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

Contents

1.Introduction

BXK16/26/50 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 BXK16/26/50 lab column:

1) User-friendly, easy operation.
2) Flared interface connector and highly elastic O-ring effectively prevent leakage.
3) Evenly distributed outflow, promoting column efficiency after packing.
4) 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
BXK16/20	16	20	4-34	2-17	5 (Max)	2-60	1-14	10	Common aqueous solutions
BXK16/40	16	40	44-76	22-37					
BXK16/40 Plus	16	40	16-54	8-32					
BXK16/70	16	70	104-134	52-67					
BXK16/70 Plus	16	70	68-128	34-64					
BXK16/100	16	100	164-194	82-97					
BXK16/70 Plus	16	100	128-188	64-94					
BXK26/20	26	20	10-90	2-17	5 (Max)				
BXK26/40	26	40	117-196	22-37					
BXK16/70 Plus	26	40	42-169	8-32					
BXK26/70	26	70	276-355	52-67					
BXK26/70 Plus	26	70	180-339	34-64					
BXK26/100	26	100	435-514	82-97					
BXK26/100 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
BXK50/20	50	20	39-333	2-17	3 (Max)	2-60	1-14	10	Common aqueous solutions
BXK50/30	50	30	235-529	12-26					
BXK50/30 Plus	50	30	0-471	0-24					
BXK50/60	50	60	823-1117	42-57					
BXK50/60 Plus	50	60	470-1058	24-54					
BXK50/100	50	100	1607-1901	82-97					
BXK50/100 Plus	50	100	1256-1845	64-94					

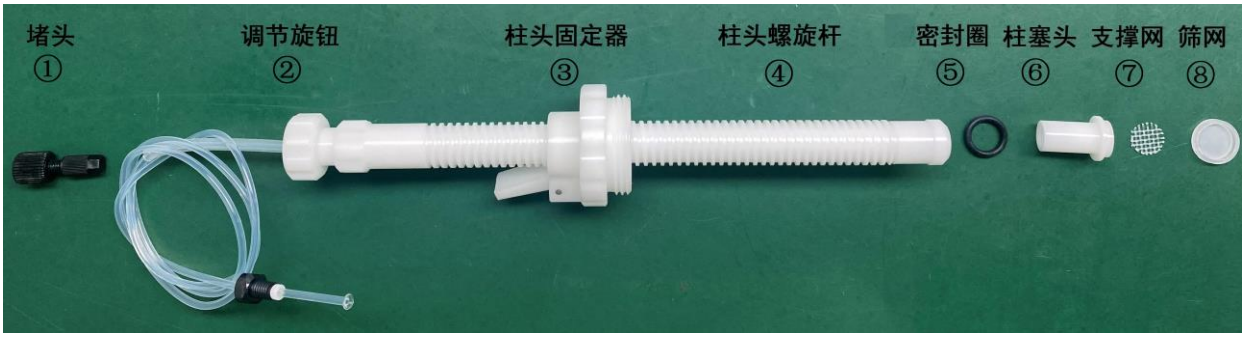
2.2 Materials

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

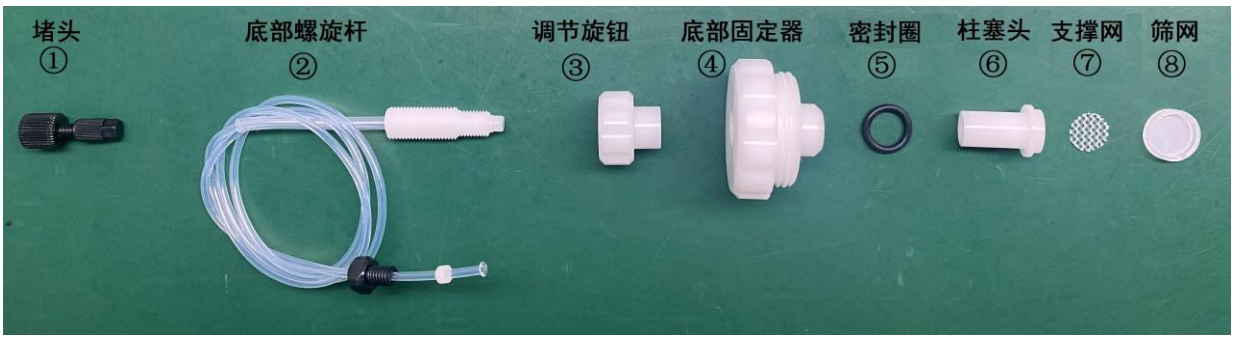
BXK tube consists of upper adaptor、Tube and lower adaptor.

