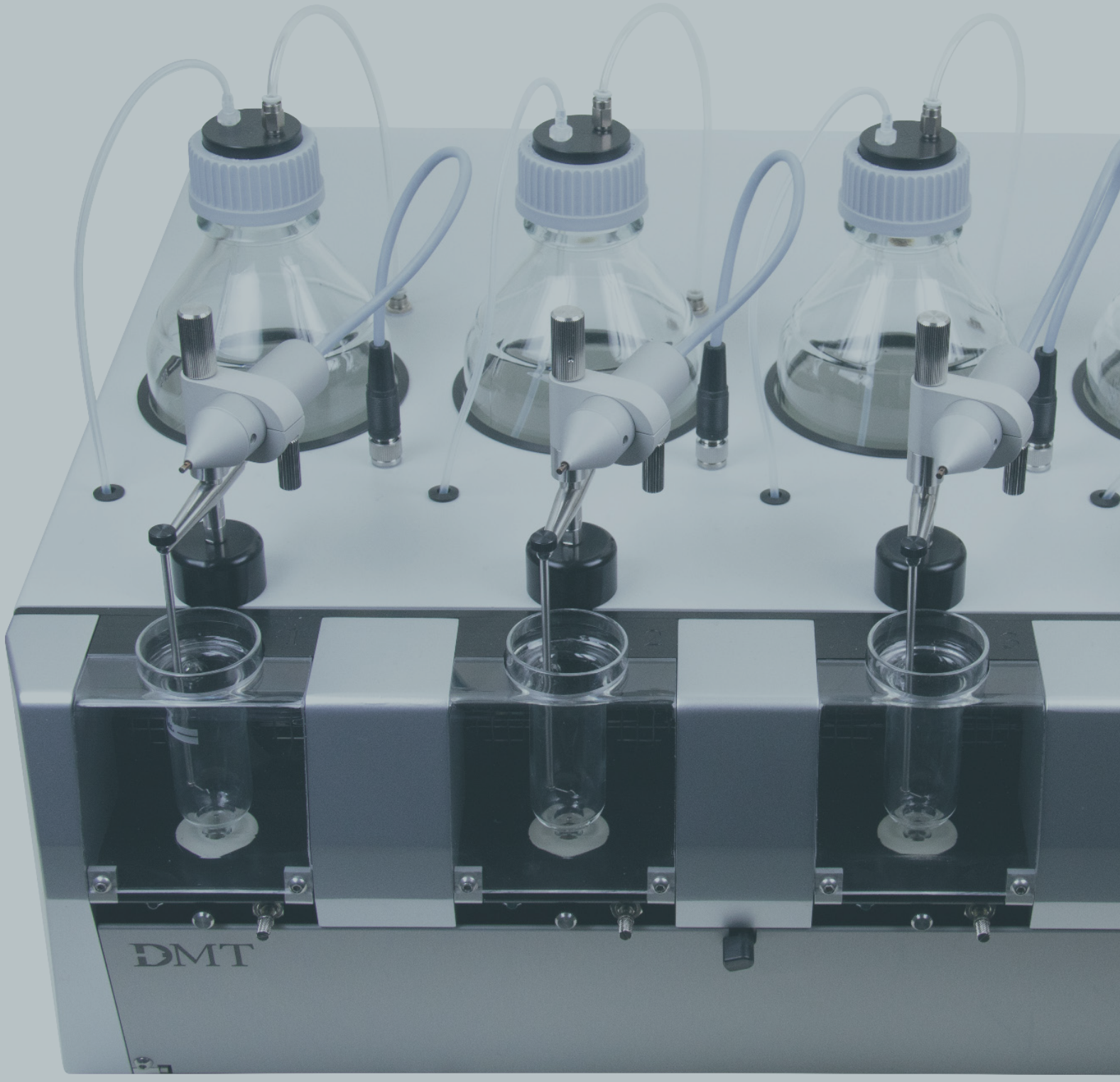
USER GUIDE, VOL.2.0

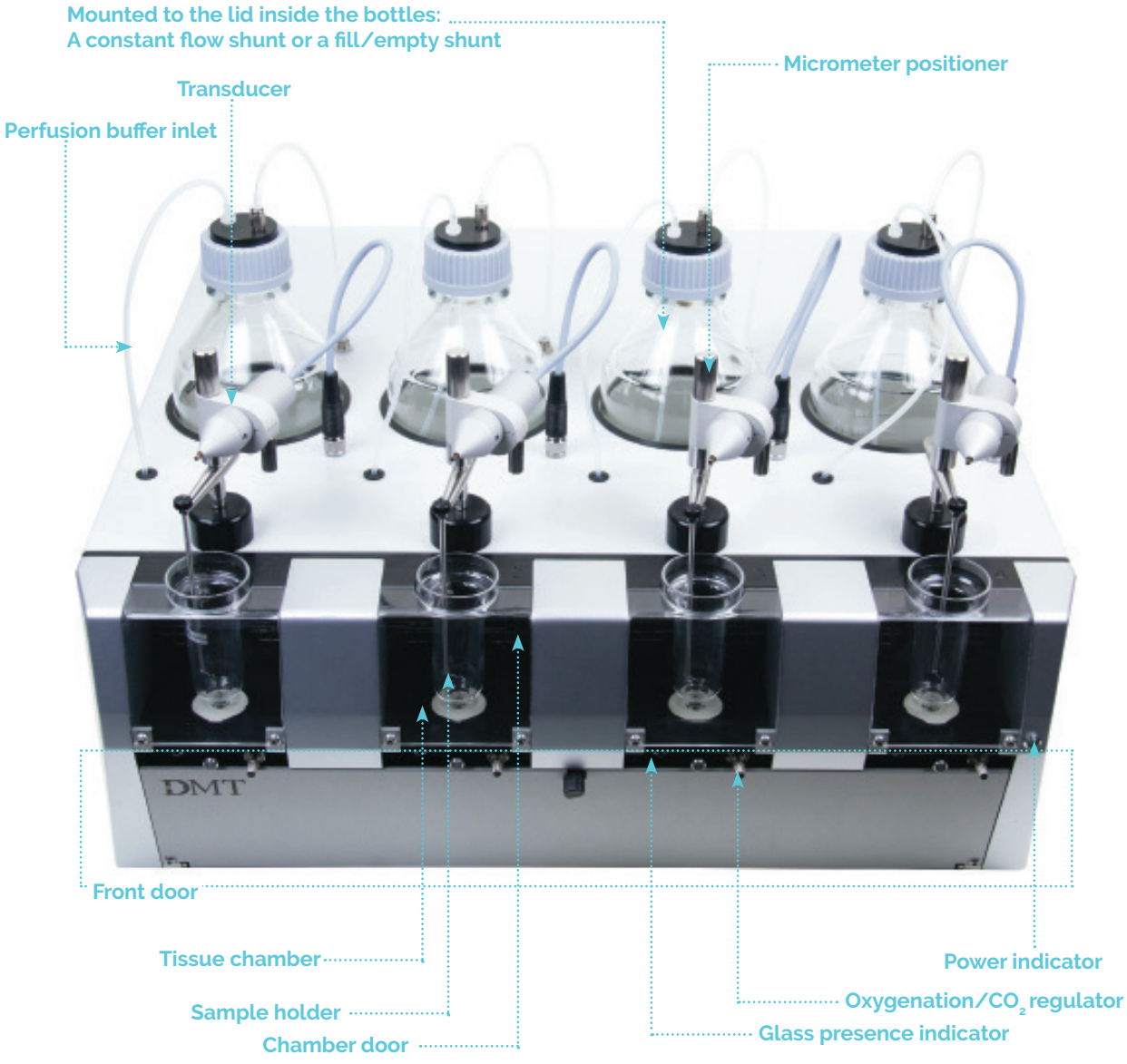
TISSUE ORGAN BATH SYSTEM - 750TOBS


DMT

CONTENTS

CHAPTER 1 - SYSTEM OVERVIEW

1.1 TISSUE ORGAN BATH SYSTEM - 750 TOBS: FRONT PANEL



