



BESTCHROM

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BHG High Pressure Glass Chromatography Column Instruction for use



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1. Introduction

BHG5.0/6.6/10 is high pressure lab scale chromatography columns designed according to customer preference. The column has smaller CV compared to BXX columns, which enables its application in the R&D stages of macromolecules including recombinant protein, antibody, vaccine, blood products, as well as micro-molecules such as antibiotics, peptides, synthetic drugs and other natural substances. The column can be packed with agarose based resins (i.e. Bestarose, Diamond resins) and polymer based resins, facilitating easier connection to AKTA systems. The column is composed of high borosilicate glass tube and PEEK accessories, enabling good tolerance towards various types of acid/alkaline solutions and organic solvents, as a result of its excellent bio-compatibility and chemical stability.

Advantages:

- 1) BHG5.0/6.6 columns use high precision glass and provide higher accuracy in measurement of CV.
- 2) All plastic parts used in upper/lower adaptors are made of PEEK, the design of double O-rings ensures good sealing.
- 3) Rotatable adaptor, offering easier height adjustment and more convenience in column packing.

2. Technical specifications and materials

2.1 Technical Specifications

Column	ID (mm)	Height (mm)	Column Volume (mL)	Bed height (cm)	Operating pressure (bar)	Operating temperature (°C)	pH Stability	Sieve plate aperture (μm)	Chemical stability
BHG5.0/15	5	150	1.2-2.4	6.0-12.0	90	2-40	1-14	10	Common aqueous solution
BHG5.0/25	5	250	3.1-4.3	16.0-22.0	90				
BHG5.0/33	5	330	4.7-5.9	24.0-30.0	90				
BHG5.0/15 Plus	5	150	0-2.4	0-12.0	90				
BHG5.0/25 Plus	5	250	2.0-4.3	10.0-22.0	90				
BHG5.0/33 Plus	5	330	3.5-5.9	18.0-30.0	90				
BHG6.6/15	6.6	150	1.2-4.1	3.5-12.0	62				
BHG6.6/25	6.6	250	4.6-7.5	13.5-22.0	62				
BHG6.6/33	6.6	330	7.4-10.3	21.5-30.0	62				
BHG6.6/40	6.6	400	9.7-12.7	28.5-37	62				
BHG6.6/15 Plus	6.6	150	0-4.1	0-12.0	62				
BHG6.6/25 Plus	6.6	250	1.5-7.5	4.5-22.0	62				
BHG6.6/33 Plus	6.6	330	4.3-10.3	12.5-30.0	62				
BHG6.6/40 Plus	6.6	400	6.7-12.7	19.5-37.0	62				

Column	ID (mm)	Height (mm)	Column Volume (mL)	Bed height (cm)	Operating pressure (bar)	Operating temperature (°C)	pH Stability	Sieve plate aperture (μm)	Chemical stability
BHG10/20	10	200	6.3-13.0	8-16.5	40	2-40	1-14	10	Common aqueous solution
BHG10/30	10	300	14.1-20.8	18-26.5	40				
BHG10/40	10	400	22.0-28.7	28-36.5	40				
BHG10/20 Plus	10	200	0-13.0	0-16.5	40				
BHG10/30 Plus	10	300	7.5-20.8	9.5-26.5	40				
BHG10/40 Plus	10	400	15.3-28.7	19.5-36.5	40				

Note: An additional 25μm hydrophilic sieve plate is included with the shipment.

2.2 Materials

Upper/Lower tubing	Adapter shaft	Connection cap	Connection cap	Column tube	O-ring	Upper/Lower Frit
ETFE	PEEK	PEEK	PEEK	Borosilicate glass	FKM	PP

3. Column structure

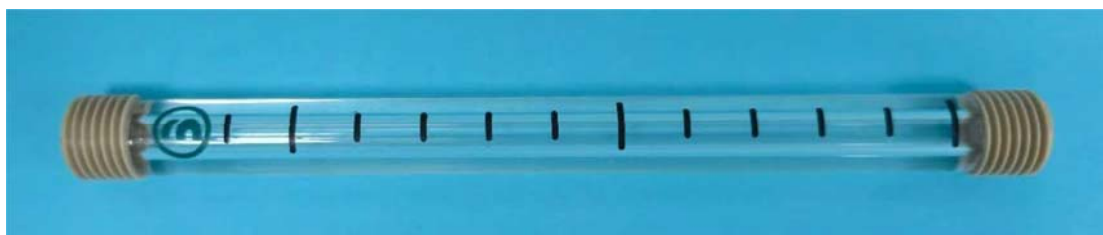
BHG column consists of three parts: Upper adaptor, column tube, lower adaptor.

