

Original-

Manual



YMC HT

High temperature chromatography column

In order to avoid personal injuries and damage to equipment, please read this manual carefully and act on the recommendations given. Keep this manual accessible for the user at all times.



 Schöttmannshof 19
46539 Dinslaken, Germany

 +49 02064 427-0

 info@ymc.eu

 www.ymc.eu

Contents

1	Information on manual	5
1.1	General.....	5
1.2	Explanation of warnings.....	5
1.3	Structure of instructions	6
1.4	Guidelines	6
1.5	Figures	6
2	Safety.....	7
2.1	Intended use	7
2.2	General safety instructions	7
2.3	Personnel requirements.....	7
2.4	Personal protective equipment	8
3	Specifications and overview	9
3.1	Fields of application	9
3.2	Specifications.....	9
3.3	Chemical resistance.....	11
3.4	Overview	12
3.4.1	Variable end piece	13
4	Delivery	14
4.1	Delivery contents.....	14
4.2	Delivery inspection	14
4.3	Accessory bag.....	15
5	Requirements for operation	17
5.1	Safety instructions for use.....	17
5.2	Glass column set up.....	18
5.3	Pressure test	18
6	Operation	20
6.1	Opening and closing the column.....	20
6.2	Tightening and loosening the O-ring.....	20
6.3	Piston adjustment.....	21
6.4	Assembly and disassembly of the end pieces	21
6.5	Glass column packing.....	22
6.5.1	Slurry preparation	23
6.5.2	Slurry column packing under flow	24
6.5.3	Slurry column packing with sedimentation.....	26
6.6	Column test and release criteria	28
6.7	Packing adapter	29
6.8	Glass column unpacking.....	30
7	Cleaning and maintenance.....	31
7.1	Notes for Cleaning-In-Place (CIP)	32
7.2	Cleaning and maintenance plan	33
7.3	Cleaning.....	34
7.4	Replacement of spare parts.....	35
7.4.1	Frit replacement	35
7.4.2	O-ring replacement	36
7.4.3	Replacing the glass body.....	36
8	Troubleshooting.....	37
9	Storage, packaging and transport.....	39

9.1	Storage.....	39
9.2	Transport.....	39
10	Disposal	40
11	Appendix	41
11.1	Spare parts glass body	41
11.2	Spare parts end pieces	42
11.3	Spare parts coupling unit	42
11.4	Index.....	43

1 Information on manual

1.1 General

This manual includes important messages and information for the intended use of this glass column. The manual must be kept accessible for the user at all times.

In order to avoid injuries and damages, this manual should be read carefully prior to use and the recommendations given should be acted upon.

1.2 Explanation of warnings

Warnings are highlighted by coloured signal word fields. Always read the full text of the warning in order to effectively protect yourself from damage!

The following signal word fields are marked by different colours and signal word for distinct security levels:

 **DANGER**

Disregarding this warning will result in death or serious injuries.

 **WARNING**

Disregarding this warning may result in death or serious injuries.

 **CAREFUL**

Disregarding this warning may result in minor or moderate injuries.

ATTENTION

Disregarding this warning can result in damage to surrounding property.

Warnings are always in the same structure. They include word, type and source of danger, consequences of neglect and security measures / avoidance.

1.3 Structure of instructions

The instructions relate directly to an activity. They are structured in a task oriented manner. Always perform the individual steps in the order in which they are given.

Instructions are structured as follows and are marked by the corresponding levels:

▶ Aim of the instruction

1. Action step

- ✓ Impact of the action step to check whether the step has been performed correctly.

2. Another action step.

Figures within instructions:

A figure is only related to the action step which is listed directly below the figure.

1.4 Guidelines

The following guidelines are available in this manual:

- Contents at the beginning
- Index in the end
- Name of the main Chapter in the header
- Links to other Chapters, for example "Information on manual" (▶ Chapter 1, p. 5)
- Additional information and hints indicated with an i-symbol



Example for additional information

1.5 Figures

Figures can differ in the graphical presentation from the product.

2 Safety

This section gives an overview of all the important safety aspects for the protection of personnel and for the safe and trouble-free handling of the glass column.

Other task-specific safety requirements are contained in the sections relating to the respective operation. In addition, the national and local legal and other applicable safety regulations must be observed.

2.1 Intended use

The glass columns are only to be used for the separation of substances using liquid chromatographic techniques.

The intended use of this product must be performed with regards to the specifications ►Chapter 3.2, p. 9. The product is only for use with liquids that have a pressure <0,5 bar above the atmospheric pressure at the intended maximum temperature (70 °C). Every use which is outside these specifications is defined as not intended use. The intended use includes the compliance with the advice and terms in the manual.

2.2 General safety instructions

In order to avoid accidents and injuries,

- operate the glass column only within its intended use.
- follow all general and special safety instructions as well as all warnings in this manual.
- follow danger signs and signs giving instructions.
- follow the rules and regulations for prevention of accidents at the site of operation.
- comply with all inspection and maintenance intervals.

2.3 Personnel requirements

The various tasks described in this manual make different demands on the qualifications and training of the persons carrying them out.





The user must:

- have competent and proficient training.
- be trained in dealing with pressure devices.
- read and fully understand this manual before using this glass column.

2.4 Personal protective equipment

In order to reduce the risks during working on the column, always use the recommended personal protective equipment.

The following protective equipment is required:

Symbol	PPE	Phase of life	Action
	Protective gloves	Operation	<ul style="list-style-type: none"> • Column packing • Column unpacking
		Initiation	<ul style="list-style-type: none"> • Cleaning
	Safety goggles	Operation	<ul style="list-style-type: none"> • All actions
		Initiation	<ul style="list-style-type: none"> • Cleaning • Pressure testing
	Dust mask	Operation	<ul style="list-style-type: none"> • Slurry preparation
	Protective clothing	Operation	<ul style="list-style-type: none"> • All actions
		Initiation	<ul style="list-style-type: none"> • Cleaning • Pressure testing

3 Specifications and overview

3.1 Fields of application

The YMC HT glass column is especially designed for high temperature applications. The column is compatible with common BioLC separation modes (IEX, SEC, Affinity, IMAC, HIC, MMC) as well as NP and RP mode in case the chemical resistance is given.

