Bio-Scale™ MT Columns

Instruction Manual

Catalog numbers
751-0081
751-0083
751-0085
751-0087

BIO-RAD

Table of Contents

Section 1
Characteristics of the Bio-Scale MT2, MT5, MT10, and MT20 Columns

1.1 Introduction

The Bio-Scale MT columns have been designed specifically to provide high resolution chromatography of bio-molecules when packed with any suitable chromatography media. Typical packings might include ion exchange, hydrophobic interaction, hydroxyapatite, or affinity chromatography media.

The Bio-Scale MT range consists of four sizes (2, 5, 10, and 20 ml), allowing easy scale-up of separation and purification protocols. The column design has been optimized to provide easy packing, equilibration, bed-height adjustment, and sample application. All column parts are bio-compatible for preservation of sample biological activity.

The Bio-Scale MT column range is designed for use with Bio-Rad's BioLogic System, as the column fittings are 1/4-28 Super-flangeless. Adaptors and unions are available from Bio-Rad for connection of these columns to other medium or high pressure systems.

1.2 Column Description

Each column consists of a borosilicate glass tube held in place by two endcaps and a plastic safety shield. The lower bed support consists of a fixed-position frit and a distribution screen assembly.

Bed height adjustment is provided by the upper bed support, which is positioned and held in place using a threaded lock-nut. A removable frit protects the top of the bed and supports a thin distribution screen which insures efficient sample application to the bed itself.

Each column can be packed with chromatography media using either dry or slurry packing methods. A minimum particle size of 5 microns is recommended.

1.3 Column Specifications

All wetted parts are bio-compatible (Tefzel, polypropylene, polyphenyl sulfide, borosilicate glass and buna-N rubber) and resistant to cleaning and sanitation solvents including 1.0 M NaOH and 25% acetic acid. The column can be used with most common aqueous buffers and certain organic solvents including alcohols and acetonitrile. Do not use halogenated hydrocarbons, aromatic solvents, or tetrahydrofuran (THF). A detailed solvent compatibility table is given in Section 4.

Table 1. Column Characteristics.

	MT2	MT5	MT10	MT20
Column Volume, ml	1.9 – 2.3	4.6 – 5.7	9.5 – 11.3	19.4 – 21.9
Column Dimension, mm	7 x 50 – 60	10 x 59 – 72	12 x 84 – 100	15 x 110 – 124
Maximum Operating Pressure, psi	1, 000	750	600	500

1145 psi = 1 MPa. 14.5 psi = 1 bar. Packing material may not withstand high pressures. See packing material manufacturer's specifications.

Section 2
Use of Bio-Scale MT Columns

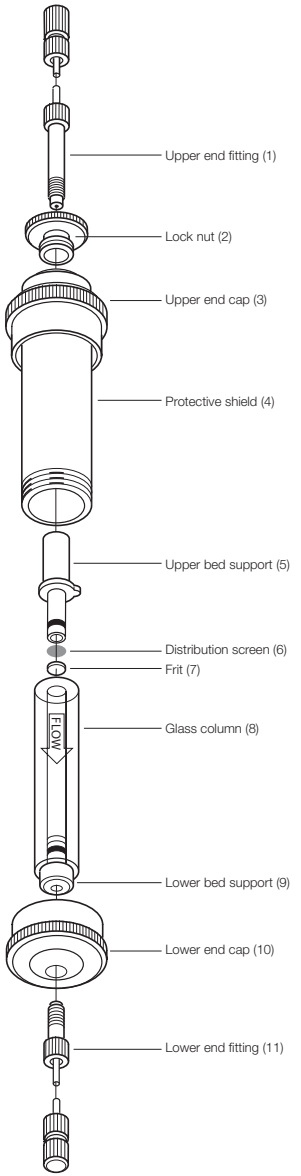

Fig. 1. Diagram of Bio-Scale MT Column.

