



BESTCHROM

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BXK100/140/200
Industry scale
chromatography column
Instruction for use



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1.Introduction

1.1 Overview

The BXX100/140/200 series chromatography column is specially designed for the pilot scale test and production of biopharma customers with high precision sanitary grade chromatography column, suitable for filling all kinds of media and low pressure chromatography media.

BXX chromatography column is composed of three parts: adaptor, column body and stand. The basic structure includes glass tube, flange, O-ring, filter nets, support nets, end-piece and rods. The rods hold the flange and end-piece to the glass tube which is sealed by O-rings. The O-ring can not only avoid the direct contact between the glass tube and the end-piece, but also ensure the sealing of the glass tube.

The adaptor and end-piece are of a single pipe design. The adaptor and end-piece are covered with a large aperture support net and a fine aperture filter net. Filter net whose pore diameter is 23 μ m (polypropylene) or 10 μ m (nylon) can be selected.

Different column heights can be set by adjusting the height of column head. The column is sealed by compressing or loosening the cylinder seal ring.

The end-piece is mounted on the stand, which is equipped with a universal wheel for easy movement. The foot brake on the universal wheel can fix the column.

1.2Material

The BXX100/140/200 series chromatography column is a series of glass chromatography columns developed for production scale by Bestchrom. All the chromatographic column materials meet the requirements of SFDA for pharmaceutical manufacturers, so it is an ideal choice for industrial production. Listed are main characteristics of this series of chromatography columns:

- ① The stainless steel parts of the chromatographic column are all made of medical grade 316L stainless steel.
- ② The glass tube body adopts the original Schott imported high-precision medical glass, the roundness error is less than 0.1mm.
- ③ The upper screen mesh, the lower screen mesh and the supporting mesh are made of original imported materials, which can withstand a variety of acid and alkali solutions and organic solvents.
- ④ O-ring using EPDM material, good airtightness, more durable.
- ⑤ Adaptor can make column bed height adjustable.

All parts of the chromatography column above have complete material certification, all meet the needs of the pharmaceutical industry.

1.3 Technical indicators and detailed materials (next page)

1.3.1 Technical Specifications

Specifications	Diameter (mm)	Floor area (cm ²)	Column Hight (cm)	Bed height(cm)		Volume(L)		Max Pressure (bar/g)	Total weight (kg)	Chromatogra phic column inlet dimension	Adaptor weight (kg)	Outline dimension (cm) D×W×H
				Min	Max	Min	Max					
BXK100/500	100	78.5	50	2	35	0.2	2.8	8	17.2	4mm	6.6	48×48×127
BXK100/750	100	78.5	75	27	57	2.1	4.5	8	18.6			48×48×152
BXK100/950	100	78.5	95	47	74.7	3.7	5.8	8	22.7			48×48×172
BXK140/500	140	154	50	2	35.6	0.3	5.5	6	29.1	6mm	10.5	59×59×127
BXK140/750	140	154	75	27	57	4.2	8.8	6	31.5			59×59×152
BXK140/950	140	154	95	47	74.7	7.2	11.5	6	33.4			59×59×172
BXK200/500	200	314	50	2	35.6	0.6	11.2	6	36.5	6mm	20.5	59×59×127
BXK200/750	200	314	75	27	57	8.5	18	6	40.1			59×59×152
BXK200/950	200	314	95	47	74.7	14.8	23.5	6	42.2			59×59×172

1.3.2 Material

Specifications	Supporting net	Screen	Column tube	Seal ring	Holder
BXK100/500	PP	10μm nylon (PA) 23μm PP	Glass	EPDM	316L
BXK100/750					
BXK100/950					
BXK140/500	PP	10μm nylon (PA) 23μm PP	Glass	EPDM	316L
BXK140/750					
BXK140/950					
BXK200/500	PP	10μm nylon (PA) 23μm PP	Glass	EPDM	316L
BXK200/750					
BXK200/950					

