Mitocell: Instructions for Use

1 Introduction

The Mitocell (MT200) is designed for the measurement of the respiration rate of either 50 or 100µl sample volumes of mitochondria and cell suspensions. The larger Mitocell (MT200A) is designed for volumes of 0.3, 0.5 and 1.0ml sample volumes. The glass top with a central channel forms the water jacket. It is secured on to the grey base unit with a black plastic clamp ring. When the polycarbonate plunger is inserted into the central channel of the glass top and the electrode is inserted in the bottom of the base unit the small respiration chamber is formed. The plunger has a central capillary, through which substrates or inhibitors may be injected during a respirometry run. The grey base unit contains the built in magnetic stirrer which rotates the spinbar in the respiration chamber. (Diagram 1).

Both mitocells are supplied with stir bars, volume adjustment rod(s) (setting pieces) and hexagon key. The spinbars for the MT200 should be kept on the magnet in order to maintain their magnetic properties when not in use. The MT200 is supplied with a red volume adjustment rod, and is supplied pre-assembled with the volume set to 50 microlitres (allowing for a stir bar in the chamber). (Diagram 2). The MT200A is supplied with three volume adjustment rods. The red rod is for volume of 0.3ml, the white rod is for a volume of 0.5ml and the black rod is for volumes of 1.0ml. The MT200A is supplied pre-assembled with the volume set to 0.5ml. (Diagram 3)

Both Mitocells are designed to work with the standard Strathkelvin oxygen electrode (not supplied).

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Diagram 1

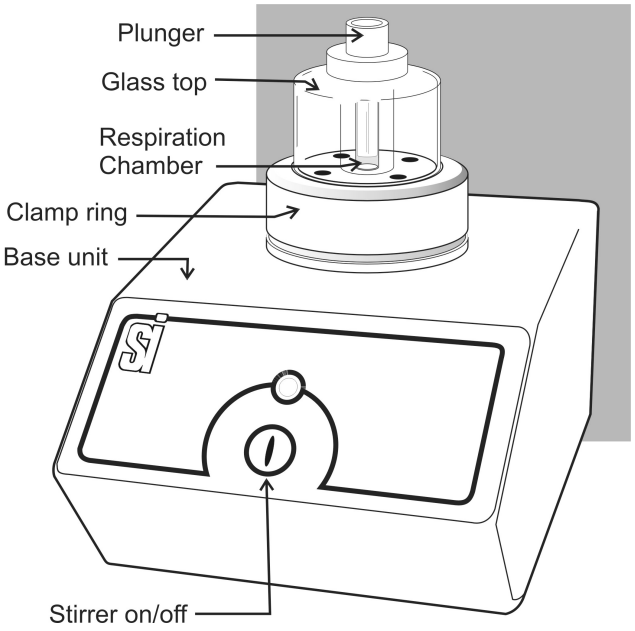


Diagram 2

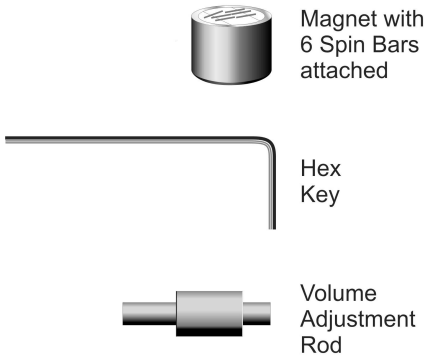
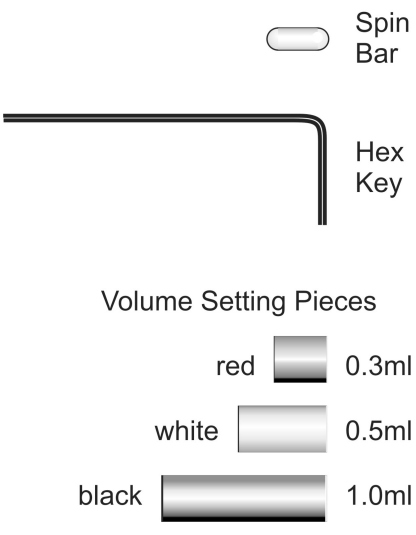


Diagram 3



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2 Assembly

Connect a pumped source of constant temperature water (regulated to ± 0.1°C) to the two tubes on the rear of the Mitocell. Allow the water to enter the water jacket slowly at first, to avoid the production of small air bubbles, which can impair visibility of the respiration chamber. Invert the Mitocell until all air bubbles have been flushed away. Unscrew the black cap of the electrode holder at the base of the unit. Ensure that the electrode and inside of the electrode holder are thoroughly dry. Insert the electrode and pass the cap over the electrode cable and screw it on tightly. The electrode tip now forms the base of the respiration chamber. Remove the plunger from the cell and drop a stir bar into it.

If you have a 782 or 929 oxygen meter, connect the link power cable between the '5Vdc out' socket on the rear of the 782 meter and the lower power socket on the back of the Mitocell. Switch the power unit on. The indicator on the Mitocell panel will illuminate. The integral stirrer can then be turned on and off by pressing the stirrer on/off membrane button, showing a stir bar symbol, just below the indicator light. When the stirrer is operating the light flashes.