2.1 Column Disassembly (part given are in parenthesis)

1. Unscrew and remove the upper (1) and lower (11) end fittings.
2. Unscrew the bottom end cap (10) from the protective shield (4).
3. Withdraw the borosilicate glass column assembly (8) from the protective shield.
4. Remove the upper (5) and lower (9) bed supports.
5. If the column is packed, remove the upper and lower distribution screens (7) and frits (6) using the frit removal tool. Do not reuse these parts. Rinse the packing material away with deionized water.
6. Wash all column parts with deionized water to remove dust and particulates.

2.2 Column Assembly

1. Insert a frit (7) and then a distribution screen (6) into the bottom of the glass tube (8) (note the orientation of the flow direction label).
2. Wet the o-ring of the lower bed support (9) with water and insert it fully into the bottom of the glass tube.
3. Insure that the frit and distribution screen are touching the lower bed support.
4. Pack the column with the media of choice until a stable bed is obtained (see Section 2.3).
5. Insert a frit and then a distribution screen into the glass tube, followed by the upper bed support (5). Be careful not to force the frit into the top of the bed.
6. Thread the lock nut (2) into the upper end cap (3). Thread the protective shield (4) onto the upper end cap.
7. Holding the upper end cap assembly upside down, insert the glass tube and gently rotate it until the upper bed support locks in place.
8. Attach the lower end cap to the protective shield.

9. Gently turn the lock nut until the upper bed support is in the desired position.
10. Attach upper (1) and lower (11) end fittings.

2.3 Column Packing

Always follow the manufacturer's instructions for packing a particular type of media. Generally, slurry packing will give better chromatographic performance than dry packing. The following guidelines may be useful:

Dry packing	Particle size Ž 20 µm
Slurry packing	Particle size < 20 µm

Dry Packing

Assemble the column according to the following figure.

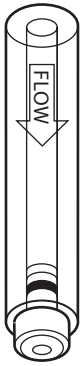

Fig. 2 Column tube assembly.

1. Holding the column tube vertically, fill one half with the packing media.
2. Tap the side of the column until the media settles.
3. Fill the column with the remainder of the media and repeat step 2.
4. When the column is filled to the desired level, complete the column assembly as described in Section 2.2, making sure the flow direction arrow on the glass tube is pointing in the current direction.

5. Attach the column to a pump and start flow at 50% of the desired operating flow rate.
6. When all the air is expelled from the column, increase the flow rate to 20% higher than the desired operating flow rate for at least 5 column volumes.
7. Stop the flow and lower the upper bed support via the lock nut to remove any head space that might have formed between the frit and the top of the bed.
8. Equilibrate the column at the desired flow rate prior to sample application.

Slurry Packing

Slurry Preparation

Dry media should be swollen according to the manufacturer's instructions.

Always degas the buffers used for column packing. Adjust the packing slurry to a 50% (V/V) volume using packing buffer.

Column Packing

1. Assemble the column according to the diagram in Figure 2 and seal the bottom outlet with Parafilm® laboratory film.
2. Fill about 25% of the column with buffer and add the packing slurry to the desired level.
3. Uncap the bottom outlet and allow the slurry to settle. Remove the film from the outlet, leaving some liquid above the settled slurry.
4. Add more slurry to the desired level.
5. Without inserting the upper frit and distribution screen, assemble the column and connect to a pump. Begin the flow at 50% of the desired operating flow rate for 3 column volumes.
6. Stop the pump, remove the upper bed support, and repeat the above steps until a stable bed at the desired level is obtained.
7. Add 5 mm of buffer to the top of the bed and insert the upper frit and distribution screen. Assemble the column.

8. Pass 5 bed volumes of buffer through the column at a flow rate 20% higher than the operating flow rate.
9. If necessary, stop the flow and lower the upper bed support via the lock nut to remove any head space that might have formed between the frit and the top of the bed.
10. Equilibrate the column at the desired flow rate.