2. Installation and alignment




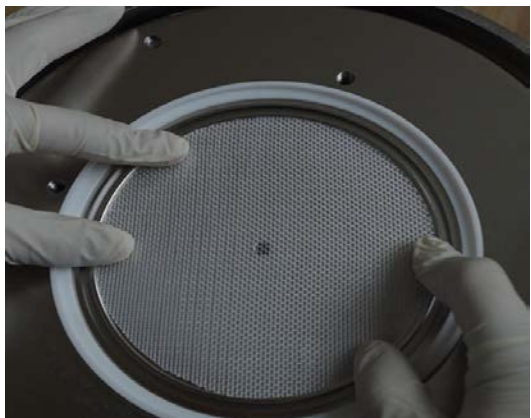


2.1 Installation and inspection of new chromatography column

- Unpacking the carton, check whether the goods are complete and in good condition according to the packing list, for any problem, please contact the Bestchrom sales representative immediately.
- Since the column and column frame are transported separately, it is necessary to first install the wheel foot on the column frame, then ,mount the column on the column frame and tighten the fixing screws with a wrench.
- Wet the seal ring with 20% ethanol and loosen the seal ring.
Note: When moving the cylinder inside the cylinder, the sealing ring must be kept wet. Otherwise, due to the greater friction between the dry sealing ring and the glass tube, it is difficult to move and may damage the sealing ring.
- Remove the adaptor and check whether the flange nut fixing the adaptor is loose. If so, please use a torque wrench with torque distance, set the torque to 4N/M, and tighten it.
Note: Force exceeding 4N/M may damage the glass tube.
- Check and ensure that the stopper under the top plate is in the navigation groove of the outer adaptor. If the stopper is loose, tighten the two bolts above the top plate with an inner hexagon wrench.
Note: If the stopper is not in the navigation groove, it can lead to serious consequences. The stopper may scratch the outer adaptor.
- Connect column fittings, such as valves and pipes.




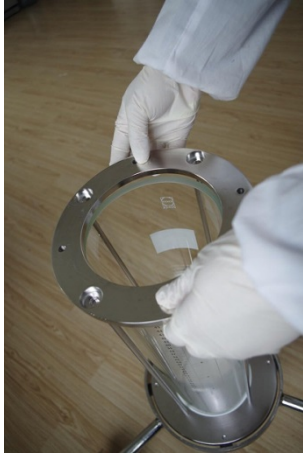


2.2 Assembly and disassembly of chromatography column

The following column mounting method is the column mounting process before leaving the factory. Users can refer to this method for assembly and disassembly in reverse order.

1) Assembly of column frame

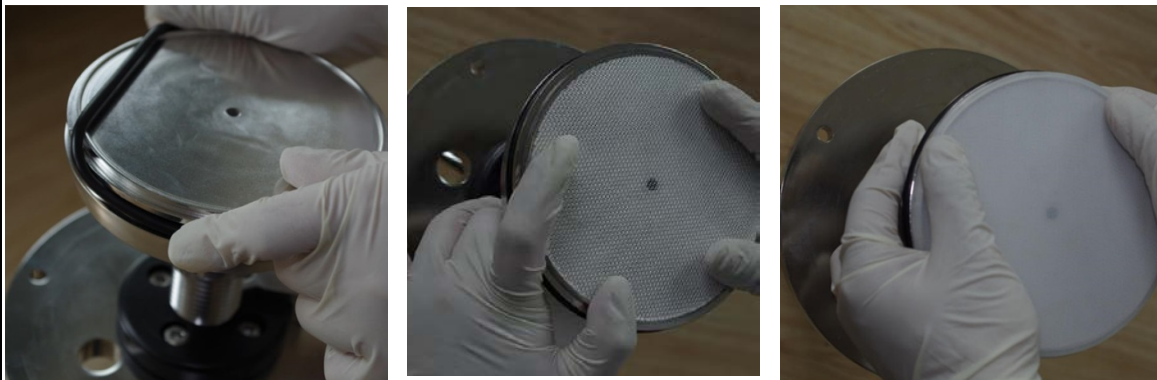
a. Install the three universal wheels on the stand and adjust the level of the stand.	b. Install the end-piece on the assembled stand, add the gasket and tighten it with the stand fixing screws.
	
c. After fixing the end-piece, the white guide ring is installed in the end-piece card slot.	d. Place the support net plate flat in the center of the end-piece.
	
e. Install the lower filter net.	f. Install the lower O-ring between the guide ring and the lower net.
	

2) Assembly of glass tubes

a. Screw the 4 rods for tube into the installed stand. Make sure insert each rod at the same depth.	b. Put in glass tube, glass tube should be directly opposite the lower O-ring.
	
c. Buckle the upper O-ring into the flange.	d. Put the flange upside down and flat on the glass tube, and make the 4 wire holes on the flange correspond to the 4 rods.
	
e. Put in the gasket and tighten with a torque wrench.	
	