1.2 TISSUE ORGAN BATH SYSTEM - 750 TOBS: REAR PANEL

1.3 TISSUE ORGAN BATH SYSTEM - 750 TOBS: CHAMBER

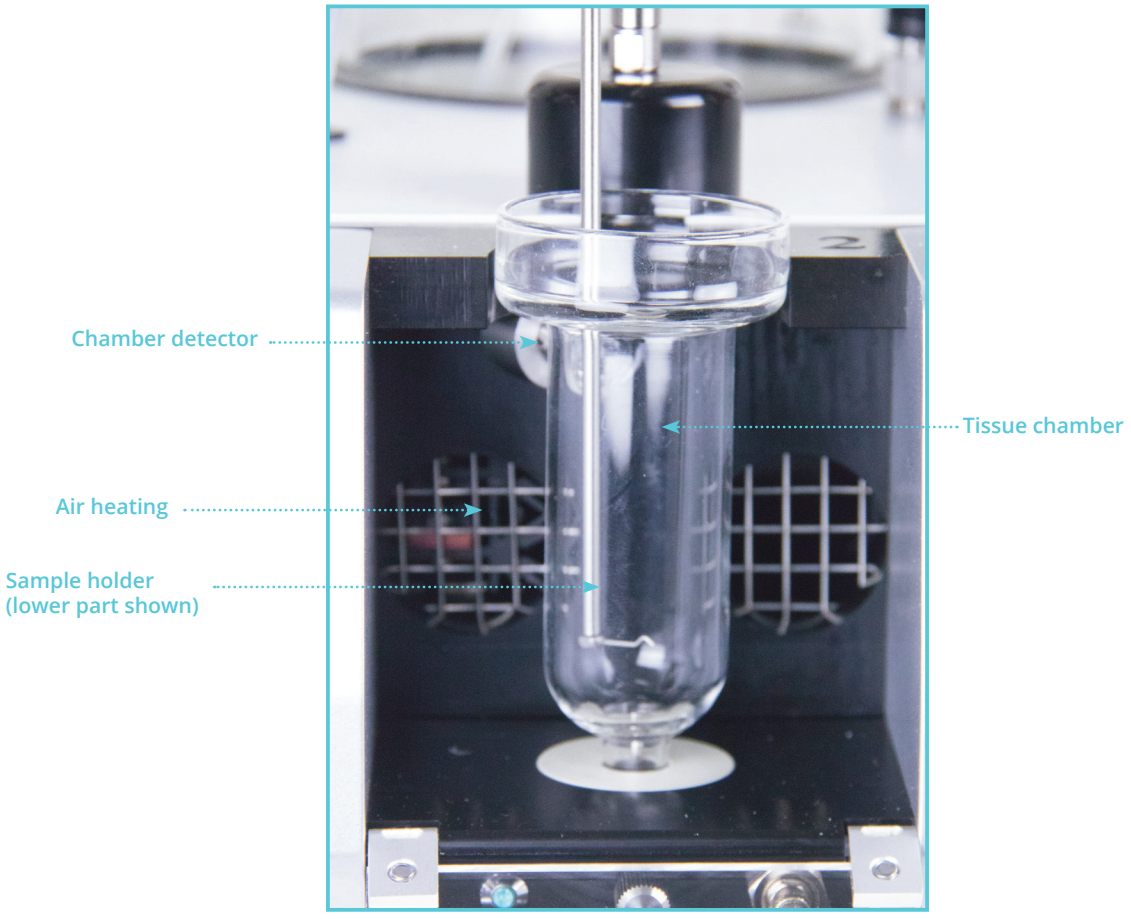
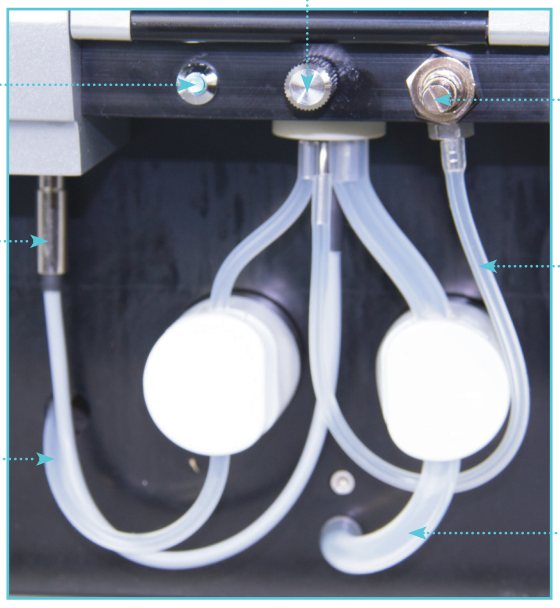


Figure A: 20ml Tissue chamber

NOTE: The Chamber Detector registers when the tissue chamber is placed. If the chamber is placed correctly the blue indicator lamp will be lit and the tissue organ bath will work.