3.2 Specifications

ID [mm]	Pressure limit [bar]	Bed length range [mm]	Column volume range [mL]
10	30	15-120	1.5-9
		95-200	9-15
		195-300	17-23
		345-750	30-35
		645-750	55-58
		905-1000	77-78
15	25	10-120	2-19
		90-200	18-32
		190-300	37-49
		335-450	65-73
		635-750	121-122
		885-1000	163-170
25	15	10-120	6-60
		90-200	55-100
		195-300	113-156
		345-450	200-230
		645-750	375-390
		895-1000	515-520
50	10	20-120	45-235
		100-200	225-390
		205-300	453-593
		355-450	785-885
		655-750	1450-1480
		905-1000	1970-2000

PED* Fluidgroup:	Group 1 referred to EU Guideline 2014/68/EU
PED* safety requirement:	Article 4.3 of EU Guideline 2014/68/EU
Glass body:	Borosilicate glass 3.3
Frits:	Glass
Seals:	FFKM
Connection:	1/4"-28G thread FEP tubing 10-15 mm ID: 0.8 x 1.6 mm 25-50 mm ID: 1.6 x 3.2 mm
Wetted parts:	PEEK, Glass, FFKM, Borosilicate glass 3.3
Approved temperature range:	4 – 70 °C

*Pressure Equipment Directive

3.3 Chemical resistance

The chemical resistance depends on the wetted materials used ▶Chapter 3.2, p.10.

From the resistance of the individual materials the chemical resistance of the column can be concluded. Such statements are based on our current technical knowledge and experiences as well as on existing literature and the information from the commodity producers. These do not exempt the users of our products from their own testing.



If desired solvent is not listed, YMC will be pleased to advise you.

ATTENTION

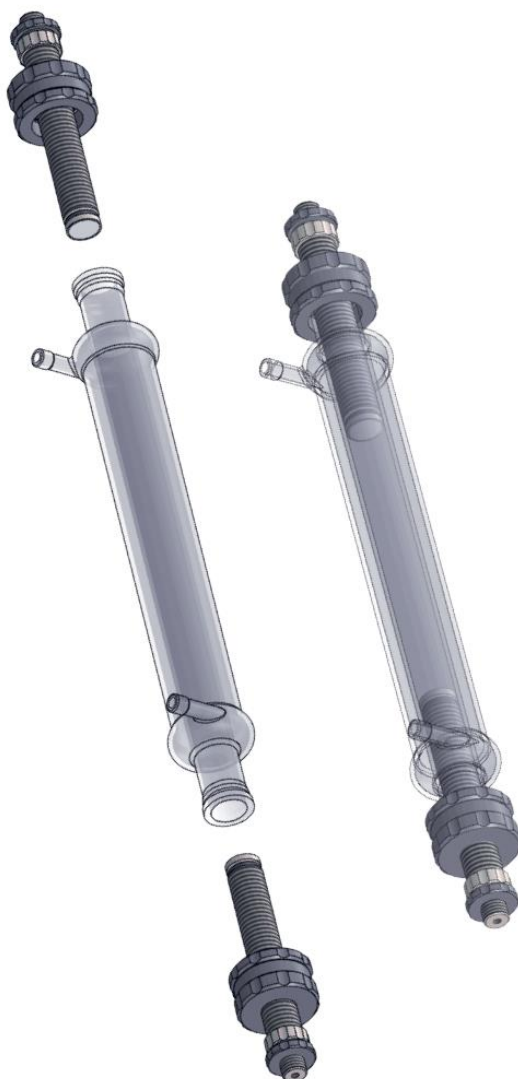
When using solvent mixtures, the resistance may differ significantly from those of the pure solvent.

Material	Borosilicate glass 3.3	PEEK	FFKM	FEP
Solvent				
Acetone	Good	Good	Good	Good
Acetonitrile	Good	Good	Good	Good
Dihydrogen phosphate	Good	Good	Good	Good
Cyclohexane	Good	Good	Good	Good
Dichlormethane	Good	Limited	Good	Good
0.1 M EDTA (3%)	Good	Good	Good	Good
1 M Acetic acid (6%)	Good	Good	Good	Good
Ethanol	Good	Good	Good	Good
Ethyl acetate	Good	Good	Good	Good
n-Hexane	Good	Good	Good	Good
Isopropanol	Good	Good	Good	Good
Methanol	Good	Good	Good	Good
2 M NaOH (8%)	Good	Good	Good	Good
1 M HCl (4%)	Good	Good	Good	Good
8 M Urea (36%)	Good	Good	Good	Good
1 M NaCl	Good	Good	Good	Good
0.5 M Na ₂ SO ₄	Good	Good	Good	Good

3.4 Overview

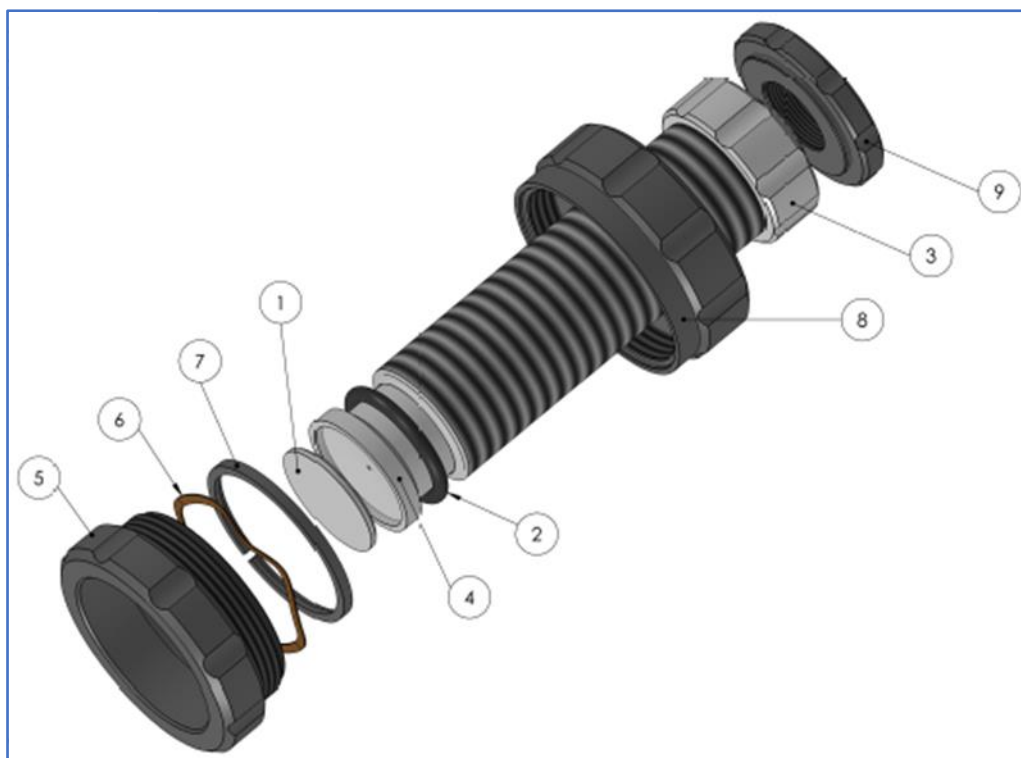


All parts listed for the column body and the end pieces are in the appendix.