3) Adaptor assembly

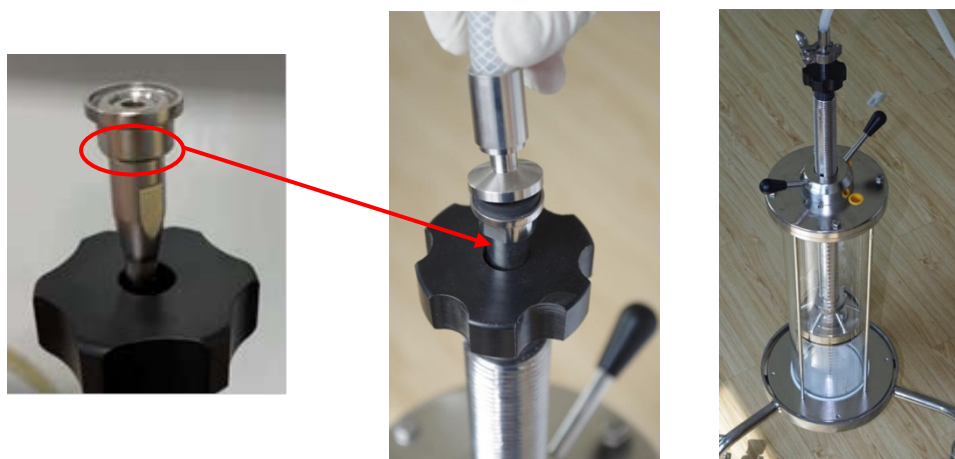
a. Put the adaptor upside down, and then put the adaptor O-ring into the slot between the sealing unit and the adaptor plate, Keep the upper support net flat, and buckle into the upper filter net.



b. Install the installed adaptor into the glass tube and install the dust plug.



c. Before use, tighten the nut of feed port to avoid the risk of leakage (if the sealing ring of feed port is damaged, it needs to be replaced in time). If external pipe connection is required, place gasket evenly between pipe and cylinder, then lock it with clamp and tighten it.



3. Column packing

3.1 Wash and rinse

- The cylinder can be filled with 0.5M NaOH and maintained for more than 1 hour. Then rinse with purified water.
- The chromatography column is injected with purified water about 10cm high, and the bubbles under the sieve at the bottom of the column are pumped out. For the small bubbles that are difficult to be discharged, the ear ball can be used to suck them out from above, or the small bubbles can be drawn out by connecting the peristaltic pump with a hose.
- The excess purified water is pumped away and purified water with a height of about 2cm is retained in the cylinder.
- Close bottom valve.

3.2 Loading step

- Use a level meter to detect and adjust the column to level and fix it.
- Tighten the valve at the bottom, slowly pour the slurry into the column at one time (use a packing reservoir if necessary). Do not bring any air bubbles into the column.

Packing reservoir: Empty glasstube with same diameter as the BXX column.

- After pouring, stir well again with a glue stick, and then wash the media particles on the inner wall of the column from top to bottom with the packing solution, and let the media settle naturally until there is about 1cm of clarifying solution on the suspension.

Note: Flushing the inner wall to reduce the media particles sticking between the seal ring and the column wall, avoiding the risk of leakage.

- Mount the adaptor, connect upper cylinder to the chromatography system or peristaltic pump by installing a four-way double-pass valve. Adjust the adaptor to descend to contact the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. With the valve at the top of the chromatography column open, slowly move the adaptor down until all bubbles are drained.

Note: one person is responsible for fixing the adaptor, and one person is responsible for rotating the regulator. If there is difficulty in the process of rotation, the regulator should be rotated in the opposite direction to ensure that it is not stuck and then continue to fall or rise to adjust, otherwise it is easy to cause damage to the outer rod thread of the adaptor.

- Open the bottom valve, set the flow rate of the pump, start the pump, and start the column loading.

Note: Different media have different best methods of column loading. Please refer to the media manual to set the column loading rate or pressure.

In addition. In the process of column loading, alarm pressure must be set to prevent damage to medium or chromatographic column caused by overpressure.