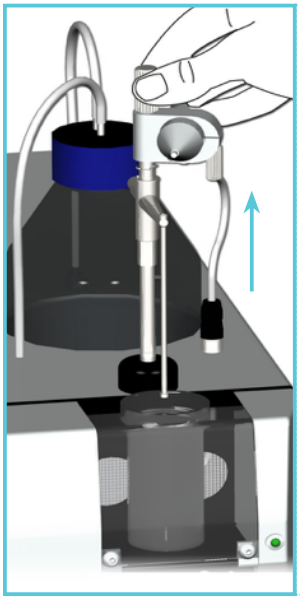


NOTE: Regarding the oxygenation/CO_2 regulator: the regulators need to be greased AT LEAST twice a year. Also, make sure that the regulators are turned at regular intervals to prevent them from getting stuck. Use ONLY the grease that came with the system.

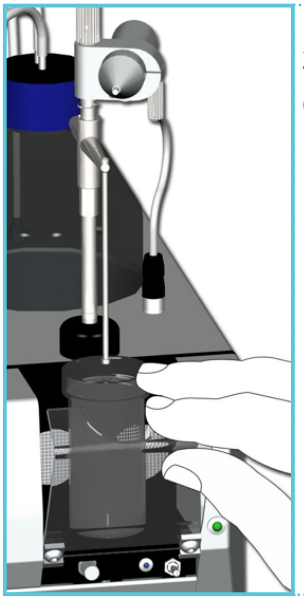
CHAPTER 2: SETTING UP THE TISSUE ORGAN BATH

2.1 TISSUE CHAMBER HANDLING PROCEDURE

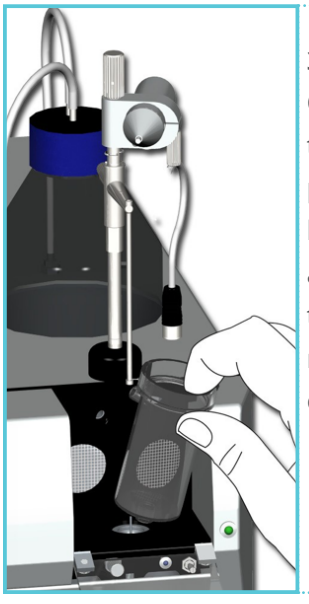
The following is a description of how to handle the tissue chambers and the different kinds of sample holders available.



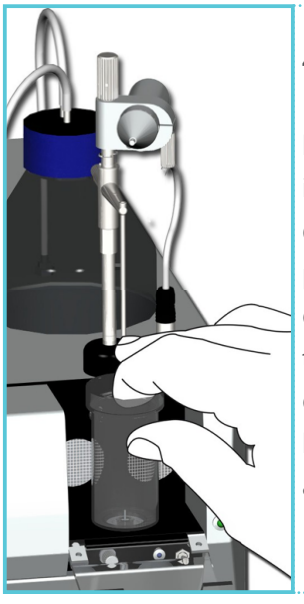
1: Move the transducer and mounting supports/ holders upwards. Ensure the sample holder is free of the chamber.



2: Open the chamber door.



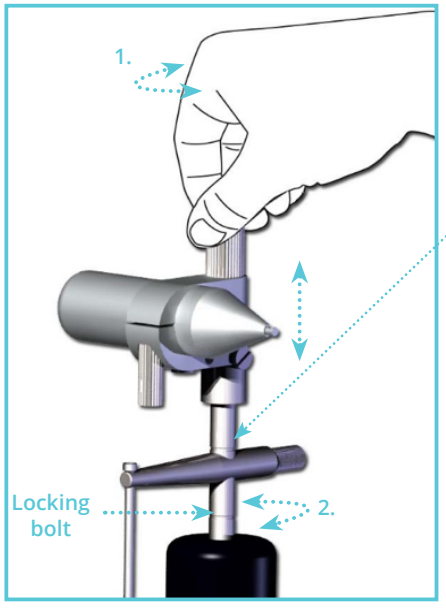
3. Removing the glass: Carefully pull the top of the chamber outwards pressing down on the bottom of the chamber a few millimeters until the chamber is free to move upwards. Then lift out the chamber.



4. Placing the glass: Place the pipe stub at the bottom of the chamber inside the chamber disc whilst aligning the pipe stub to the stub opening. Gently place the pipe stub situated on the inside of the heating compartment and secure in.

