



BESTCHROM

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BXK450
Industry scale
chromatography column
Instruction for use



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1. Introduction

1.1 Overview

BXK450 series chromatographic column is a high precision sanitary chromatographic column specially designed for the large-scale production of biomolecule drugs. It is suitable for the packing of resins in low pressure chromatographic columns.

BXK chromatography column is composed of three parts: adaptor, column body and stand. The basic structure includes glass tube, flange, O-ring, screens, support nets, deflector, 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 designed for single pipe use. The adaptor and end-piece are covered with a large pore support net, a fine pore screen and deflector. When choosing screen, screen with different pore sizes (23 μ m (polypropylene) or 10 μ m (nylon)) are available.

Different column heights can be set by adjusting the height of adaptor. The column can be sealed by compressing or loosening the 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.

1.2 Material

The BXK450 series chromatography column is a glass chromatography columns developed for industrial scale purification. All materials used meet the requirements of SFDA for pharmaceutical manufacturers, so it is an ideal choice for industrial production. Listed are main characteristics the column:

- ① Column body is made of medical grade 316L stainless steel.
- ② The glass part of tube body is made of imported high-precision medical glass with better smoothness and roundness.
- ③ Screen, support nets and deflector are made of imported materials, enable tolerance with a variety of acid /alkali solutions and organic solvents.
- ④ O-ring made of EPDM material, offering better sealing and durability.
- ⑤ Adaptor which enables adjustable bed height.

All parts of the chromatography column have complete material certification, meeting requirements of pharmaceutical industry.

1.3 Technical specifications and materials (next page)

1.3.1 Technical Specifications

Specifications	Diameter (mm)	Floor area (cm ²)	Column Height (cm)	Bed height (cm)		Column Volume(L)		Max Pressure (bar/g)	Total weight (kg)	Chromatographic column inlet dimension	Adaptor weight (kg)	Outline dimension (cm) D×W×H
				Min	Max	Min	Max					
BXK450/500	446	1562	50	3	42	4.7	65.6	2.5	200	10mm	100	80×80×140
BXK450/750	446	1562	75	22	58	56.2	90.6	2.5	215			80×80×165
BXK450/950	446	1562	100	47	83	95.3	131.2	2.5	230			80×80×190

1.3.2 Material

Specifications	Upper/lower supporting net	Upper/lower Filter	Column tube	Seal ring	Deflector	Hardware
BXK450/500	PP	10μm nylon (PA) 23μm (PP)	Acrylic	(EPDM)	PP	316L stainless steel
BXK450/750						
BXK450/950						

Note: Refer to 5.1 for material chemical stability.

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.
- Place column on the ground. Wet the O-ring with 20% ethanol and loosen the sealing ring.

Note: Due to the strong friction between the dry sealing ring and glass tube, keep the sealing ring wet when moving the adaptor inside the column to prevent damaging the sealing ring.

- Rotate the adaptor counter-clockwise, raise the adaptor to 30CM scale mark, remove the adjustor of O-ring, connect adaptor to column stand.
- Loosen 8 fixed nuts (connecting adaptor and column tube), switch the adaptor on the stand, move the adaptor outwards, check to make sure all screws are tightly fixed. Use a wrench to tighten them in case of any loose screw.

Note: Force exceeding 3N/M may damage the glass tube.

- Check and ensure that the anti-switch key is in the socket of the adapter's outer tube.
- Connect column fittings, such as valves and pipes.

2.2 Assembly and disassembly of chromatography column

The following steps are 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 four universal wheels on the stand and adjust the level of the stand. Place the guide plate face up in the middle of the base plate and press to let it fit into the base plate groove.	b. Place the bottom supporting net to the center of the base plate.
	
c. Fit the filter in the O-ring, insert the O-ring and filter to the base plate groove.	
	

2) Assembly of tubes

- a. Insert the O-ring to the groove of the flange. Tighten the 10 rods and fix them on flange(see the picture).
- b. Put in tube, tube should be directly opposite the lower O-ring. Tighten the left 6 rods.