- **Column tube:** The column tube is double-layer design, the inner tube material is high borosilicate glass, the outer tube material is acrylic, which can protect the inner tube, and the jacket layer formed at the same time can be heated by water bath. The length of the column and tube is 20cm, 30cm, 40cm, 60cm, 70cm and 100cm, and the diameter is 16mm, 26mm and 50mm.
- **Upper adaptor components:** It is composed of plug, hose, adjusting knob, cylinder head fixer, cylinder head screw rod, sealing ring, screen, supporting net and plunger head.



Note：①plug②adjusting knob③cylinder head fixer④cylinder head screw rod ⑤sealing ring⑥plunger head⑦supporting net⑧screen

- **Lower adaptor components:** It is composed of plug, hose, bottom screw rod, adjusting knob, bottom fixer, sealing ring, screen, supporting net and plunger head.



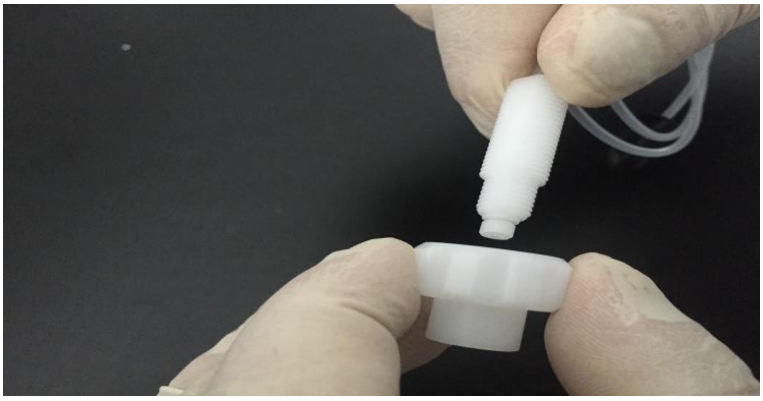
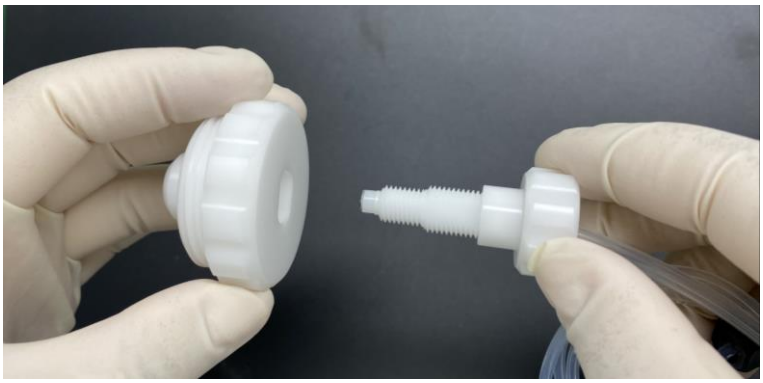
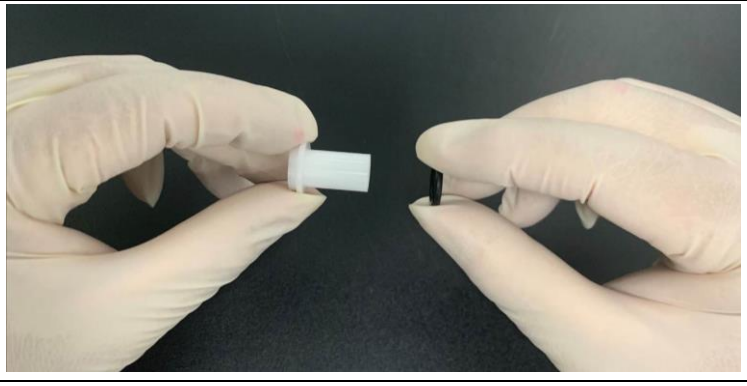
Note：①plug②bottom screw rod③adjusting knob④bottom fixer⑤sealing ring⑥plunger head⑦supporting net⑧screen