The YMC HT column consists of a glass body equipped with a tempering jacket and two variable end pieces. The end pieces can be removed from the column body.

3.4.1 Variable end piece



- | | |
|-----------------|-----------------|
| 1 Frit | 6 Spring washer |
| 2 O-ring | 7 Snap ring |
| 3 Outer spindle | 8 Counter nut |
| 4 Inner spindle | 9 Screw nut |
| 5 Counter screw | |

The variable end piece consists of the counter screw connection and the piston. The piston consists of the inner (4) and outer spindle (3). The pistons are attached to a counter screw (5) and counter nut (8) held in place by the snap ring (7) and the spring washer (6). The frit (1) is inserted in the inner spindle. A tubing can be connected to the end piece externally. By turning the screw nut (9), the O-ring is compressed or relaxed and this way tightened or loosened.

4 Delivery

The YMC HT glass columns are shipped as pre-assembled units. A quickguide, accessory bag and Certificate of Quality Control is delivered with every glass columns. Using the serial number, the column can be identified clearly. The product code includes information of inner diameter, length and frit porosity.

4.1 Delivery contents

- Glass column
- Quickguide
- Accessory bag
- YMC Quality Control / Certificate of Quality Control
- If ordered especially / separately:
 - Material certificates / statements of conformity

4.2 Delivery inspection



YMC recommends retaining and storing the original packaging and the foam cushion so that the column can always be shipped safely.

Delivery inspection

- ▶ Delivery inspection should be performed as follows:
 1. Check the delivery for visible transport damage.
 2. Check the delivery for the completeness of parts.
 3. Compare the column description on the glass body with the data of the delivery note.

Transport damages

- ▶ Procedure, if you observe any visible transport damage:
 1. Refuse or reserve acceptance of the delivery.
 2. Record the transport damage on the delivery note of the transporter or in the transport documents.
 3. Inform YMC Europe GmbH and / or the responsible supplying agent.

4.3 Accessory bag


YMC HT glass columns are shipped with an accessory bag containing different fittings for connecting the column to the system. The tubing is connected to the piston outlet using a ferrule and a nut. For connecting the tubing to the system, 10-32 or M6 fittings are included depending on the column inner diameter. The size of the tubing is also dependent on the column dimension.

ATTENTION





The accessory bag includes fittings for the column and the system

- Use only fittings with 1/4"-28G thread for connection with the piston.


Connection Principle






Piston Thread: 1/4"-28G Ferrule Nut Tubing 1/16" (0.8 x 1.6)

<p>Connection to Column 1/4"-28G</p>  <p>K.P316</p>	<p>Connection to System 10-32 HPLC</p>  <p>JR-58000</p>
<p>Connection to Column/System 1/4"-28G</p>  <p>JR-CFL-CB1PK-S JR-55050</p>	<p>Connection to System M6</p>  <p>K.P200N K.P207</p>

Connection Principle



Piston Thread: 1/4"-28G Ferrule Nut Tubing 1/8" (1.6 x 3.2)

<p>Connection to Column 1/4"-28G</p>  <p>K.P316</p>	<p>Connection to System M6</p>  <p>K.P300N K.P307</p>
<p>Connection to Column/System 1/4"-28G</p>  <p>JR-CFL-CB2PK-S JR-55051</p>	

Accessory bag 10-15 mm ID		
Part number	Description	Quantity
PT0,8FE1,6	1/16" FEP tubing (0.8 x 1.6 mm)	1
JR-55050	Nut, 1/4"-28G for 1/16" tubing	4
JR-CFL-CB1PK-S	Ferrule, collapsible, for 1/16" tubing	4
JR-58000	10-32 nut (one-piece-fitting) for 1/16" tubing	2
K.P207	M6 nut for 1/16" tubing	2
K.P200N	Ferrule for 1/16" tubing	2
K.P316	Plug PTFE (1/4"-28G)	2
	Quickguide	1
	Frit removal tool	1
	Certificate of Quality Control	1
	Overview Accessories	1

Accessory bag 25-50 mm ID		
Part number	Description	Quantity
PT1,6FE3,2	1/8" FEP tubing (1.6 x 3.2 mm)	1
JR-55051	Nut, 1/4"-28G for 1/8" tubing	4
JR-CFL-CB2PK-S	Ferrule, collapsible, for 1/8" tubing	4
K.P307	M6 nut for 1/8" tubing	2
K.P300N	Ferrule for 1/8" tubing	2
K.P316	Plug PTFE (1/4"-28G)	2
	Quickguide	1
	Frit removal tool	1
	Certificate of Quality Control	1
	Overview Accessories	1

5 Requirements for operation

5.1 Safety instructions for use

⚠ WARNING

Use correctly matched protective clothing when working with the glass column.

⚠ WARNING

Do not put the glass column under gas pressure.

⚠ CAREFUL

Operate the column only with pressures within the stated specifications. Otherwise there is a risk of injury and material damage.

⚠ CAREFUL

The temperature range which is used must not exceed / fall below the given specifications.

ATTENTION

Use the column only with chemicals that are proven applicable with the wetted materials.

ATTENTION

Make sure that the fluids used are always clean and free from particles, as this can lead to blockages.

5.2 Glass column set up



For columns with 25 mm and 50 mm inner diameter, column stands are available to set up the column.

- ▶ The glass column is set up as follows:
 1. Make sure that the glass column is fixed in the stand / holder.
 2. Make sure that the glass column is standing vertical in the final operating place.

5.3 Pressure test

WARNING

Operation of pressure tests

Eye or skin injuries possible due to solvent leakage or incorrectly sealed connectors.