- Make the glue surface stable at the set flow rate, and mark the height position of the column bed.
- Stop the pump and seal the bottom outlet of the column.
- Loosen the O-ring, and quickly adjust the position of the upper net to 0.5cm above from the rubber surface.
- Tighten the O-ring, open the outlet at the bottom of the column, and open the pump at the same time.
- Continue to press the glue until the glue surface does not change. Mark the bed height position and stop the pump.
- At the same time, the other end of the four-way double-way valve is put into a beaker filled with liquid column (to prevent bubble suction), at the same time, the switch is rotated, the sealing ring of the cylinder head is slightly relaxed, and the cylinder head is pressed 3-5mm below the marked position (note: the liquid is drained from the four-way double-way valve at this time).
- Tighten the O-ring, close the bottom valve, and complete column loading.

Note: After column loading, the column loading solution above the adaptor can be sucked out by peristaltic pump and washed with purified water for 2~3 times to prevent the potential corrosion of salt-containing solution on the column, 20% ethanol or 0.1M NaOH can be filled above the adaptor to avoid bacteria breeding in the chromatography column.

4. Evaluation of Packing

- Test the column efficiency to check the quality of packing. The tests are required after the column packing, during the column working life and when the separation and purification performance is deteriorated. The method of the expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As).
- 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):

$$\text{HETP} = L/N$$

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

Note :

V_R = retention volume

W_h = half-peak width

L = column height

N = the number of theoretical plates

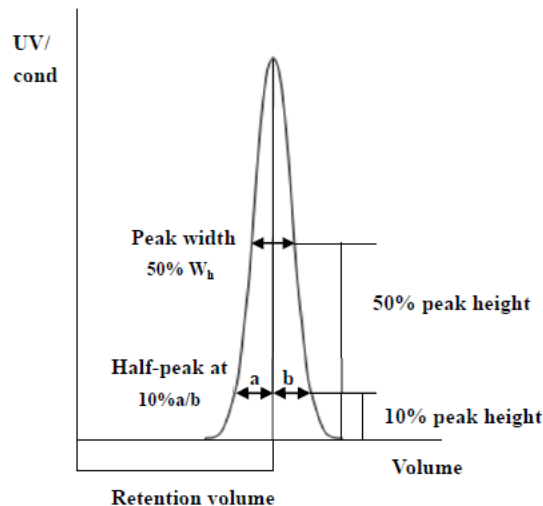
(The units of V_R and W_h should be the same)

$$\text{As} = b/a$$

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 (d_{50}) 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.

For fillers of different particle sizes, the column efficiency is considered to be ideal when the following values are reached:

Particle size (μm)	Product	N/m	As
34	Bestarose HP	> 8000	0.8-1.5
34	Chromdex	> 10000	0.7-1.3
90	Bestarose HF	> 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

5. Maintenance and servicing

Chromatographic column is a sophisticated scientific research and production equipment, and a good chromatographic state is very important to the experimental results or the quality of the products produced.

- All liquids and samples used in the column need to be filtered through a membrane (pore diameter smaller than $0.45\mu\text{m}$) to remove particulate matter and prevent blocking column screen.
- After the chromatography column is used for a long time, the permeability of the net may decrease due to protein denaturation and other reasons, which shows that the back pressure of the chromatographic column increases. In that case, it is necessary to clean or replace the net separately. It is recommended to replace the net once a year to keep the chromatography column in good condition.
- EPDM O-ring (especially the O-ring of adaptor) may be aged and deformed under long-term compression, so it is recommended to replace it once a year as preventive maintenance.
- The packed column should avoid direct sunlight or sudden changes in temperature.
- The chromatography column with medium needs to be treated with antibacterial treatment when it is not used for a long time. Usually, 20% ethanol is used as the preservation solution. It is recommended to replace the fresh 20% ethanol every three months.
- Bubble needs to be prevented to enter the chromatography column filled with medium during storage. Once bubbles enter, it has to be refilled.
- Although most of the stainless steel components of the chromatography column are made of 316L stainless steel, there will still be rusting and corrosion under wet and high salt conditions, so the surface of the chromatography column should be kept away from the wet and high salt environment.

5.1 Chemical tolerance

The components of industrial BXK series chromatography columns contacting with feed liquid are made of four kinds of materials: 316L stainless steel, high borosilicate glass, EPDM, polypropylene and nylon (only limited to 10μm screen), and chemical reagents harmful to these four materials should be avoided during use. The following table lists some chemical reagents that may be used for reference.