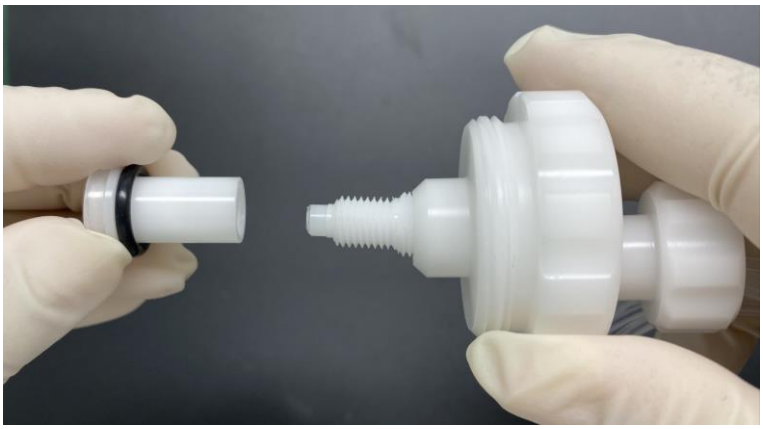
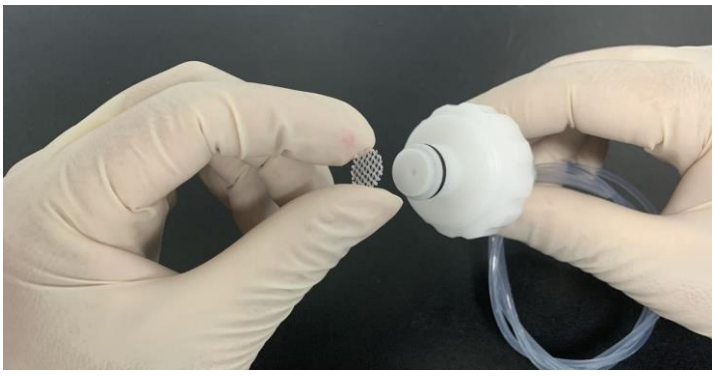
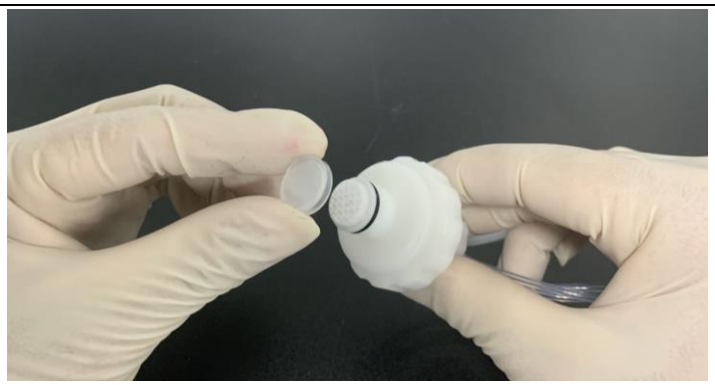
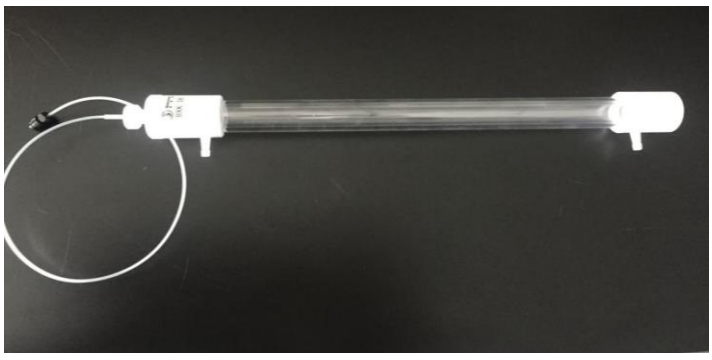
***No lower adaptor is available. Both adaptors are upper ones.**