- ▶ Perform the pressure test behind a safety screen.
 - ▶ During work on the glass column, wear the prescribed personal protective equipment.
-
-

WARNING

Glass column under high pressure

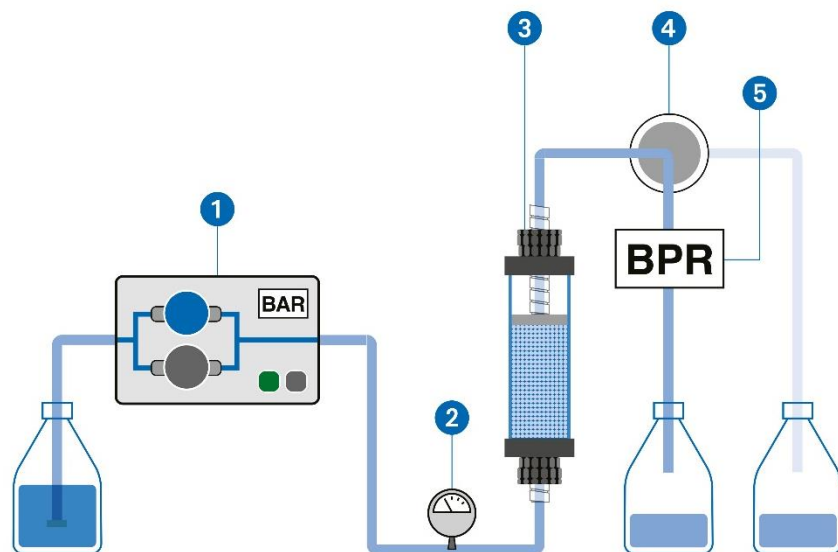
Eye or skin injuries possible due to remaining pressure in the tubing or in the glass column.

- ▶ Stop the pump during work on the glass column.
 - ▶ During work on the glass column, wear the prescribed personal protective equipment.
-

A pressure test is used to check the tightness of both the seals in the glass column and also the tubing connectors. Before using the column for chromatographic applications, it is recommended that a pressure test is performed to verify the correct assembly.

Prior to delivery of the column, the column has been tested according to the standard procedure described below. The relevant certificate is located in the documentation folder.

Standard procedure pressure test



- | | |
|------------------|---------------------------|
| ① Pump | ③ Glass column |
| ② Pressure gauge | ④ 3-way valve |
| | ⑤ Back pressure regulator |

► The pressure test should be performed as follows:

1. Assemble the system according to the schematic overview of a pressure test. The 3-way valve must not be set to the back pressure regulator.
2. Tighten the O-rings on both end pieces.
3. Fill the glass column with water / isopropanol (bottom-up).
4. Switching the 3-way valve to the back pressure regulator
5. Increase the testing pressure by slow and stepwise adjustment of the flow rate at the pump.
 - ✓ The testing pressure is the pressure limit of the columns plus 10%.
6. Keep the testing pressure constant for 10 minutes.
7. If leakage occur, check the connections. If required, replace tubing and / or connectors.

If the testing pressure was constant for 10 minutes, the column is ready for use.

i If a back pressure regulator is used, the maximum pressure is reached easier.

i Leakages at the piston of the adjustable end piece are often related to impurities trapped in the seal.

6 Operation

WARNING

Risk of explosion

Severe injuries possible due to glass splinters.

- ▶ Never put the glass column under gas pressure!
-

In order to guarantee safe operation, the glass column must be used for the intended use only (▶Chapter 2.1, p. 7).

6.1 Opening and closing the column

- ▶ To open the column, proceed as follows:
 1. Loosen the screw nut by turning it anti-clock-wise. Hold the outer spindle during loosening.
 - ✓ The O-ring is loosened.
 2. Fix the counter screw and turn the counter nut anti-clock-wise.
 - ✓ The end piece can be removed now from the glass body.
- ▶ To close the column, proceed as follows:
 1. Insert the end piece with loosened O-ring.
 2. Tighten the counter nut and counter screw.
 3. Tighten the screw nut by turning it clock-wise. Hold the inner spindle during tightening.
 - ✓ The O-ring is now sealed.

6.2 Tightening and loosening the O-ring

- ▶ The O-ring is tightened as follows:
 1. Hold the inner spindle.
 2. Turn the screw nut clockwise in the direction of the outer spindle. The O-ring is squeezed.
 - ✓ O-ring is sealed when a constant black line appears in the glass body.
- ▶ The O-ring is loosened as follows:
 1. Hold the outer spindle.
 2. Turn the screw nut anticlockwise in the direction of the inner spindle. The O-ring is loosened.
 - ✓ O-ring is not sealed anymore if the black line disappears.

6.3 Piston adjustment

- ▶ The piston is adjusted as follows:
 1. O-ring seals by turning the screw nut clockwise.
 - ✓ O-ring is sealed when a constant black line appears in the glass body.
 2. Hold the closed counter screw connection.
 3. By turning the counter screw connection clockwise, the piston moves up. By turning the counter screw connection anti-clockwise, the piston moves down.

6.4 Assembly and disassembly of the end pieces

- ▶ The end piece is assembled at the glass body as follows:
 1. Fit the counter screw (8) onto the glass body (11).
 - ✓ The thread is in direction of the open end of the glass body.
 2. Attach the spring washer (7) to the glass body.
 - ✓ Push the spring washer over the glass body sideways.
 3. Attach the snap ring (6) to the glass body.
 - ✓ The cone of the 50 mm ID snap ring shows to the open end of the glass body.
 4. Insert the piston with the attached counter nut (5) in the glass body.
 5. Screw the counter screw (8) into the counter nut (5).

- ▶ The end piece is disassembled as follows:
 1. Open the counter screw connection.
 2. Remove the piston from the glass body.
 3. Remove the snap ring from the glass body.
 4. Remove the spring washer from the glass body.
 5. Remove the counter screw.



6.5 Glass column packing

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personal protective equipment.
-

ATTENTION

Adapt the packing method to the manufacturer's packing material information. Improper packing conditions can cause damage or completely destroy the material.

ATTENTION

Avoid the use of a magnetic stirrer! Depending on the nature of the packing material, stirring can cause mechanical damage to the material. Refer to the information provided by the manufacturer of the packing material.

Two different procedures are common for slurry packing the column:

- under flow,
- with sedimentation.

6.5.1 Slurry preparation

⚠ CAREFUL

Formation of dust with dry packing material

Lung irritation possible if packing material is inhaled.

- ▶ Read the material safety data sheet from the packing material manufacturer.
 - ▶ Wear a dust mask.
-



The use of a more dilute liquid slurry simplifies the column packing. The total column volume of the slurry should not exceed the maximum volume of the column.



The removal of fines decreases the column back pressure and so improves the performance of the packed column.

- ▶ The slurry is prepared as follows:
 1. Mix the solvent or buffer with the appropriate amount of packing material in a suitable container (see manufacturer's recommendations).
 - ✓ The total volume of the slurry must be less than the maximum column volume.
 - ✓ The amount of packing material determines the final bed length.
 2. Mix the slurry with a stirring rod until the slurry is homogenous and avoid introduction of air bubbles.

