mesh if necessary.</li> <li>4. Hose is folded or blocked by foreign objects.</li> </ol>

Trouble	Cause and solution
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 bottom adaptor is correctly mounted. 2. Make sure the sieving mesh specification matches the resin particle size.

## 9.Order information

Product	Item code	Pack/pcs
BXK16/20	BC234231	1
BXK16/40	BC236231	1
BXK16/40 Plus	BC236241	1
BXK16/70	BC238231	1
BXK16/70 Plus	BC238241	1
BXK16/100	BC239231	1
BXK16/100 Plus	BC239241	1
BXK26/20	BC334231	1
BXK26/40	BC336231	1
BXK26/40 Plus	BC336241	1
BXK26/70	BC338231	1
BXK26/70 Plus	BC338241	1
BXK26/100	BC339231	1
BXK26/100 Plus	BC339241	1
BXK50/20	BC434231	1
BXK50/30	BC435231	1
BXK50/30 Plus	BC435241	1
BXK50/60	BC437231	1

Accessories	Item code	Pack/pcs
BXK50/100	BC439231	1
BXK50/100 Plus	BC439241	1

Accessories	Item code	Pack/pcs
Top adaptor (16column)	BS250011	1
Column stopper (16column)	BS200011	1
Bottom adaptor (16column)	BS250021	1
Gasket (16/26column)	BS200025	5
1/16" hose connector (16/26column)	BS200035	5
10µm sieving mesh (16column)	BS220045	5
23µm sieving mesh (16column)	BS220055	5
Supporting mesh (16column)	BS220035	5
Tube (16/20column)	BS214001	1
Tube (16/40column)	BS216001	1
Tube (16/70column)	BS218001	1
Tube (16/100column)	BS219001	1
Plastic tube (16/20column)	BS214011	1
Plastic tube (16/40column)	BS224011	1
Plastic tube (16/70column)	BS218011	1
Plastic tube (16/100column)	BS219011	1
16 packing reservoir	B-16R	1
16 packing reservoir bottom assembly parts (with O-ring)	BS-16R	1
16/26 packing reservoir plastic casing	BS214001	1
BXK16 tube connector	BA400031	1
Top adaptor (26column)	BS350011	1
Bottom adaptor (26column)	BS300011	1

Accessories	Item code	Pack/pcs
10µm sieving mesh (26column)	BS350021	1
23µm sieving mesh (26column)	BS320015	5
23µm sieving mesh (26column)	BS320025	5
Supporting mesh (16column)	BS320035	5
Tube (26/20column)	BS314001	1
Tube (26/40column)	BS316001	1
Tube (26/70column)	BS318001	1
Tube (26/100column)	BS319001	1
Plastic tube (26/20column)	BS314011	1
Plastic tube (26/40column)	BS316011	1
Plastic tube (26/70column)	BS318011	1
Plastic tube (26/100column)	BS319011	1
26 packing reservoir	B-26R	1
26 packing reservoir bottom assembly parts (with O-ring)	BS-26R	1
BXK26 tube connector	BA400041	1
26 Column stand	BA5312001	1
Top adaptor (50column)	BS450021	1
Column stopper (50column)	BS400011	1
Bottom adaptor (50column)	BS450031	1
Gasket (50column)	BS400025	5
10µm sieving mesh (50column)	BS420015	5
23µm sieving mesh (50column)	BS420025	5
Supporting mesh (50column)	BS420035	5
Tube (50/20column)	BS414001	1
Tube (50/30column)	BS415001	1
Tube (50/60column)	BS417001	1

Accessories	Item code	Pack/pcs
Tube (50/100column)	BS419001	1
Plastic tube (50/20column)	BS414011	1
Plastic tube (50/30column)	BS415011	1
Plastic tube (50/60column)	BS417011	1
Plastic tube (50/100column)	BS419011	1
50 packing reservoir plastic casing	BS416001	1
50 packing reservoir	B-50R	1
50 Column stand	BA5412001	1
Column disassembling tool	BS200041	1