2.2 ADJUSTING THE TRANSDUCER

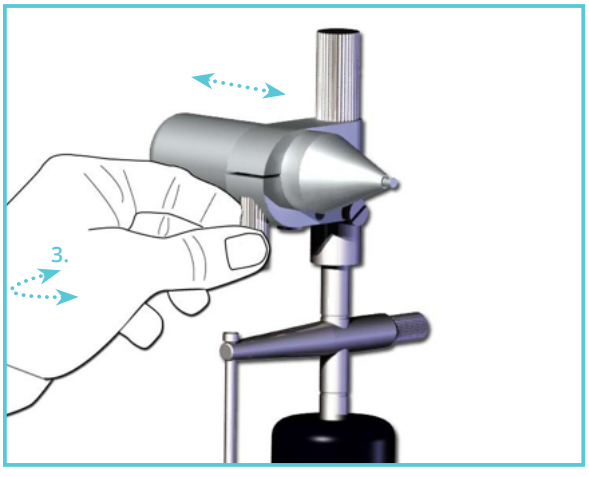
When tissue is placed in the sampleholder do a course adjustment on the tissue holder to fit the tissue just between the holder and the transducer without stretching it. With the tissue submerged in the buffer set the force to 0. Then use the micrometer positioner to stretch the tissue to the disired resting tension.



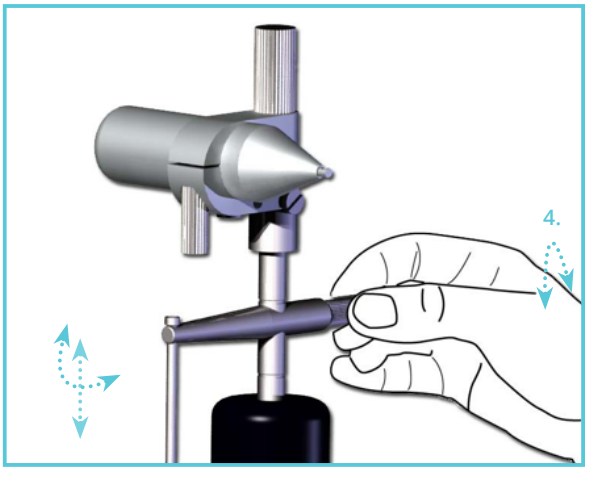
1. Vertical fine adjustment of the transducer:
Turn the micrometer screw clockwise to lower the transducer. Turn counter clockwise to lift the transducer. The marked line is the lowest position of the transducer. The micrometer positioner has a working range of 0 - 26 mm above the line.

NOTE: The micrometer positioner has a slag of 1 to 2 turns when shifting from clockwise to counterclockwise rotation and vice versa. It is easy to feel when the micrometer positioner starts to move the transducer.

2. Final alignment of the transducer:
The transducer can be finally aligned in parallel to the tissue holder and secured by rotating the locking bolt.



3. Horizontal adjustment of the transducer:
Turn the screw clockwise to free the transducer. The transducer can now be moved horizontal. Turn the screw counterclockwise to lock the transducer.



4. Course adjustment of the lower tissue holder:
Turn the screw counter clockwise to unlock the tissue holder. When unlocked, it is possible to move the holder in 4 orientations, up, down, left and right. Turn the screw clockwise to lock.

2.3 DIFFERENT TYPES OF TISSUE SAMPLE HOLDERS

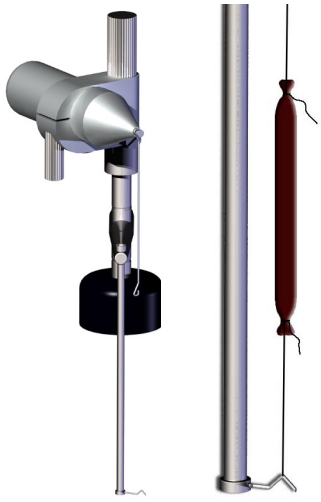


Figure A: Triangle hooks (standard)

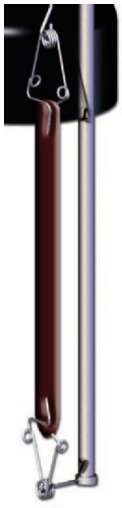


Figure B: Closing clips (optional)

