BESTCHROM
博 格 隆

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

Contents

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 16/100 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 26/40 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 (℃)	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

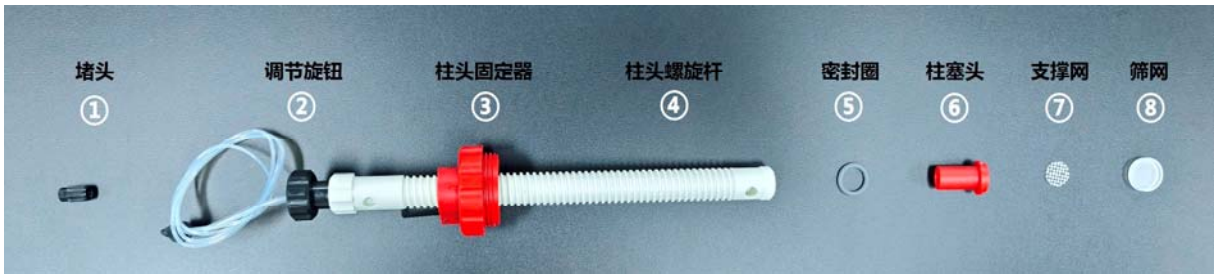
Upper/Lower thin tubing	Upper/Lower plug	Gasket	Tube	Seal ring	Upper/Lower Support net	Upper/lower net
Fluorinated ethylene propylene copolymer(FEP) Ethylene tetrafluoroethylene copolymer (ETFE)	Polypropylene(PP)	Polyformaldehyde (POM)	Borosilicate glass (inner tube) Acrylic(outer tube)	Ethylene-Propylene-Diene Monomer (EPDM)	Polypropylene(PP)	10μm Nylon(PA) 23μm Polypropylene(PP)

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

3. Column structure

BXK tube consists of three parts: upper adaptor, tube and lower adaptor.

- **Tube body:** The column tube is of a double-layered. Inner tube is made of borosilicate glass while its outside is made of acrylic. The outer tube 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) :** stop plug[1], tubing, adjusting knob, top end cap, adaptor shaft, O-ring , plunger , supporting net and net.



Note：①stop plug ②adjusting knob ③top end cap ④adaptor shaft ⑤O-ring ⑥plunger ⑦supporting net ⑧net

- **Lower adaptor consists of the following parts[2]:** stop plug, tubing, bottom shaft, adjusting knob, bottom end cap, O-ring, net , supporting net and plunger.

Note：①stop plug ②bottom shaft ③adjusting knob ④bottom end cap ⑤O-ring ⑥plunger ⑦supporting net ⑧net

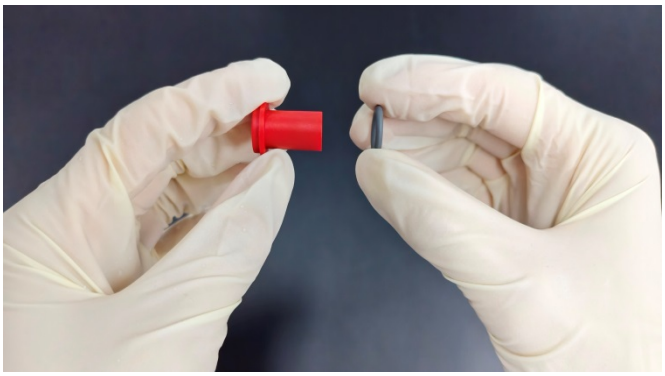
1. For the connection of BXK50 adaptor stop plug, use M6 connector and M6 1/16 connector for connection.