6.5.2 Slurry column packing under flow

- ▶ The column is packed with the slurry method under flow as follows:
 1. Flush the system for packing with the packing solvent.
 2. Check the column parts with regards to cleanness and function.
 3. Insert the lower piston into the column body ▶Chapter 6.1, p. 20.
 - ✓ Adjust the piston length so that the required bed length can be reached.
 4. Tighten the O-ring of the lower end piece ▶Chapter 6.2, p. 20.
 5. Fixe the assembled column on a packing stand or tripod.
 6. Assemble the packing adapter (▶Chapter 6.7, p. 29). For shorter bed lengths, a packing adapter is not required.
 7. Connect the pump to the lower piston.
 8. Pump approx. 1-2 cm of liquid into the column from below.
 - ✓ This way, air in the frits is removed. Take care that the liquid and the O-ring are free of air bubbles.
 9. Stop the pump and disconnect it from the lower piston.
 10. Close the lower piston with a plug.
 11. Adjust the vertical alignment of the column with a level.
 - ✓ The adjustment of the column is important for a good packing result.
 12. Connect the upper piston (outside the column) with the pump.
 13. Flush the upper piston with liquid.
 - ✓ This way, air is removed from the upper piston and frit.
 14. Stop the pump and disconnect the upper piston from the pump.
 15. Homogenise the slurry.
 - ✓ Stir constantly in one direction in order to avoid the entry of air.
 - ✓ A mixing duration of 5 minutes is common.
 - ✓ For air removal, the slurry can be put in an ultrasonication for 5 minutes. Afterwards, the slurry needs to be homogenised again.
 16. Pour the homogenised slurry into the open column.
 - ✓ Pour the slurry down the opposite side of the glass wall in order to avoid the entry of air.
 17. When the material is completely transferred into the column, clean the glass wall with packing solvent to remove any remaining particles.
 - ✓ Only in this way can optimum sealing of the O-ring be guaranteed.
 18. Fill the column completely with packing solvent until it reaches the upper edge.
 19. Put the upper piston in a slight angle on the glass edge of the packing adapter.
 20. Bring the piston slowly into a vertical position.
 - ✓ This way it ensures that no air is trapped below the piston.
 21. Close the counter screw and tighten the O-ring.
 22. Lower the upper piston until it is filled completely with liquid.
 - ✓ Liquid comes out of the upper piston.

23. Remove the air from the tubing.
24. Connect the tubing to the upper piston.
25. Remove the plug at the lower piston.
26. Connect the tubing to the lower piston.
27. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
 - ✓ Wait after every step until the pressure is constant and only increase the flow rate once the pressure has stabilised.
28. Wait until the column bed has formed and the bed length does not change anymore.
 - ✓ For checking, mark the bed length under flow.
 - ✓ If no packing adapter is used, continue with step 45.
29. Decrease the flow rate stepwise until the pump can be stopped.
30. Remove the tubing from the lower piston.
31. Close the lower piston with a plug.
32. Remove the upper piston from the packing adapter.
33. Remove the liquid in the packing adapter with a syringe.
34. Remove the packing adapter.
35. Fill the column with packing solvent up to the upper edge.
36. Insert the upper piston at an angle to the glass edge of the glass column.
37. Bring the piston slowly into a vertical position.
 - ✓ This way will ensure that no air remains below the piston.
38. Close the counter screw and tighten the O-ring.
39. Lower the upper piston until you reach the column bed.
 - ✓ Take care that the upper piston is filled with packing solvent completely and that liquid comes out.
40. Remove the air in the tubing of the system.
41. Connect the tubing to the upper piston.
42. Remove the plug from the lower piston.
43. Connect the tubing to the lower piston.
44. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
45. Wait until the bed length does not change anymore.
 - ✓ If the packed bed compresses further, mark the new position of the bed under flow. Continue with steps 46-56.
 - ✓ If the packed bed does not compress further, continue with steps 46-50 and step 57 afterwards.
46. Decrease the flow rate stepwise until you can stop the pump.
47. Remove the tubing from the lower piston.
48. Close the lower piston with a plug.
49. Disconnect the pump from the upper piston.

50. Lower the upper piston until you reach the column bed.
 - ✓ Take care that the upper piston is completely filled with liquid and solvent comes out.
51. Remove the air from the tubing of the system.
52. Connect the tubing to the upper piston.
53. Remove the plug from the lower piston.
54. Connect the tubing to the lower piston.
55. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
56. Go back to step 45.
57. Lower the piston until you reach the last mark (mark under flow).
58. Connect the column to the system for testing and qualification.

6.5.3 Slurry column packing with sedimentation

- ▶ The column is packed via the sedimentation slurry method as follows:
 1. Flush the system for packing with the packing solvent.
 2. Check the column parts with regards to cleanness and function.
 3. Insert the lower piston into the column body ▶Chapter 6.1, p. 20.
 - ✓ Adjust the piston length so that the required bed length can be reached.
 4. Tighten the O-ring of the lower end piece ▶Chapter 6.2, p. 20.
 5. Fix the assembled column on a packing stand or tripod.
 6. Assemble the packing adapter ▶Chapter 6.7, p. 29. For shorter bed lengths, a packing adapter is not required.
 7. Connect the pump to the lower piston.
 8. Pump approx. 1-2 cm of liquid into the column from below.
 - ✓ This way, air in the frits is removed. Take care that the liquid and the O-ring are free of air bubbles.
 9. Stop the pump and disconnect it from the lower piston.
 10. Close the lower piston with a plug.
 11. Adjust the vertical alignment of the column with a level.
 - ✓ The adjustment of the column is important for a good packing result.
 12. Connect the upper piston (outside the column) with the pump.
 13. Flush the upper piston with liquid.
 - ✓ This way, air is removed from the upper piston and frit.
 14. Stop the pump and disconnect the upper piston from the pump.
 15. Homogenise the slurry.
 - ✓ Stir constantly in one direction in order to avoid the entry of air.
 - ✓ A mixing duration of 5 minutes is common.
 - ✓ For air removal, the slurry can be put in an ultrasonic for 5 minutes. Afterwards, the slurry needs to be homogenised again.