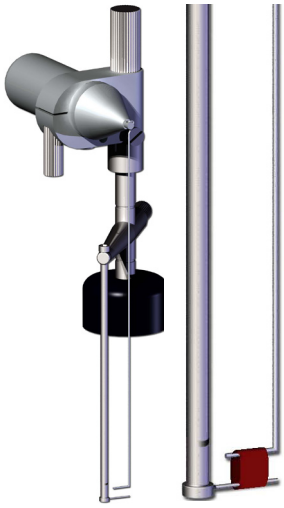


Figure C: Pins (optional)

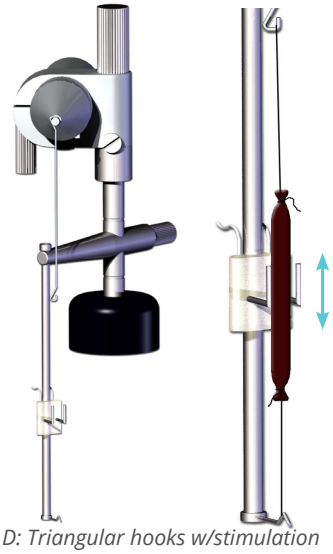


Figure D: Triangular hooks w/stimulation electrodes clips (optional)

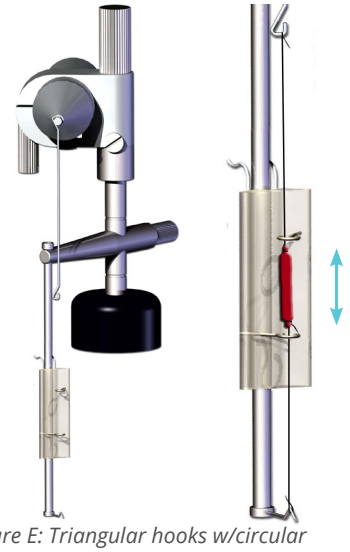


Figure E: Triangular hooks w/circular stimulation electrodes (optional)

NOTE: The above mounting supports are only examples of supports delivered by DMT. If other mounting supports are needed ask your DMT sales representative.

2.4 ISOMETRIC TRANSDUCER:

Prior to shipment the 750 TOBS has gone through 2 days of continuous testing, including a final force transducer calibration. However, to ensure that the Tissue Organ Bath is working at its highest performance, DMT recommends that a new force transducer calibration is performed before the first use of the 750 TOBS system.

As part of the general maintenance of the 750 TOBS system, DMT also recommends that the bath is weight calibrated at least once every month, every time the system has been moved or has not been used over a long period.

The weight calibration procedure is described in detail in chapter 4 in the User Manual.

Figure 4.1.1: Transducer with calibration weight.

CHAPTER 3: CLEANING AND MAINTENANCE

3.1 CLEANING THE TISSUE ORGAN BATH SYSTEM

The 750TOBS is a very delicate and sophisticated piece of research equipment. In order to keep it working at its best, DMT recommend that the following sections are read carefully and that the instructions are followed at all times. DMT strongly recommends that the Tissue Organ Bath and surroundings should be cleaned after each experiment. After a "normal" experiment use the following procedure to clean the Tissue Organ Bath, chamber, tubing and tissue holders.

1. Turn pressure OFF
2. Use clean Schott Duran bottles
3. Fill with distilled water
4. Turn Pressure ON
5. Rinse the silicone tubing with double distilled water by activating the Fill & Empty Control several times.

NOTE: Only works when a tissue chamber is in place

6. Change the tubing after use of unknown or toxic chemicals, or if any kind of hydrophobic reagent has been used.

IMPORTANT:
It is VERY important that the tubing being used is the original tubing from DMT. The quality, diameter and length are VERY important in order to insure the correct draining and filling of the chamber.

7. Carefully dismount the tissue bath chamber and perform a normal glass wash.
8. If aggressive solution or drugs have been used it is possible to disconnect the temperature probe (see figure 3.1 below) and take out the chamber disc. Clean both with double distilled water, and dry with a paper towel.

IMPORTANT:
In exceptional cases it may be necessary to demount the sample holders for cleaning to make sure that all surfaces are cleaned.