2.4 Connections to Chromatography Systems

Bio-Scale MT columns can be used with any chromatography system that can provide the appropriate flow rate and pressure. Each column is supplied with 1/4-28 fittings for connection to Bio-Rad's BioLogic System, M6 fittings for connection to FPLC® systems, and 10-32 fittings for connection to HPLC systems. Inlet and outlet tubing must be 1/16" OD. Inner diameters of 0.020" or 0.030" in Tefzel®, PTFE or PEEK material are recommended.

Section 3
Care of the Bio-Scale MT Columns

3.1 Frit Removal

The top frit may need to be replaced after extensive column use or if increasing backpressures are noticed. Each column is supplied with a frit removal tool, polyethylene frit, and distribution screen. The screen assists in distributing the sample over the entire column surface and also acts as a pre-filter. The screen should be replaced every time the frit is changed, refer to Figure 1.

1. Remove the upper and lower end fittings from the column
2. Remove the upper lock nut from the top end cap.
3. Unscrew the lower end cap from the protective shield and slide out the glass column.
4. Remove the upper bed support from the glass column.
5. Using the frit removal tool, remove the distribution screen and frit by pressing the hook into the frit in a sideways motion with slight downward pressure.
6. Add 5 mm of buffer to the top of the resin bed. Place a new frit and screen into the tube. Insert the upper bed support and use it to carefully push down the frit and screen until it just touches the top of the resin bed.
7. Holding the upper end cap assembly upside down, insert the glass tube and gently rotate it until the upper bed support locks in place.
8. Attach the lower end cap to the protective shield.
9. Gently turn the lock nut until the upper bed support is in the desired position.
10. Equilibrate the column at the desired flow rate.

3.2 Column Bed Top-Off

If the top of the bed becomes fouled and column performance is not restored by a cleaning procedure and frit change, then a few millimeters of the bed should be removed and replaced with fresh media.

Section 4
Solvent Compatibility Table

Table 2. Solvent Compatibility.

Solvents
Acetic acid (50%)
Acetonitrile
Butanol
Citric acid
Dimethyl formamide (DMF)
Dimethylsulfoxide (DMSO)
Ethanol
Elthylenediamine tetraacetic acid (EDTA)
Formaldehyde (40%)
Formic acid
Glycerol
Goods buffers
Guanadine-HCl (6 M)
Hydrocloric acid (2 M)
Isopropanol
Lactic acid (85%)
Methanol
SDS
Sodium acetate (sat.)
Sodium hydroxide (1 M)
Sodium thiocyanate (3 M)
Sulfuric acid (dilute)
Trifluoroacetic acid (0.1%)
Triton® X-100 detergent
Urea (7 M)

Do not use halogenated hydrocarbons, aromatic solvents, or ethers.

Section 5
Product Information

Catalog Number	Product Description	Bed Dimension, mm	Bed Volume, ml	Pressure Limit, psi
751-0081	**Bio-Scale MT2**	7 x 52	2	1.000
751-0083	**Bio-Scale MT5**	10 x 64	5	750
751-0085	**Bio-Scale MT10**	12 x 88	10	600
751-0087	**Bio-Scale MT20**	15 x 113	20	500

All columns come with 5 extra frits, 5 distribution screens, frit removal tool, 2 o-rings, 2 1/4 x 28 fittings, 2 M6 fittings, and 2 10-32 fittings.

Catalog Number	Product Description
751-0091	**Bio-Scale 2 Replacement Parts Kit**, includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit removal tool
751-0093	**Bio-Scale 5 Replacement Parts Kit**, includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit removal tool
751-0095	**Bio-Scale 10 Replacement Parts Kit**, includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit removal tool
751-0097	**Bio-Scale 20 Replacement Parts Kit**, includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit removal tool
751-0099	**Bio-Scale Fittings kit**, include 2 Super Flangeless Nuts (1/4 x 28 threads) and 6 ferrules, 2 Flangeless M6 nuts, 4 ferrules and 2 caps, 2 Fingertight II fittings (10-32 threads)

BIO-RAD

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