16. Pour the homogenised slurry into the open column.
 - ✓ Pour the slurry down the opposite side of the glass wall in order to avoid the entry of air.
17. When the material is completely transferred into the column, clean the glass wall with packing solvent to remove any remaining particles.
 - ✓ Only in this way can optimum sealing of the O-ring be guaranteed.
18. Do not move the glass column until packing is completed.
 - ✓ YMC recommends covering the open column body during sedimentation with some plastic film.
19. Wait until the packing material has settled (sedimentation).
 - ✓ Depending on the packing material, this can take several hours.
 - ✓ If no packing adapter is used, continue with step 22.
20. Remove the liquid in the packing adapter with a syringe.
21. Remove the packing adapter.
22. Fill the column with packing solvent up to the upper edge.
23. Insert the upper piston at an angle to the glass edge of the glass column.
24. Bring the piston slowly into a vertical position.
 - ✓ This way will ensure that no air remains below the piston.
25. Close the counter screw connection and tighten the O-ring.
26. Lower the upper piston until you reach the column bed.
 - ✓ Take care that the upper piston is filled with packing solvent completely and that liquid comes out.
27. Remove the air in the tubing of the system.
28. Connect the tubing to the upper piston.
29. Remove the plug from the lower piston.
30. Connect the tubing to the lower piston.
31. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
32. Wait until the bed length does not change anymore.
 - ✓ If the packed bed compresses further, mark the new position of the bed under flow. Continue with steps 33-43.
 - ✓ If the packed bed does not compress further, continue with steps 33-37 and step 44 afterwards.
33. Decrease the flow rate stepwise until you can stop the pump.
34. Remove the tubing from the lower piston.
35. Close the lower piston with a plug.
36. Disconnect the pump from the upper piston.
37. Lower the upper piston until you reach the column bed.
 - ✓ Take care that the upper piston is completely filled with liquid and solvent comes out.
38. Remove the air from the tubing of the system.
39. Connect the tubing to the upper piston.

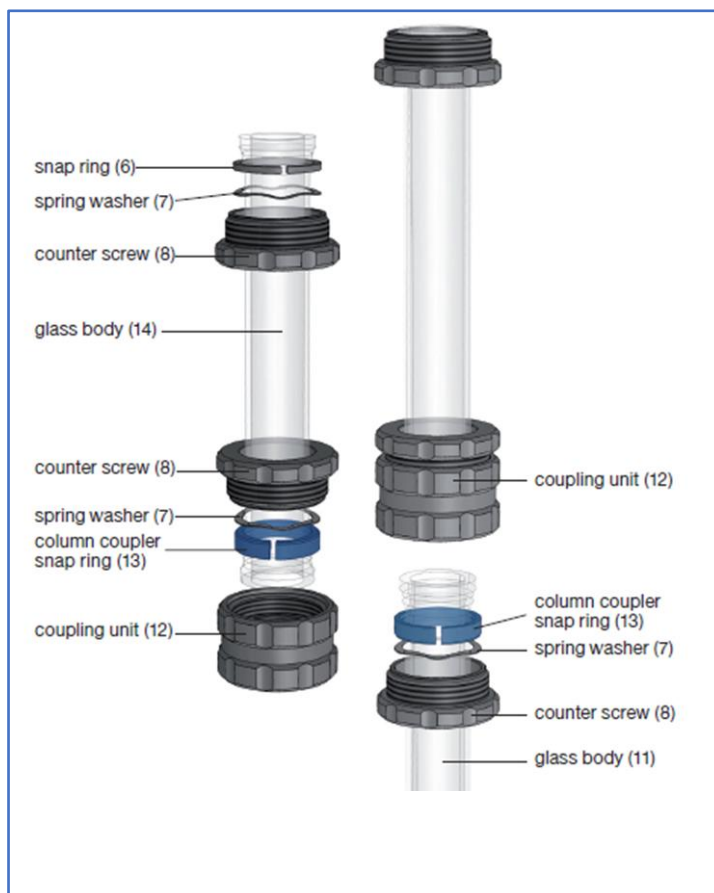
40. Remove the plug from the lower piston.
41. Connect the tubing to the lower piston.
42. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
43. Go back to step 30.
44. Lower the piston until you reach the last mark (mark under flow).
45. Connect the column to the system for testing and qualification.

6.6 Column test and release criteria

The column test and the release criteria are selected with regards to the later application requirements. The preparation of the required specifications for the final use of a column or for the purification of a target molecule is determined individually. YMC will gladly support you.

6.7 Packing adapter

Due to the increased maximum column volume, the slurry concentration can be reduced. This way, the packing process is simplified. The packing adapter consists of a coupling unit and an additional glass body.



► The packing adapter is assembled as follows:

1. Remove the upper end piece ►Chapter 6.1, p. 20.
2. Remove the snap ring (6) of the column from the glass body (11).
3. Assemble the blue column coupler snap ring (13) at the open column.
 - ✓ The chamfer of the column coupler snap ring shows in direction of the open end of the column.
4. Put the counter screw (8) over the glass body (14).
 - ✓ The thread of the counter screw shows in direction of the open end of the glass body.
5. Assemble the spring washer (7) at the glass body (14).
6. Assemble the blue column coupler snap ring (13) on the glass body.
 - ✓ The chamfer of the column coupler snap ring shows in direction of the open end of the column.
7. Connect the coupling unit (12) with the counter screw (8) of the glass body and the counter screw of the column. Take care that the O-rings in the coupling unit are in the right position.
8. Assemble the end piece on the open end of the packing adapter. ►Chapter 6.1, p. 20.

6.8 Glass column unpacking

WARNING

Glass column under high pressure

Eye or skin injuries possible due to remaining pressure in the tubing or in the glass column.

- ▶ Stop the pump during working on the glass column.
 - ▶ While working on the glass column, wear the prescribed personal protective equipment.
-

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personal protective equipment.
-

- ▶ The glass column is unpacked under flow as follows:

1. Remove the lower end piece ▶Chapter 6.1, p. 20.
2. Put a collection vessel below the column body.
3. Connect the pump to the tubing from the end piece.
4. Start the pump.
 - ✓ Because of the flow, the packing material is pressed out of the column body into the collection vessel.
5. Clean the glass column ▶Chapter 7.3, p. 34.

7 Cleaning and maintenance

WARNING

Incorrect maintenance

Severe injuries when used after incorrect maintenance are possible, if the column or the column parts do not withstand the operating pressure.

- ▶ Only authorised and qualified personnel should carry out the maintenance.
-

WARNING

Incorrect replacement of spare parts

Severe injuries due to breakage possible if unauthorised spare parts are used.

- ▶ Use only the manufacturer authorised spare parts.
-

WARNING

Faulty maintenance of the pilot column

Severe injuries due to deterioration possible.

- ▶ Meet maintenance intervals.
 - ▶ Perform the maintenance as described.
-



Consumable parts lists are included in the appendix.

7.1 Notes for Cleaning-In-Place (CIP)

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personal protective equipment.
-

ATTENTION

Cleaning with inappropriate solvents

Material damage possible due to corrosion, leaching or deformation.