Substance and concentration	Tolerance	Potential impact	Remarks
Common aqueous solution	Usable	/	/
8M urea	Usable	/	/
Low concentration stain remover (Triton/TBP) ,1%	Usable	/	/
2M NaOH	Usable	/	/
Ethanol	Usable	/	/
10% acetone	Usable	/	can be used for long time with lower than 10% acetone
50% glycol	Usable	/	/
100% isopropyl alcohol	Usable	/	/
50% acetonitrile	Short-term use	Seal ring to harden	/
2M NaCl	Short-term use	Lead to rust	Chloride ions can cause corrosion of stainless steel, especially in the environment with pH lower than 4. NaCl solution with concentration lower than 2M can be used normally in chromatography. After use, it should be washed with purified water in time. The preservation solution of chromatographic column should not contain NaCl.
25% HAC	Short-term use	/	Not recommended for use with 10μm nylon mesh
0.1M HCl	Short-term use	/	Cannot be used with 10μm nylon mesh
0.1M HNO ₃	Short-term use	/	Not recommended for use with 10μm nylon mesh

Substance and concentration	Tolerance	Potential impact	Remarks
6MGuanidine hydrochloride	Short-term use	Causes corrosion of stainless steel	Rinse with purified water immediately after use
Trichloromethane Dichloromethane	Prohibition of use	Causes the seal ring to dissolve	/
10%H ₂ O ₂	Prohibition of use	Lead to aging of sealing ring	/
Toluene	Prohibition of use	Lead to aging of sealing ring	/

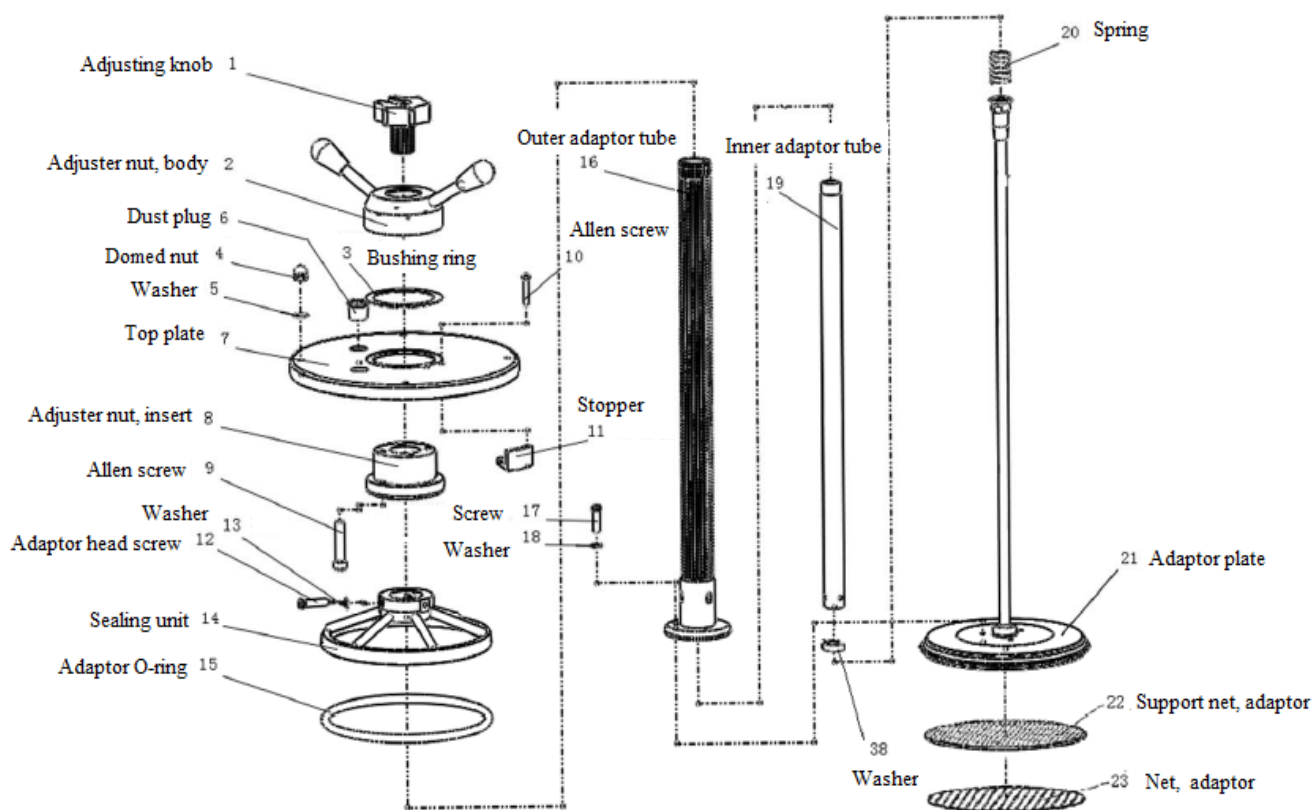
Note: Short-term use can be used during chromatography, but it needs to be washed in time after use.