2. Plus series column has no lower adaptor, both adaptors are upper 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**

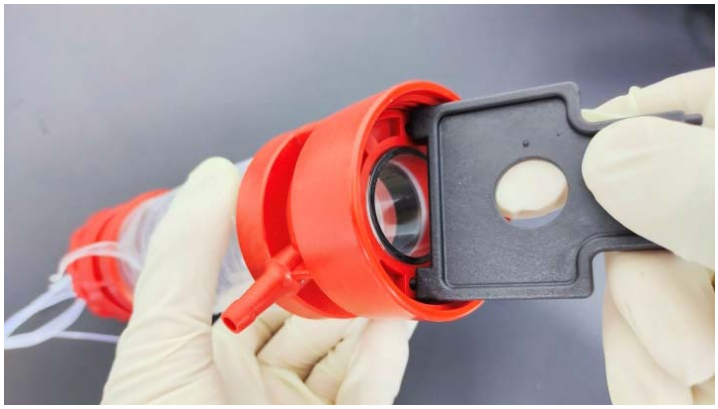
Step	Application drawing
a.Rotate the bottom shaft into the adjusting knob.	
b.Assemble the shaft and the bottom end cap.	
c.Insert the O-ring into the column plunger.	

Step	Application drawing
d.Connect the column stop plug (with O-ring) to plunger, tighten it(make sure gasket and tubing are properly connected before assembling).	
e.Place the supporting net on the column plunger.	
f.Buckle the net on the column plunger.	
g.Rotate and screw bottom end cap into the column bottom.	

- **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 top end cap. Adaptor assembling is completed.	

- **Column tube packing**

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

5. Column packing

10~15cm loading bed is recommended for adsorption chromatography. For SEC resins, column bed should be 60~90cm.

1) Pack the column (connect to adaptor reservoir if necessary), wash column with purified water or 20% ethanol.
2) Remove the lower adaptor and wash with buffer, drain the bubble under the net, mount the lower adaptor to the column bottom, tighten adjusting knob to let O-ring fully stretch out(to get best sealing effect), close the lower plug. Keep 1cm height of buffer in the column bottom, adjust column and keep it vertical to ground.
3) Add packing 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 go, make sure do not take any bubble in.

5) If a packing reservoir is available, slowly pour moderate amount of packing buffer to the reservoir. Connect upper adaptor to the chromatography system, remove the bubbles in the tubing. Mount top end cap to packing reservoir.

6) Set the flow rate[1], open bottom stop plug of lower adaptor. Turn on the pump and compress column bed.

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

8) Wash the upper adaptor with packing buffer, remove the bubble trapped in the net, remove reservoir (if available), mount the upper adaptor to column tube.

9) Adjust upper adaptor to about 0.5~1cm above the resin surface, make sure adaptor inlet is filled with packing buffer.

10) Open bottom stop plug, connect to pump, keep flow rate unchanged (make sure pressure is under max limit of resin and max pressure of column (2MPa)). Keep compressing resin bed till column bed is consolidated, mark the column bed height.

11) Stop the pump, close bottom stop plug, open top stop plug, loosen adjusting knob of upper adaptor slightly, lower the adapter to about 3~5mm below the resin bed surface , close top stop plug, tighten the adjusting knob, complete the column packing.

1：The required flow rate varies from resin types 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 samples 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
Loading	1.0% CV	1.0% CV
Mobile phase	Water	0.4M NaCl in water
Flow rate	30 cm/h	30cm/h
Monitor	UV 280 nm	Conductivity

- Calculation of HETP and As

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

HETP = L/N

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

Where: V_R = retention volume

W_h = peak width at half peak height

L = column bed height

N = number of theoretical plates

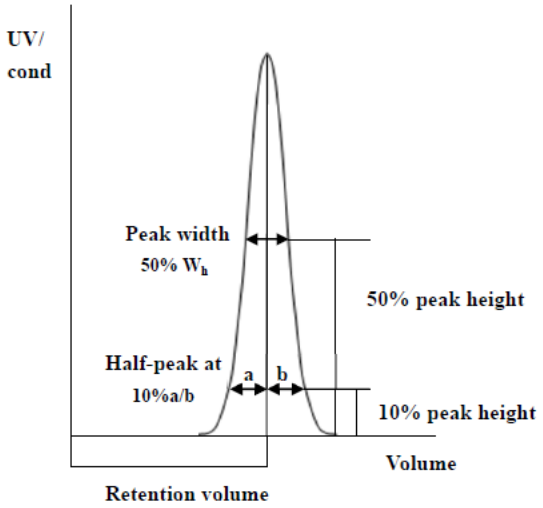
V_R and W_h are measured in the same units

As = b/a

Where:

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

b = 2nd half peak width at 10% of 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 resin bed deterioration.