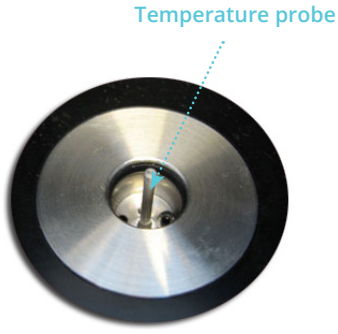


Figure 3.1
Chamber disc and temperature probe

APPENDIX 1 - BUFFER RECIPES

PHYSIOLOGICAL SALINE SOLUTION (PSS)

1x PSS (1000ml):

Solution 1:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
NaCl	58.44	118.99	6.954
KCl	74.56	4.69	0.350
$MgSO_4$ - ^7H_2O	246.48	1.17	0.289
KH2PO4	136.09	1.18	0.161

Solution 2:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
$CaCl_2$ - ^2H_2O	147.02	2.50	0.368

Solution 3:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
$NaHCO_3$	84.01	25.00	2.100
EDTA	372.24	0.03	0.010
Glucose	198.77	5.50	1.091

1. Dissolve the chemicals in 100 ml double distilled H_2O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.
2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H_2O to a final volume of 500 ml.
3. Solution 3 is added to the graduated bottle, which afterwards is filled with additional double distilled H_2O to a final volume of 850 ml.
4. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.
5. Solution 2 is added and the graduated bottle is filled with additional double distilled H_2O to reach the final volume of 1000 ml. Continue the carbogen bubbling until the pH of the buffer solution reaches 4.4.

25x Concentrated PSS (1000ml)

Solution 1:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
NaCl	58.44	118.99	173.850
KCl	74.56	4.69	8.750
$CaCl_2$ - ^2H_2O	147.02	2.50	9.200

Solution 2:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
$MgSO_4$ - ^7H_2O	246.48	1.17	7.225
KH2PO4	136.09	1.18	4.025

Solution 3:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
EDTA	372.24	0.03	0.250

1. Dissolve the chemicals for solution 1 in about 800 ml double distilled H_2O in a 1000 ml graduated bottle. Dissolve the chemicals for solutions 2 and 3 in 75 ml double distilled H_2O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.
2. Solution 2 and 3 is added to solution 1 and the graduated bottle is filled with additional double distilled H_2O to reach a final volume of 1000.0 ml.

Before use:

3. Dilute the 25 x PSS stock solution 1:25 using double distilled H_2O.
4. Add
   1.091 g/l Glucose
   2.100 g/l $NaHCO_3$
5. Aerate the solution with carbogen (95%O_2 + 5%CO_2) for at least 20 minutes. If necessary wait further for the pH of the buffer to reach pH 4.4.

A.4.1.3 KPSS

1 x KPSS (1000ml):

Solution 1

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
KCl	74.56	123.70	9.223
$MgSO_4$ - ^7H_2O	246.48	1.17	0.289
KH_2PO_4	136.09	1.18	0.161

Solution 2

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
$CaCl_2$ - ^2H_2O	147.02	2.50	0.368

Solution 3

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
$NaHCO_3$	84.01	25.00	2.100
EDTA	372.24	0.03	0.010
Glucose	198.77	5.50	1.091

1. Dissolve the chemicals in approximately 100 ml double distilled H_2O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.
2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H_2O to a final volume of 500 ml.
3. Solution 3 is added to the graduated bottle that is filled with additional double distilled H_2O to a final volume of about 850 ml.
4. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.
5. Solution 2 is added and the graduated bottle is filled with additional double distilled H_2O to reach the final volume of 1000.0 ml. Continue the carbogen bubbling until the pH of the buffer solution reaches 7.4.

25 x KPSS (1000ml)

Solution 1:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
KCl	74.56	123.70	230.575
$CaCl_2$ - ^2H_2O	147.02	2.50	9.200

Solution 2:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
$MgSO_4$ - ^7H_2O	246.48	1.17	7.225
KH_2PO_4	136.09	1.18	4.025

Solution 3:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
EDTA	372.24	0.03	0.250

1. Dissolve the chemicals for solution 1 in about 800ml double distilled H_2O in a 1000 ml graduated. Dissolve the chemicals for solutions 2 and
2. 3 in 75 ml double distilled H_2O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.
3. Solutions 2 and 3 are added to solution 1 and the graduated bottle is filled with additional double distilled H_2O to reach a final volume of 1000 ml.

Before use:

4. Dilute the 25 x PSS stock solution 1:25 using double distilled H_2O.
5. Add
   1.091 g/l Glucose
   2.100 g/l $NaHCO_3$
6. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for at least 20 minutes. If necessary wait for the pH of the buffer to reach pH 7.4.

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