- c. After mounting the O-ring on flange, buckle it on the tube, make sure the 16 screw holes can fit the 16 rods.



- d. Put in the washer and fix rods by tightening nuts. Mount the tube on base plate, fix with 8 screws, tighten the nuts.



3) Adaptor assembly

a. Put the column adaptor horizontally (avoid hard material knock against the adaptor), fit guide plate to the groove below the adapter plate, mount O-ring on adaptor.



b. Install supporting net, filter(screen).



c. Insert the adaptor to the tube with the help of stand.



d. If external pipes are required, place washers evenly between the pipes and the adaptor, then lock them with clamps and tighten them.



3. Column packing

3.1 Cleaning and moistening

- The column can be packed with 0.5M NaOH and kept for more than 1 hour. Then rinse with purified water.
- Inject about 10cm height of purified water to column, remove the bubbles in screen bottom, For tiny bubbles that are difficult to be drained, pump them out by rubber suction bulb, or by a peristaltic pump connected with a hose.
- Pump away excessive purified water, retain about 2cm height of purified water in the column.
- Close the bottom valve.

3.2 Packing step

- Use a gradienter to 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 B XK column.

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

Note: This step reduces the resin particles sticking between the sealing ring and the column inner wall, eliminating the risk of leakage.

- Mount the adaptor, connect the upper adaptor to the chromatography system or peristaltic pump by installing a four-way double-pass valve.
- Lower the adapter to contact with the clarifying solution, gently shaking the adaptor to remove bubbles under the screen from the edge of the sealing ring, drain the bubbles under the screen. Tighten the sealing ring slightly and turn the four-way double-way valve switch to bypass.
- Move the adaptor downwards by rotating the adaptor, at which point the liquid is drained via a bypass of the adaptor's four-way double-pass valve. When no bubbles are discharged from the bypass, rotate the valve until it is connected with the pump.

Note: one person is responsible for fixing the adapter, and the other is responsible for rotating the regulator. When rotation proved to be difficult, rotate the adaptor in the opposite direction, make sure that it is not stuck and then continue adjust vertically, otherwise it is easy to cause damage to the outer rod thread of the adapter.

- Open the bottom valve, set the flow rate, turn on the pump, start packing the column.

Note: Different resins have different packing methods. Please refer to the resin manual to set the packing rate or pressure.

Meanwhile, set an alarm pressure during packing process to prevent damage caused to resin and column due to overpressure.

- Pack the column till the resin surface is stabilized, mark the bed height on the column.

- Stop the pump and seal the bottom outlet of the column.
- Loosen the O-ring, and quickly adjust the upper screen to 0.5cm above resin 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 resin till its surface is stabilized. Mark the bed height and turn off the pump, close bottom valve.
- 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 adaptor is slightly relaxed, and the adaptor 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 packing.

Note: After column packing, packing 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 prevent growth of bacteria in the 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 (A_s).
- 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
Sample Volume	1.0% CV	1.0% CV
Mobile phase	Water	0.4M NaCl in water
Flow rate	30 cm/h	30cm/h
Detector	UV 280 nm	Conductivity

- Method for measuring HETP and A_s
 According the UV or the conductivity curve to calculate the column Theoretical plate height (HETP), theoretical plate number (N) and the asymmetry (A_s):

$$HETP=L/N$$

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

Note :

VR = retention volume

Wh = 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

The theoretical plate number can vary with the detection conditions, so it can only be used as a reference value. The results are comparable only when the instruments and conditions remain unchanged. Solute, solution, eluent, sample volume, flow rate, flow path, temperature and other conditions all affect the results. For best results, sample loading should be controlled to less than 2.0% of column volume and linear flow rate should be 20-30cm /h.