- ▶ Check the chemical resistance of the wetted parts for the cleaning method before the CIP procedure.
-

With the cleaning-in-place procedure, a packed glass column can be cleaned without unpacking or dismantling. Depending on the contamination, three different procedures can be used.

1. Regeneration,
2. Sterilisation,
3. Depyrogenation.

Regeneration

Regeneration removes inorganic and organic contamination, which binds to the chromatography matrix and substantially affects the capacity and resolution of the column. Commonly these are: lipids, pyrogens, protein aggregates, pigments, polyphenols or metal complexes.

Sterilisation

Sterilisation is the removal and / or destruction of microorganisms and spores that will contaminate the purified product. The most common method is the treatment with sodium hydroxide, acetic acid or ethanol solution.

Depyrogenation

Depyrogenation includes the removal of endotoxins, which can bind to the chromatographic material or the column hardware (frits, tubing, etc.) and which can contaminate the purified product.

Cleaning methods for CIP

Treatment	Type of contamination	Sterilisation	Depyrogenation
Pepsin, pH 1.5 - 2	Proteolysis of adsorbed proteins	No	No
Non-ionic detergents (Triton X-100, Tween 80)	Removal of hydrophobic proteins and lipids	No	No
Cationic detergents pH 9 - 11	Removal of hydrophobic proteins and lipids	No	Partly
Urea, 6 - 8 M	Removal of protein aggregates	No	Unknown
1 - 100 mM EDTA, pH depending on stationary phase	Removal of metal complexes	No	No
0.1 - 1 M NaOH	Removal of bounded hydrophobic proteins, lipopolysaccharides	Good	Good
0.5 - 1 M acetic acid in 60% ethanol	Removal of lipids, pigments, lipopolysaccharides, and other lipophilic substances	Very good	Good

7.2 Cleaning and maintenance plan

The specified maintenance plan here is to be regarded as a general recommendation. Depending on the frequency of use and specific application, the appropriate maintenance plan and the intervals must be defined locally.

Interval	Maintenance
After every operation	<ul style="list-style-type: none"> Clean the glass column.
Daily	<ul style="list-style-type: none"> Check the column bed, adjust the piston if necessary. Check all tubing and connections for leakage. Fix loosened cylinder screws again or replace tubing and connectors.
Every 3 months	<ul style="list-style-type: none"> Check the pistons and the O-rings. If damaged, replace it.
Every 6 months	<ul style="list-style-type: none"> Check the frit position and constitution. If damaged, replace it.

7.3 Cleaning

WARNING

Glass column under high pressure

Eye or skin injuries possible due to the remaining pressure in the tubing or in the glass column.

- ▶ Stop the pump while working on the glass column.
 - ▶ When working on the glass column, wear the prescribed personal protective equipment.
-

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personal protective equipment.
-

Devices required:

- Wetted and lint-free rags
- Solvent for column body and end piece cleaning ▶ Chapter 3.3, p. 11.

Authorised cleaning agents for cleaning the outer wall of the column body:

- Commercially available dishwashing cleaners
 - Aliphatic hydrocarbons
 - Aliphatic alcohols
 - Aromatic hydrocarbons
 - Aldehydes
 - Diluted and weak acids
 - Bases
- ▶ The glass column is cleaned as follows:
1. Remove the end piece ▶ Chapter 6.1, p. 20.
 2. Clean the end pieces with an appropriate solvent and / or flush the fixed end piece with 1 M sodium hydroxide.
 3. Clean the glass body with an authorised cleaning agent and a wetted, lint-free rag.
 4. Let the column body and the end pieces dry.

7.4 Replacement of spare parts

7.4.1 Frit replacement

ATTENTION

Faulty assembly

Damage to the pistons possible due to faulty assembly.

- ▶ Make sure that the frit fits into the opening of the inner spindle exactly. Otherwise, the pistons or the frits may be damaged.

ATTENTION

Frit replacement

Damage or structural changes of the frits possible due to replacement.

- ▶ Frits which were pushed out with the frit removal tool should be replaced by new ones.
- ▶ Even if the frits seem to be intact on first view, there can be structural changes or microfissures in the frit. This can result in damage or blockages in the process.
- ▶ As the frits are pushed in, an optimum frit fixation can be guaranteed if new frits are used only.



When frits are pushed out with the frit removal tool, they are destroyed in most cases. Therefore, always use new frits after removal. When pushing the frit in, some material can be detached from the frit. Only this way, an optimum frit fixation is guaranteed.



- ▶ The frits in the end pieces are replaced as follows:
 1. Remove the end piece ▶ Chapter 6.1, p. 20.
 2. Remove the old frit from the inner spindle using the frit removal tool supplied. For this, push the long wire through the bore in the inner spindle and push the frits with force out of the inner spindle.
 - ✓ When pushing the frit out, a strong resistance will be observed.
 3. Place the new frit on a smooth, clean surface with the wide side facing down. The chamfer on the frits faces to the piston.
 4. Place the piston with the opening over the frit and press vertically down, with care, until the frit is fully located in the piston.
 - ✓ When pushing the frit in, a strong resistance will be observed.

7.4.2 O-ring replacement

ATTENTION

Screw nut loosened completely

Damage to the inner spindle possible when the screw nut is loosened completely.

- ▶ Make sure that the inner spindle cannot slip free from the outer spindle when the screw nut is loosened completely.

- ▶ The O-ring is replaced as follows:

1. Remove the end piece ▶Chapter 6.1, p. 20.
2. Remove the screw nut from the inner spindle completely.
 - ✓ The inner spindle is not fixed anymore and free.
3. Remove the inner spindle from the outer spindle.
4. Remove the O-ring from the inner spindle.
5. Place the new O-ring over the inner spindle.
6. Put the inner spindle into the outer spindle.
7. Fix the inner spindle again by screwing on the screw nut.

7.4.3 Replacing the glass body

- ▶ The glass body is dismantled as follows:

1. Remove the end pieces ▶Chapter 6.1, p. 20.
2. Disassemble the end pieces ▶Chapter 6.4, p. 21.
3. Assemble the end pieces on the new glass body.

8 Troubleshooting

WARNING

Incorrect maintenance

Severe injuries during operation after incorrect maintenance possible if the column or column parts do not withstand the operating pressure.

- ▶ Only authorised and qualified personnel should carry out the maintenance.

WARNING

Incorrect replacement of spare parts

Severe injuries due to breakage possible, if authorised spare parts are not used.

- ▶ Use only the manufacturer authorised spare parts.