- **column tube**

Column tube material: Borosilicate glass.

Length: 15cm,20cm,25cm,30cm,33cm,40cm and other specifications.

Diameter: 5.0mm, 6.6mm, 10mm..



- **Upper adaptor:** It is composed of connection cap, shaft, tubing, adjusting nut, retaining nut, O-ring, frit, 1/16 tubing connector, stop plug.



- **Lower adaptor:** It is composed of connection cap, plunger, tubing, retaining nut, O-ring, frit, 1/16 tubing connector, stop plug.



4. Packing the column

- 1) Assemble the column (connect to a packing reservoir if it exists), wash the column tube with purified water or 20% EtOH.
- 2) Remove the lower adaptor and wash with packing buffer, remove air under the frit, mount the lower adaptor to the column bottom, tighten the stop plug. Keep 1cm height of liquid in the column bottom, adjust column and keep it vertical to ground.
- 3) Wash resin, prepare resin slurry according to the instruction.
- 4) Connect adaptor to pump, remove air in the adaptor.
- 5) Stir slurry well and pour it slowly to the column at one time, make sure do not take any bubble in. Top up tube/packing reservoir by slowly adding buffer. Mount on top adaptor.
- 6) Set the flow rate¹, open stop plug of the lower adaptor, turn on the pump to compress resin bed.
- 7) When resin bed is consolidated, keep on compress resin bed for more than 15min, turn off the pump, close the bottom stop plug.
- 8) Remove packing reservoir (if it is mounted), connect top adaptor to column tube.
- 9) Adjust top adaptor to a position 0.5~1cm above the resin surface, make sure adaptor inlet is filled with liquid.
- 10) Open lower plug, connect to pump, keep a constant flow rate (make sure pressure is under max limit of resin and column). Keep compressing resin till resin bed is consolidated, mark the resin bed height.
- 11) Stop the pump, close the lower plug, open the top plug, slightly loosen adjusting nut, compress the adapter to about 3~5mm below the resin bed, close top plug, complete column packing.

1: The required flow rate varies from resins and bed heights, please refer to user instructions or seek technical support from Bestchrom team.

5. Column efficiency testing

Efficiency of packed column can be evaluated.

- 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	Purified Water	0.4M NaCl in water
Flow rate	30 cm/h	30cm/h
Monitor	UV 280 nm	Conductivity

- HETP and As Calculaiton

Use the conductivity curve to calcu

late the Height equivalent to a theoretical plate (HETP), number of theoretical plates (N) and the asymmetry (As):

$$HETP=L/N$$

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

Where: VR=retention volume

Wh=peak width at half peak height

L=column bed height

N=number of theoretical plates

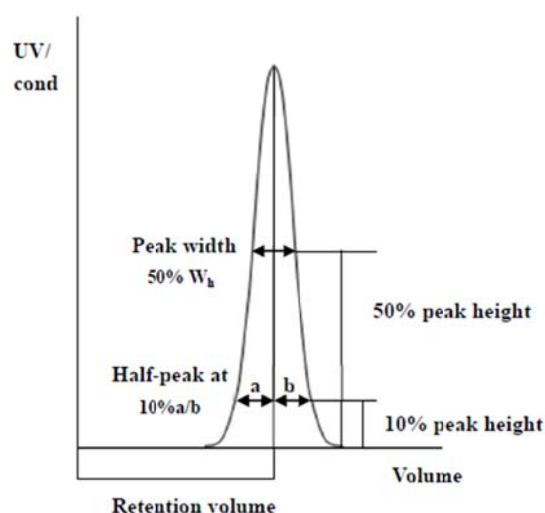
VR and Wh are measured in the same units

$$As=b/a$$

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

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)	Resin	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.