- Evaluation the column packing

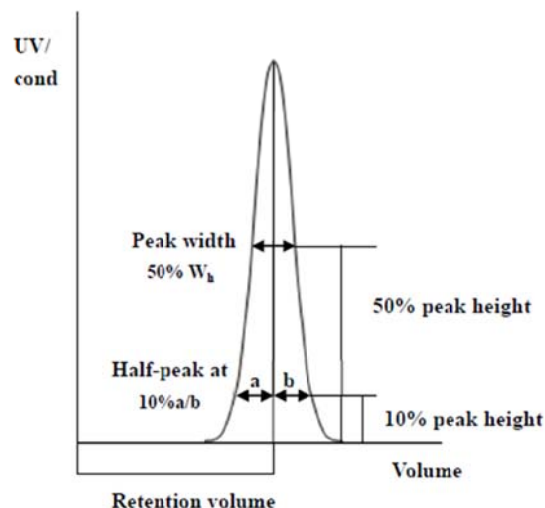
Generally, if the HETP value is less than three times the average particle size of the medium and the asymmetry factor falls within the range of 0.8 to 1.5, it indicates that the column efficiency is in good condition. For unsatisfactory column efficiency results, it is necessary to analyze the root causes and repack the column.

For packing media with different particle sizes, the column is considered to have a good packing performance when the measured column efficiency and asymmetry factor (As) meet the corresponding values specified in the table below.

Column efficiency and As for common resins

Particle size (μm)	Product	Column efficiency (N/m)	As
34	Bestarose HP	>6000	0.8-1.5
34	Chromdex	>10000	0.7-1.3
90	Bestarose FF	>2500	0.8-1.5
90	Bestarose XL	>2500	0.8-1.5
60~90	Diamond	>3000	0.8-1.5
200	Bestarose BB	>1000	0.8-1.5

Note: Column efficiency is related to the diameter and height of the chromatography column; the above column efficiency is for reference only and can be adjusted upwards based on actual conditions.



5. Maintenance

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 0.45μm membraneto remove particles to prevent blocking the column screen.
- After being used for a long time, the permeability of the screen net may decrease due to protein denaturation and other reasons, which are often indicated by the increase in back pressure. 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 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.
- The packed column should avoid exposure to direct sunlight or sudden temperature volatility.
- 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 with fresh 20% ethanol every three months.
- Prevent bubbles to enter the column during storage. Once bubble enters, repacking is necessary.
- Although most of the stainless steel components are made of 316L stainless steel, rusting and corrosion may still occur under some conditions. For example, long-term exposure to NaCl, low pH, high temperature or damaged stainless steel surface, which are all going to increase risk from NaCl corrosion.
- It is recommended to wash with water (at least five times of CV), to remove NaCl on the surface of stainless steel.

5.1 Chemical tolerance

The following table lists the chemical reagents that might be used.

Substance and concentration	Tolerance	Potential impact	Remarks
Common aqueous solution	Usable	/	/
Urea,8M	Usable	/	/
Low concentration stain remover (Triton/TBP) ,1%	Usable	/	/
NaOH,2M	Usable	/	/
Acetone, 10%	Usable	/	can be used for long time in lower than 10% acetone.
Glycol, 50%	Usable	/	/
isopropyl alcohol, 100%	Usable	/	/
Acetonitrile,50%	Short-term use	Lead to hardening of O-ring	/

Substance and concentration	Tolerance	Potential impact	Remarks
NaCl, 2M	Short-term use	Rusting	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.
HAC, 25%	Short-term use	/	Do not use with 10um nylon mesh.
HCl, 0.1M	Short-term use	/	Do not use with 10um nylon mesh.
HNO ₃ , 0.1M	Short-term use	/	Do not use with 10um nylon mesh.
GuHCl, 6M	Short-term use	Causes corrosion of stainless steel.	Wash with purified water right after use.
Trichloromethane, dichloromethane	Forbidden to use	Lead to dissolving of O-ring and cracks on acrylic tube	/
H ₂ O ₂ , 10%	Forbidden to use	Lead to aging of sealing ring.	/
Toluene	Forbidden to use	Lead to aging of sealing ring.	/
>20% ethanol	Forbidden to use	Cracks on acrylic tube	/
Strong oxidizing agent (peroxide)	Forbidden to use	Cracks on acrylic tube	/
Fluorine compounds and halides	Forbidden to use	Cracks on acrylic tube	/
Lipid	Forbidden to use	Cracks on acrylic tube	/