Fault description	Possible cause	Solution
Dead volume occurred	Exceeding the maximum allowable pressure of the column packing	▶ If dead volume occurs, proceed as follows: 1. Stop the pump. 2. Readjust the piston of the adjustable end piece.
	Stressed column packing	
Peak shape of the eluted substances deteriorates	Dead volume at the column inlet	See fault description "Dead volume occurred"
	Partially clogged frit	Frit replacement ▶ Chapter 7.4, p. 35.
	Separation performance of the stationary phase altered by impurities	Wash the stationary phase. Contact also the manufacturer of the packing material.
	Stationäre Phase mechanisch zerstört	Replace packing material
Air in the column	Degassing or evaporation of solvent during storage	New column conditioning. Possibly new packing material is necessary.
Exceptional pressure rise during operation	Valve circuit incorrect	Check valve positions
	Clogged frit	Frit replacement ▶ Chapter 7.4, p. 35.
	Over-tightened fixing screws	Replace fixing screw and clamping tubing, cut the end of the tubing.
Pressure drop during operation	Leakage from tubing or fitting between pump and column	Check tubing and connections
	Empty solvent reservoir	Refill solvents

Fault description	Possible cause	Solution
Solvent drips from the column	Piston leaks	Tighten the set screw again. Remove the piston and check the O-ring.
	Tubing connection leaks	Tighten the fixing screws. Check the tightness of tubing connection.
Piston adjustment is not possible	O-ring is not tightened	Check if the O-ring is tightened. Tighten the O-ring.
Tightening of the O-ring not possible	O-ring too old	Replace the O-ring
	O-ring not in the right position	Remove the O-ring and move it by hand. Assemble the O-ring again.



If faults occur which are not listed here, please contact YMC for support.

9 Storage, packaging and transport

WARNING

Solvents in the glass column

Dangers possible due to liquids leaking from the column during transportation.

- ▶ Unpack and clean the column before transport.
-

9.1 Storage



For storage of packed columns, the chemical resistance of the wetted parts and of the packed stationary phase (please contact the manufacturer) should be considered.

The glass column should be stored as follows:

- unpacked
- cleaned
- dry
- empty

9.2 Transport

We recommend the use of the original packaging for transportation. If you are no longer in possession of the box, please pack the column in a similar way.

- ▶ The column is packaged as follows:
 1. Unpack the glass column ▶Chapter 6.8, p. 30.
 2. Clean the glass column ▶Chapter 7.3, p. 34.
 3. Insert the end pieces.
 4. Put the glass column in a suitable packaging.
 5. Make sure that there is a minimum distance of 50 mm between the column and the packaging.
 6. Protect the glass column with bubble wrap or similar packaging materials.
 7. Attach appropriate warning signals to the box (e. g. "Caution breakable glass").

10 Disposal

For the disposal of the glass column it has to be disassembled and disposed according to the national and other applicable environmental regulations.

11 Appendix

11.1 Spare parts glass body

Pos.	ID [mm]	Max. bed length [mm]	Part No.	Description	Material
1	10	120	HT10/120/K	Glass body	Borosilicate glass 3.3
2	10	200	HT10/200/K	Glass body	Borosilicate glass 3.3
3	10	300	HT10/300/K	Glass body	Borosilicate glass 3.3
4	10	450	HT10/450/K	Glass body	Borosilicate glass 3.3
5	10	750	HT10/750/K	Glass body	Borosilicate glass 3.3
6	10	1000	HT10/999/K	Glass body	Borosilicate glass 3.3
8	15	120	HT15/120/K	Glass body	Borosilicate glass 3.3
9	15	200	HT15/200/K	Glass body	Borosilicate glass 3.3
10	15	300	HT15/300/K	Glass body	Borosilicate glass 3.3
11	15	450	HT15/450/K	Glass body	Borosilicate glass 3.3
12	15	750	HT15/750/K	Glass body	Borosilicate glass 3.3
13	15	1000	HT15/999/K	Glass body	Borosilicate glass 3.3
14	25	120	HT25/120/K	Glass body	Borosilicate glass 3.3
15	25	200	HT25/200/K	Glass body	Borosilicate glass 3.3
16	25	300	HT25/300/K	Glass body	Borosilicate glass 3.3
17	25	450	HT25/450/K	Glass body	Borosilicate glass 3.3
18	25	750	HT25/750/K	Glass body	Borosilicate glass 3.3
19	25	1000	HT25/999/K	Glass body	Borosilicate glass 3.3
20	50	120	HT50/120/K	Glass body	Borosilicate glass 3.3
21	50	200	HT50/200/K	Glass body	Borosilicate glass 3.3
22	50	300	HT50/300/K	Glass body	Borosilicate glass 3.3
23	50	450	HT50/450/K	Glass body	Borosilicate glass 3.3
24	50	750	HT50/750/K	Glass body	Borosilicate glass 3.3
25	50	1000	HT50/999/K	Glass body	Borosilicate glass 3.3

11.2 Spare parts end pieces

Pos.	ID [mm]	Part No.	Description	Material
1	10	ECO1011K/P2	O-rings Pack of 2	FFKM
2	15	ECO1511K/P2	O-rings Pack of 2	FFKM
3	25	ECO2511K/P2	O-rings Pack of 2	FFKM
4	50	ECO5011K/P2	O-rings Pack of 2	FFKM
5	10	ECO10G10/P2	Frits, 10-16 µm Pack of 2	Glass
6	15	ECO15G10/P2	Frits, 10-16 µm Pack of 2	Glass
7	25	ECO25G10/P2	Frits, 10-16 µm Pack of 2	Glass
8	50	ECO50G10/P2	Frits, 10-16 µm Pack of 2	Glass

11.3 Spare parts coupling unit

Pos.	ID [mm]	Part No.	Description	Material
1	10	ECO10KU11K/P2	O-rings Pack of 2	FFKM
2	15	ECO15KU11K/P2-2	O-rings Pack of 2	FFKM
3	25	ECO25KU11K/P2-2	O-rings Pack of 2	FFKM
4	50	ECO50KU11K/P2-2	O-rings Pack of 2	FFKM

11.4 Index

C

CIP	32
Cleaning	32
Cleaning-In-Place	32

D

Delivery contents	14
Disposal	40

F

Frit replacement	35
------------------	----

G

Glass body replacement	36
------------------------	----

P

Packaging	39
Packing	22
Packing adapter	29
Personal protective equipment	8
Pressure test	18
Protective equipment	8

R

Resistance, chemical	11
----------------------	----

S

Signal word	5
Signal word field	5
Slurry	23
Slurry preparation	23
Slurry method sedimentation	26
Slurry method under flow	24
Storage	39

T

Transportation	39
----------------	----

U

Unpacking	30
-----------	----

W

Warnings	5
Warning notices	5