For resins 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(μm)	Resin matrix	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

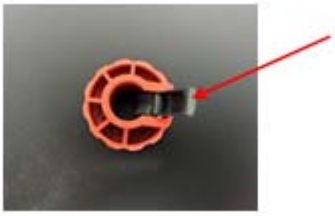
- The outer tube of the chromatography column is made of acrylic material, which will not tolerate organic solvents with concentrations greater than 40% (ethanol, acetonitrile, acetone, etc.). Otherwise, it may cause cracks on tube.
- Make sure the column plunger and shaft are tightened to avoid leakage.
- Keep the protective tubing in the adaptor when using column, do not fold connecting tubing 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 of glass tube.
- Since fractional force between O-ring and glass tube is small, when the O-ring is fully stretching out, don’t rotate adaptor in counter-clockwise direction to prevent the loose of plunger and shaft,

which will cause leakage in adaptor.

- The bottom end cap of BXK50 column is designed offers fixing grooves. When loosening/tightening column plungers, first loosening the adjusting knob to detach the plunger and bottom end piece, in order to prevent damages from friction between plunger screw threads and fixing grooves.
- Assembly of tubing and gasket: first sharpen the tubing, let the tubing go through gasket, cut the surplus tubing and make sure no tubing is exposed from the gasket (See the following picture).

8. Trouble shooting

Trouble	Cause and solution
Leakage from O-ring	1.O-ring is damaged, replace with a new one. 2.Hard object is stuck between O-ring and glass tube. Wash the O-ring and tube. Mount the adaptor after supernatant appearing on the resin bed surface.
Leakage from the connector of adaptor and tubing when using	1.Loose connection between column plunger and adapter shaft. 2.M6 connector is not tightened when connecting to tubing.

Trouble	Cause and solution
Upper adaptor slides when O-ring is tightened (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. 2.Too many broken/shattered beads in resin. 3.Sample is not appropriately treated. Adaptor net is blocked by protein precipitation. Wash the net in absolute ethanol or 1M NaOH for 30min in ultrasonic cleaner. Replace a net if necessary. 4.tubing is folded or blocked by foreign objects.
Flow rate is lower than setting rate	1.Check for the existence of air in pipes and tubing. 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 net specification matches the resin particle size.

9. Order information

Product	Cat.No.	PCS/Pack
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

Product	Cat.No.	PCS/Pack
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
BXK50/100	BC439231	1
BXK50/100 Plus	BC439241	1

Accessories	Cat.No.	PCS/Pack
Upper adaptor (16column)	BS250011	1
Column plunger (16column)	BS200011	1
Lower adaptor (16column)	BS250021	1
Gasket (16/26column)	BS200025	5
1/16" tubing connector (16/26column)	BS200035	5
10μm net (16column)	BS220045	5
23μm net (16column)	BS220055	5
Supporting net (16column)	BS220035	5
O-ring（16 column）	BS230015	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

Accessories	Cat.No.	PCS/Pack
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
Upper adaptor (26column)	BS350011	1
Plunger（26 column）	BS300011	1
Lower adaptor (26column)	BS300011	1
10μm net (26column)	BS350021	1
23μm net (26column)	BS320015	5
23μm net (26column)	BS320025	5
Supporting net (16column)	BS320035	5
O-ring（26 column）	BS330005	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

Accessories	Cat.No.	PCS/Pack
Upper adaptor (50column)	BS450021	1
Column plunger (50column)	BS400011	1
Lower adaptor (50column)	BS450031	1
Gasket (50column)	BS400025	5
10μm net (50column)	BS420015	5
23μm net (50column)	BS420025	5
Supporting mesh (50column)	BS420035	5
O-ring (50 column)	BS430005	5
Tube (50/20column)	BS414001	1
Tube (50/30column)	BS415001	1
Tube (50/60column)	BS417001	1
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 dismantling tool	BS200041	1
Column stand (BXK50 column)	BA500121	1
ETFE tubing fittings 0.75mm 60cm	BS200051	1
ETFE tubing fittings 0.75mm 80cm	BS200061	1
ETFE tubing fittings 0.75mm 100cm	BS200071	1