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

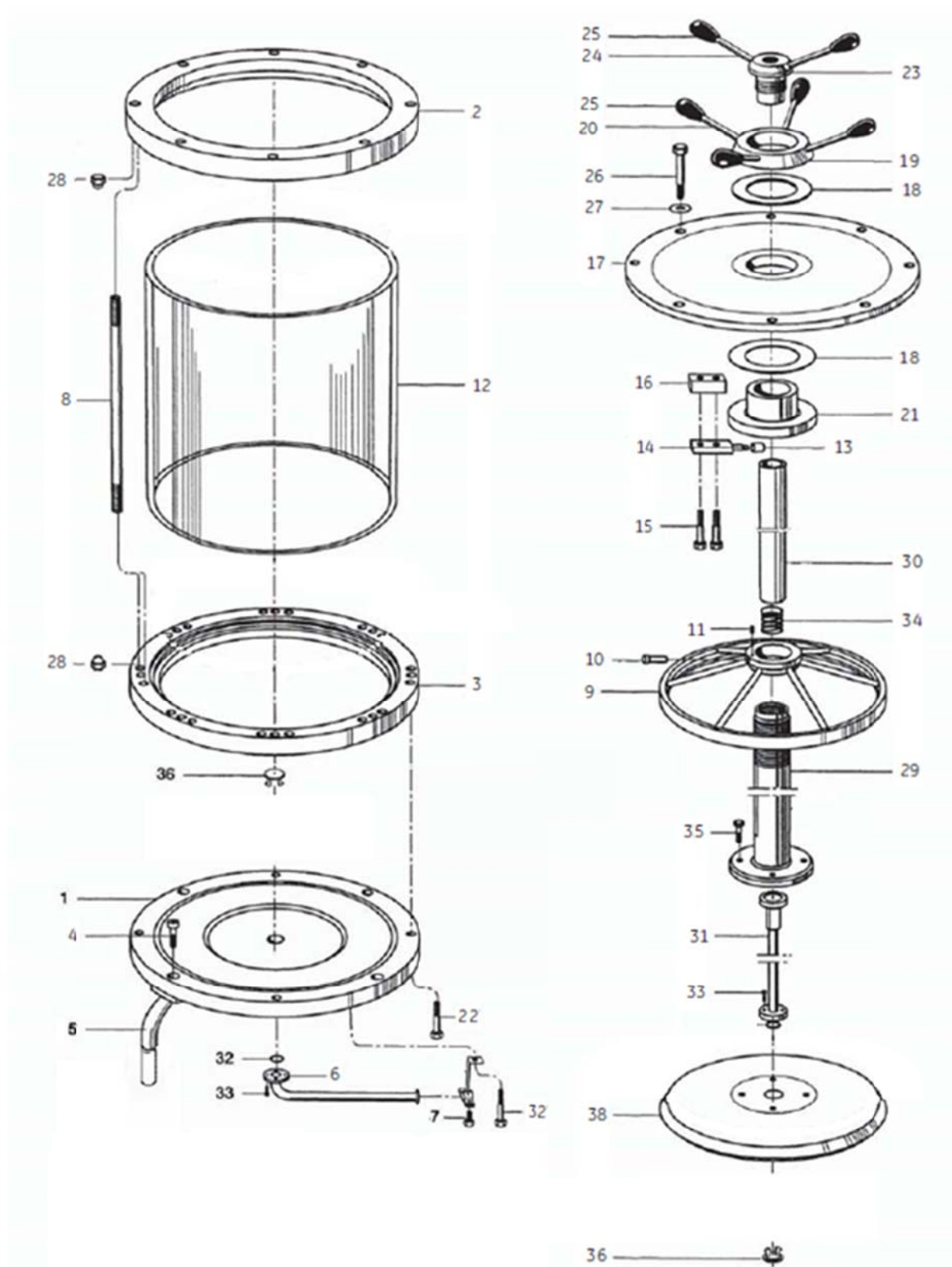
6. Handling Information

- 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.
- 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 mounting/dismounting the adaptor, slowly move adaptor downward/upward when the O-ring is loosening. If the O-ring is stuck, gently rotate the adaptor leftwards and rightwards. Do not push/pull it forcibly or shake it to avoid damage to the glass tube.
- Except for the chemicals that have been proven harmless to the column, do not use other chemicals to the column.