6. Handling Information

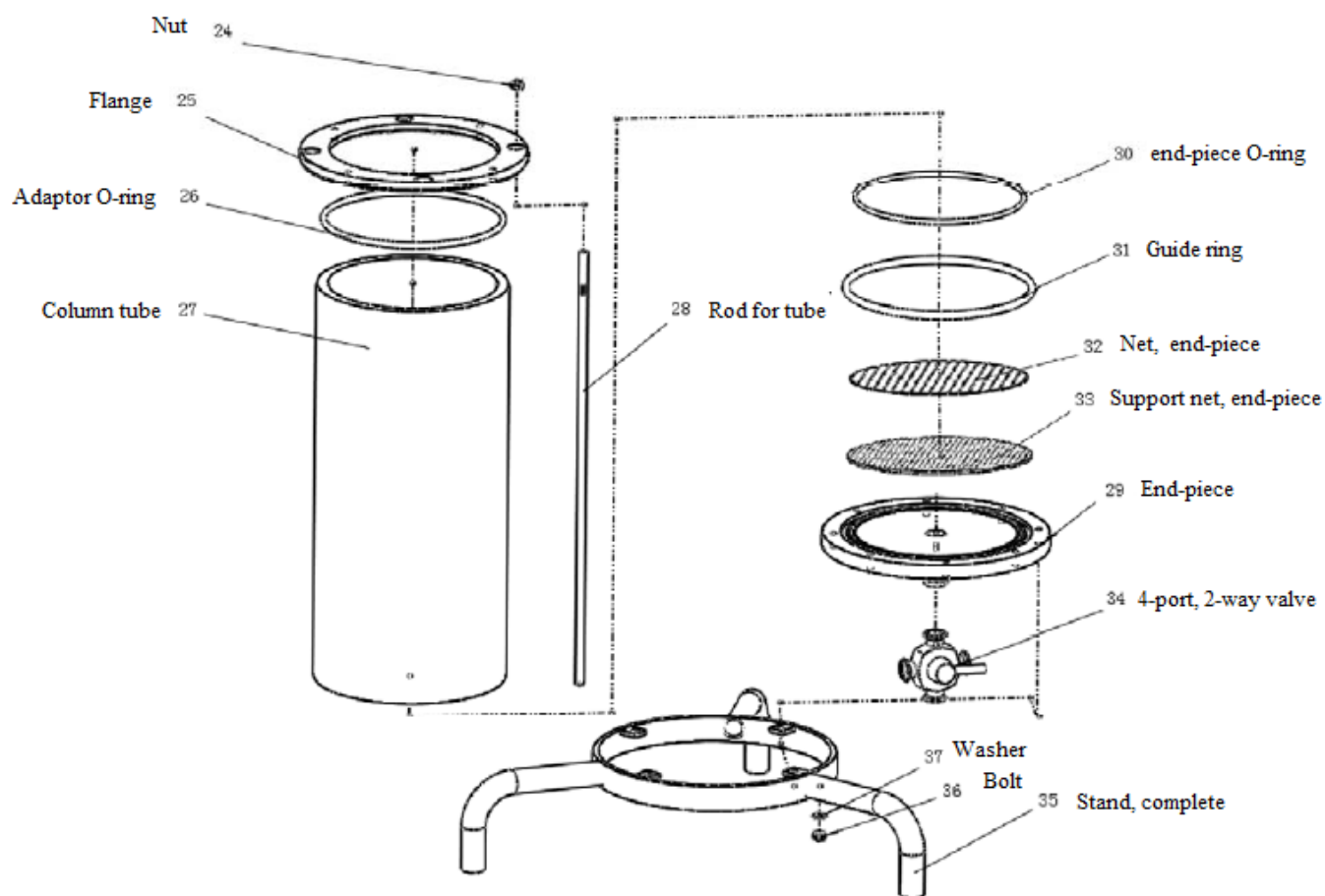
- Chloride ion can easily corrode stainless steel parts, when there is a saline solution sprinkled on the surface of stainless steel, it is necessary to wash with a large amount of purified water in time, and dry with a soft cloth .
- The column is glass product, should be handled with care, do not use hard objects to hit the column.
- Make sure installation, maintenance, operation and testing are carried out by fully trained personnel who understand the operating instructions.
- Personnel should always wear appropriate protective clothing to ensure safety during operation. Special attention should be paid when working near the column.
- The working pressure of the column must not exceed the designed pressure, otherwise there will be danger of injury and column destruction. Appropriate safety equipment must be installed.
- The operating temperature of the column should not exceed the designed temperature range.
- When installing/removing the adaptor, slowly vertically downward/upward under the condition of loosening the O-ring.If the O-ring is stuck, gently rotate the cylinder left and right.Do not push/pull it forcibly or shake it left and right to avoid the rupture of the glass tube.
- Before use, please use a socket wrench with torque pitch, set the torque to 5.5-6N /M, and tighten the feed port nut.
- Do not use chemicals other than those that have been proven harmless to the column.