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

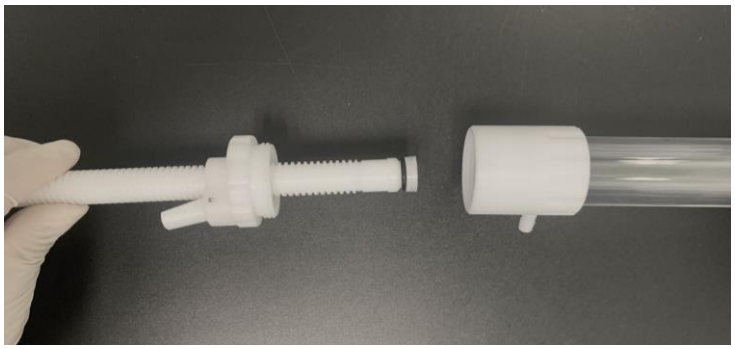
4.Column packing

- **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	

Step	Application drawing
d. Connect the column stopper with O-ring to screw rod, tighten it.	
e.Place the supporting sieve to the column stopper	
f. Fasten the sieve on the column stopper	
g. Screw bottom fastener to 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 adaptor fastener. Adaptor assembling is completed	

5.Loading column

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[1], 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

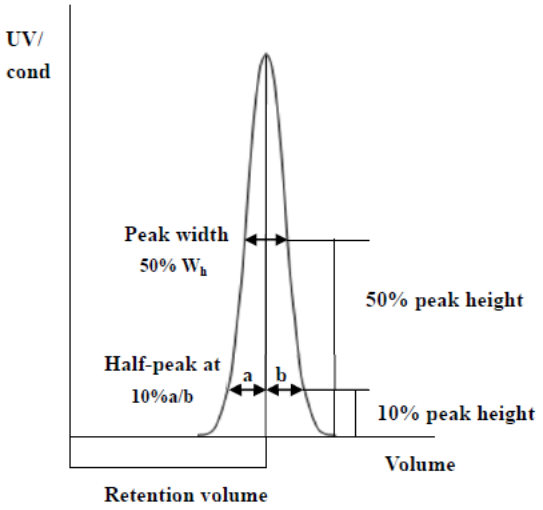
N = the number of theoretical plates

(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:

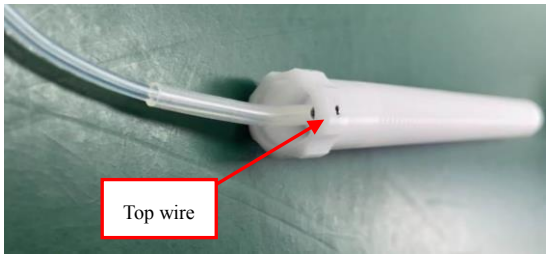
Column efficiency and As for common resins：

Particle size（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

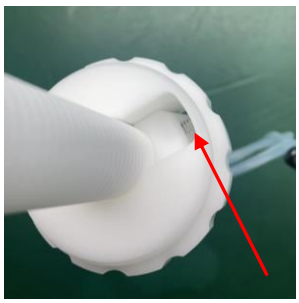
- The outer tube of the chromatography column is made of acrylic material, which will not tolerate organic solvents with concentrations greater than 40%(ethanol, acetonitrile, acetone, etc.) to avoid cracks.
- Make sure the column stopper and screw rod are tightened to avoid leakage.
- For seriously 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 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.

8.Trouble shooting

Trouble	Cause and solution
Leakage from seal 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	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. 

Trouble	Cause and solution
Back pressure is unusually high	1.Flow rate is higher than the max flow rate of resin during column packing. 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 specification matches the resin particle size.

9.Order information

Product	Item code	Pack/pcs
BXK16/20	B-1620	1
BXK16/40	B-1640	1
BXK16/40 Plus	BC226221	1
BXK16/70	B-1670	1
BXK16/70 Plus	BC228221	1
BXK16/100	B-16100	1
BXK16/100 Plus	BC229221	1
BXK26/20	B-2620	1
BXK26/40	B-2640	1
BXK26/40 Plus	BC326221	1
BXK26/70	B-2670	1
BXK26/70 Plus	BC328221	1
BXK26/100	B-26100	1
BXK26/100 Plus	BC329221	1
BXK50/20	B-5020	1
BXK50/30	B-5030	1
BXK50/30 Plus	BC425221	1
BXK50/70	B-5070	1
BXK50/70 Plus	BC427221	1
BXK50/100	B-50100	1
BXK50/100 Plus	BC429221	1

Accessories	Item code	Pack/pcs
adaptor（16column）	B-16A	1
adaptor O-ring（16column）	BS230015	5
10μm sieve（16column）	BS220045	5
23μm 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
10μm sieve（26column）	BS320015	5
23μm 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
10μm sieve（50column）	BS420015	5
23μm sieve（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