7. Appendix

7.1 BXK 450 chromatography column structure decomposition Figure 1



7.2 Column parts, material and Cat.No

S/N	Parts	Quantity per set	Material
1	Base plate	1	316
2	Upper flange	1	316L
3	Lower flange	1	316L
4	Bolt	4	Stainless steel
5	Universal wheel	4	Stainless steel
6	Feed flow out pipe	1	316L
7	Bolt	8	Stainless steel
8	Rod	16	316
9	seal adjusting unit	1	316L
10	Adjusting unit screw	1	316
11	Adjusting unit latch	1	PTFE
12	Column tube	1	Acrylic
13	Anti-rotation key	1	PTFE
14	Anti-rotation key latch	1	316
15	Anti-rotation screw	2	316
16	Anti-rotation cover plate	1	316
17	Upper column plate	1	316L
18	PTFE washer	2	PTFE
19	Bed height adjustor	1	316L
20	Handle bar	4	316
21	PEEK nut	1	PEEK
22	Bolt	4	Stainless steel
23	Sealing adjustor	1	316
24	Handle bar	2	316
25	Handle	6	POM(Black)
26	Adaptor nut	8	316
27	Flat washer	8	316

S/N	Parts	Quantity per set	Material
28	Rod fixing screw	16	316
29	Outer pipe of adaptor	1	316L
30	Inner pipe of adaptor	1	316L
31	Feed flow in pipe	1	316L
32	Bolt	8	Stainless steel
33	Screw	8	316L
34	Spring	1	Stainless steel
35	Bolt	4	Stainless steel
36	Guide plate buckle	2	PP

8. Ordering information

Parts	Cat.No	Pcs/Package
BXK450/500 (10μm)	BC916211	1
BXK450/500	BC916411	1
BXK450/750 (10μm)	BC918211	1
BXK450/750	BC918411	1
BXK450/1000 (10μm)	BC919211	1
BXK450/1000	BC919411	1
Adaptor O-ring (450 column)	BS930012	2
10μm upper filter (450 column)	BS920012	1
10μm lower filter (450 column)	BS920032	1
23μm upper filter (450 column)	BS920022	1
23μm lower filter (450 column)	BS920042	1
Upper support net (450 column)	BS920052	1
Lower support net (450 column)	BS920062	1
Column tube (450/500 column)	BS916001	1
Column tube (450/750 column)	BS918001	1
Column tube (450/950 column)	BS919001	1



Parts	Cat.No	Pcs/Package
450 column adaptor lift up tool	B-S009	1
450 Lifting Nut	BS900011	1
Guide plate (450 column)	BS900021	1
Four-point two-way valve, 10mm	B-010	1
Four-point four-way valve, 10mm	BA220021	1
1 "stainless steel clamp	B-0011	1
	BA500016	10
Washer (TC25,12.5mm)	BA500052	2
	BA500056	10
Blind plate (TC25)	B-019	2
Blind plate (TC25)	B-019	2
Blind plate (TC50)	BA500072	2
Pressure gauge (TC25 connector , 6bar)	BA500081	1
Pressure gauge (TC25 connector , 10bar)	BA500091	1
Three-way (TC25Stainless steel T type)	BA500101	1
Three-way (2 TC25Stainless steel T type,1 TC50 T type)	BA500111	1
Pressure gauge (TC50 connector , 6bar)	BA500191	1
Pressure gauge (TC50 connector , 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
Stirrer (150mm plate)	BA500021	1