7. Appendix

7.1 BXK100/140/200 chromatographic column structure decomposition Figure 1



7.2 Structural Decomposition Diagram of BXK100/140/200 Chromatography Column 2



7.3 List of column components, materials, item number.

Number	Product	Material
1	Adjusting knob	Polyoxymethylene
2	Adjuster nut, body	316L
3	Bushing ring	POM
4	Domed nut	316L
5	Washer	316L
6	Dust plug	POM
7	Top plate	316L
8	Adjuster nut, insert	PEEK
9	Allen screw	316L
10	Allen screw	316L
11	Stopper	316L
12	Adaptor head screw	316L
13	Washer	Teflon
14	Sealing unit	316L
15	Adaptor O-ring	EPDM
16	Outer adaptor tube	316L
17	Screw	316L
18	Washer	316L
19	Inner adaptor tube	316L
20	Spring	304
21	Adaptor plate	316L
22	Support net, adaptor	Polypropylene
23	Net, 23 µm adaptor	Polypropylene
	Net, 10 µm adaptor	Nylon
24	Nut, M6	316L
	Nut, M8	316L
25	Flange	316L
26	Adaptor O-ring	EPDM
27	Column tube 500	Borosilicate glass
	Column tube 750	
	Column tube 950	
28	Rod for tube 500	316L
	Rod for tube 750	
	Rod for tube 950	

Number	Product	Material
29	End-piece	316L
30	end-piece O-ring	EPDM
31	Guide ring	PTFE
32	Net, 23 μ m, end-piece	Polypropylene
	Net, 10 μ m, end-piece	Nylon
33	Support net, end-piece	Polypropylene
34	4-port, 2-way valve	316L
		Teflon
35	Stand, complete	304
36	Bolt	316L
37	Washer	316L
38	Washer	POM

8. Ordering Information

Product	Batch No	Pack/unit
BXK100/500 (10 μ m)	BC536211	1
BXK100/500	B-100500	1
BXK100/750 (10 μ m)	BC538211	1
BXK100/750	B-100750	1
BXK100/950 (10 μ m)	BC539211	1
BXK100/950	B-100950	1
BXK140/500 (10 μ m)	BC636211	1
BXK140/500	B-140500	1
BXK140/750 (10 μ m)	BC638211	1
BXK140/750	B-140750	1
BXK140/950 (10 μ m)	BC639211	1
BXK140/950	B-140950	1
BXK200/500 (10 μ m)	BC736211	1
BXK200/500	B-200500	1
BXK200/750 (10 μ m)	BC738211	1
BXK200/750	B-200750	1