6. Cautions

- ◆ Connection cap should be mounted on shaft and plunger correctly, make sure it is tightly screwed, to prevent leakage.
- ◆ Adaptor dismantling/assembling should be done in axial direction, avoid shake brutally in right and left directions, to prevent damage to tube.
- ◆ Prior to assembling shaft and plunger, wet the O-ring, slowly push it into tube in a vertical direction, avoid packing column when it is on the tilt, so as to prevent damage to O-ring or glass tube.
- ◆ It is strictly prohibited to use excessively high pressure.
- ◆ Shaft has a left-hand thread. To prevent thread stripping, when disassembling the upper adaptor for cleaning, the adjusting nut should be unscrewed from one end of the shaft (the end towards O-ring); during installation, the adjusting nut should be screwed in from the other end of the shaft (the end towards connection cap).
- ◆ Scale marks on glass tube should be used as a reference, use real measured data in practice.
- ◆ After fully tighten the adjusting nut, screwing backwards by 15° to slightly loosen it, so as to prevent damage to glass tube caused by excessively tightened nut.

7. Trouble shooting

Trouble	Cause and solution
Leak from connection cap	Check whether connection cap and 1/16 tubing connector are tightly screwed.
Excessively high pressure	1) Packing flow is higher than max flow of resin, confirm column packing condition. 2) Tubing is folded or has kink mark.
Flow is lower than setting rate.	1) Check for the existence of air in tubing. 2) Check for leakage. 3) Make sure device is operating normally.
Resin leakage from the column lower outlet	1) Make sure the lower adaptor is correctly mounted. 2) Make sure the frit specification matches the resin particle size.

8. Order Information

Column	Cat.No	Pack/pcs
BHG5.0/15	BC033601	1
BHG5.0/25	BC030601	1
BHG5.0/33	BC030602	1
BHG5.0/15 Plus	BC033602	1
BHG5.0/25 Plus	BC030603	1
BHG5.0/33 Plus	BC030604	1
BHG6.6/15	BC033603	1
BHG6.6/25	BC030605	1
BHG6.6/33	BC030606	1
BHG6.6/40	BC036601	1
BHG6.6/15 Plus	BC033604	1
BHG6.6/25 Plus	BC030607	1
BHG6.6/33 Plus	BC030608	1
BHG6.6/40 Plus	BC036602	1
BHG10/20	BC134601	1
BHG10/30	BC135601	1
BHG10/40	BC136601	1
BHG10/20 Plus	BC134602	1
BHG10/30 Plus	BC135602	1
BHG10/40 Plus	BC136602	1

Accessories	Item code	Pack/pcs
Tube (BHG5.0/15)	BS013001	1
Tube (BHG5.0/25)	BS014001	1

Accessories	Item code	Pack/pcs
Tube (BHG5.0/33)	BS015001	1
Tube (BHG6.6/15)	BS013011	1
Tube (BHG6.6/25)	BS014011	1
Tube (BHG6.6/33)	BS015011	1
Tube (BHG6.6/40)	BS016011	1
Tube (BHG10/20)	BS114021	1
Tube (BHG10/30)	BS115021	1
Tube (BHG10/40)	BS116021	1
Column connector(for packing reservoir) (BHG5.0/6.6)	BA500007	1
Column connector(for packing reservoir) (BHG10)	BA500008	1
Adaptor O-ring (BHG5.0)	BS030015	5
Adaptor O-ring (BHG6.6)	BS030025	5
Adaptor O-ring (BHG10)	BS130035	5
Complete upper adaptor (BHG5.0)	BS050011	1
Complete lower adaptor (BHG5.0)	BS050021	1
Complete upper adaptor (BHG6.6)	BS050031	1
Complete lower adaptor (BHG6.6)	BS050041	1
Complete upper adaptor (BHG10)	BS150021	1
Complete lower adaptor (BHG10)	BS150031	1
10µm sieve plate (BHG5.0)	BS020075	5
10µm sieve plate (BHG6.6)	BS020085	5
10µm sieve plate (BHG10)	BS120035	5
25µm sieve plate (BHG5.0)	BS020095	5
25µm sieve plate (BHG6.6)	BS020105	5
25µm sieve plate (BHG10)	BS120045	5