Product	Batch No	Pack/unit
BXK200/950 (10μm)	BC739211	1
BXK200/950	B-200950	1
Adaptor O-ring (100 column)	BS530012	2
10μmNet,adaptor (100)	BS520012	2
10μm Net, end piece (100 columns)	BS520032	2
23μmNet,adaptor (100 columns)	BS520022	2
23μm Net, end piece (100 columns)	BS520042	2
Support net,adaptor (100columns)	BS520052	2
Support net, end piece (100columns)	BS520062	2
Glass tube (100/500columns)	BS516001	1
Glass tube (100/750columns)	BS518001	1
Glass tube (100/950columns)	BS519001	1
BXK100 Stand (With wheels)	BS540001	1
Adaptor O-ring (140columns)	BS630012	2
10μm Net,adaptor (140columns)	BS620012	2
10μm Net, end piece (140columns)	BS620032	2
23μm Net,adaptor (140columns)	BS620022	2
23μm Net, end piece (140columns)	BS620042	2
Support net,adaptor (140columns)	BS620052	2
Support net, end piece (140columns)	BS620062	2
Glass tube (140/500columns)	BS616001	1
Glass tube (140/750columns)	BS618001	1
Glass tube (140/950columns)	BS619001	1
BXK Stand (With wheels)	BS640001	1
Adaptor O-ring (200columns)	BS730012	2
10μm Net,adaptor (200columns)	BS720012	2
10μm Net, end piece (200columns)	BS720032	2
23μm Net,adaptor (200columns)	BS720022	2
23μm Net, end piece (200columns)	BS720042	2
Support net,adaptor (200columns)	BS720052	2
Support net, end piece (200columns)	BS720062	2
Glass tube (200/500columns)	BS716001	1
Glass tube (200/750columns)	BS718001	1
Glass tube (200/950columns)	BS719001	1
BXK200 Stand (With wheels)	BS740001	1



Product	Batch No	Pack/unit
100 Reservoir	B-100-01	1
140 Reservoir	B-140-01	1
200 Reservoir	B-200-01	1
Four-point two-way valve,6mm	B-006	1
Four-point two-way valve,10mm	B-010	1
Four-point two-way valve,6mm	BA210021	1
Four point four way valve,10mm	BA220021	1
1"Stainless steel clamp	B-0011	1
	BA500016	10
Blind board (TC25)	B-019	2
Blind board (TC50)	BA500072	2
Gasket (TC25, 6.9mm)	B-018	2
	BA500036	10
Gasket (TC25, 12.5mm)	BA500042	2
	BA500046	10
Pressure gauge (TC25 interface, 6bar)	BA500081	1
Pressure gauge(TC25 interface, 10bar)	BA500091	1
Three-way (TC25Stainless steel T type)	BA500101	1
Three-way (TC25/TC50Stainless steel T type)	BA500111	1
Pressure gauge (TC50 interface, 6bar)	BA500191	1
Pressure gauge(TC50 interface, 10bar)	BA500181	1
TC joint PVC pipe, 6mm, 1.5M	B-0013	1
TC joint PVC pipe, 6mm, 2M	BA187001	1
TC joint PVC pipe, 10mm, 1.5M	B-0014	1
TC joint PVC pipe, 10mm, 2M	BA197001	1
TC connector silicone pipe, 6mm, 1.5M	B-0015	1
TC connector silicone pipe, 6mm, 2M	BA187011	1
TC connector silicone pipe, 10mm, 1.5M	B-0016	1
TC connector silicone pipe, 10mm, 2M	BA197011	1
Glue stick (80mmplate)	BA500011	1
Glue stick (150mmplate)	BA500021	1
end-piece O-ring (100columns)	BS530021	2



Product	Batch No	Pack/unit
Adaptor O-ring (100columns)	BS530031	2
end-piece O-ring (140columns)	BS630021	2
Adaptor O-ring (140columns)	BS630031	2
end-piece O-ring (200columns)	BS730021	2
Adaptor O-ring (200columns)	BS730031	2
End-piece (100columns)	B100500-12	1
End-piece (140columns)	B140500-12	1
End-piece (200columns)	B200500-12	1
Outer adaptor tube (100columns)	B100500-31	1
Outer adaptor tube (140columns)	B140500-31	1
Outer adaptor tube (200columns)	B200500-31	1
Adjuster nut, insert (100columns)	B100500-22	1
Adjuster nut, insert (140columns)	B140500-22	1
Adjuster nut, insert (200columns)	B200500-22	1
Adaptor plate (100columns)	B100500-34	1
Adaptor plate (140columns)	B140500-34	1
Adaptor plate (200columns)	B200500-34	1