Connect the oxygen electrode cable to the socket on the back of the 782 and 929 oxygen meter. You are now ready to calibrate the electrode.

3 Calibration of the electrode

The electrode should be recalibrated each day, and should be left connected to the meter, with the meter switched on, if in daily use.

Read the section in the 782 or 929 manual on electrode calibration.

High point calibration: Circulate water at the temperature of the experiment, through the water jacket. Remove the plunger and half fill the respiration chamber with aerated water. Switch on the stirrer. Calibrate the high point, then suck out the water and dry the chamber with a wick of absorbent tissue.

Zero calibration: Half fill the respiration chamber with sodium sulphite solution. It is not necessary to stir. Complete the zero calibration, then suck out the solution and wash out several times with distilled water to remove all traces of the sulphite solution. Finally dry the chamber with a wick of absorbent tissue.

4 Running an experiment

To run an experiment, pipette the required volume of the respiring suspension plus 10μl into the respiration chamber. (i.e for a volume of 50μl pipette 60μl). The suspension should already be at the same temperature as the Mitocell. (The extra volume allows liquid to partially fill the central channel of the plunger, which minimises the diffusion of oxygen from the air). Make sure no air bubbles are introduced with the sample during this procedure.

Insert the plunger into the respiration chamber, displacing the air and excess liquid into the capillary of the plunger. Turn on the stirrer and start recording the oxygen change.

During the course of an experiment, substrates or inhibitors can be injected directly into the chamber, using the Strathkelvin modified syringe supplied. (spares can be purchased if required). These syringes have a spacer which ensures that the needle does not touch and possibly damage the membrane of the electrode. During injection hold the plunger in place to avoid the introduction of air bubbles.

At the end of the run remove the plunger and spin bar. Return the stir bar to its magnet in order to keep it magnetised and ready for use (MT200 only). Between runs, the contents of the respiration chamber can be removed by suction, followed by rinsing with distilled water. Finally remove any residual moisture with a wick of absorbent tissue. Take care not to damage the electrode membrane during this process.

5 Changing the volume of the chamber

1. Drain the water from the Mitocell as in Section 6 below. Remove the electrode. Remove the plunger.

2. Unscrew the black plastic clamp ring to release the glass top.

3. (i) For the MT200 remove and invert the glass top.
   (ii) For the MT200A remove the glass top

4. (i) For the MT200 insert the longer end of the red volume rod into the base of the central channel for a 100 μl sample volume or the shorter end for 50 μl.
   (ii) For the MT200A drop the red rod into the central channel for 0.3 ml, white for 0.5 ml and black for 1ml

5. (i) For the MT200 - while holding the rod in place insert the plunger into the other end of the channel until it seats on the rod.
   (ii) For the MT200A – Insert the plunger into the central channel on top of the rod.
   For both the MT200 and MT200A. Insert the hexagonal key into the small hole on the side of the circular adjustment collar on the plunger. Use the key to loosen the set screw inside the collar. Slide the collar until it rests on the of the glass top.

6. Secure the collar to the plunger by tightening the collar set screw with the hexagon key, just tight enough to prevent the collar moving. Take care not to over-tighten the screw as this may fracture the plunger.

7. Remove the volume rod, refit the glass top and secure with the plastic clamp ring. **Before inserting the electrode ensure that electrode and inside the electrode holder have been dried thoroughly**. Re-insert the electrode and screw its cap on tight.

6 Cleaning the chamber and water jacket

If you need to clean the complete unit, proceed as follows:

Switch off the pumped water supply. Disconnect the outlet and inlet water supply tubes and drain the water jacket. Remove electrode (**it is important that the electrode is removed before disassembly**). Unscrew the clamp ring and remove the glass top. Wash the glass top in detergent solution, followed by several rinses. Ensure that the glass top is completely dry before reassembly.

7 Electrode Care

See 782 or 929 manual.

8 Setting stirring speed

The default speed for the stirrer is 1100 rpm, which is optimal for the small stainless steel stir bar of the MT200. It is possible to select a different speed by carrying out the following steps:

To set selected speed:

1. Ensure that the unit has been powered off for at least 1 minute.
2. Hold down the button on the top panel and turn on the power (turn on at wall, or plug in the power cable).
3. Continue to hold down the button until the rapid indicator flashes stop (after 80 flashes).
4. The indicator will then flash slowly 10 times. Release the button after the number of flashes that corresponds to the speed desired.

No. flashes	Speed (rpm)
1	400
2	500
3	600
4	700
5	800
6	900
7	1000
8	**1100** (default)
9	1200
10	1300

To reset default speed:

1. Ensure that the unit has been powered off for at least 1 minute.
2. Hold down the button on the top panel and turn on the power (turn on at wall, or plug in the power cable).
3. The indicator will flash rapidly. Release the button while it is doing so.

9 Tips and troubleshooting

- The stir bar may be most easily removed from the respiration chamber by using a syringe needle or straightened paper clip to pick it up magnetically. Take care not to touch the electrode membrane.

- An increase in noise level on the oxygen traces when the stirrer is switched on usually indicates that the electrode membrane is damaged. A new membrane should be fitted.

- If the stirrer does not start when the stirrer on/off button is pressed (ie. the indicator does not begin to flash), unplug the power lead from the back of the Mitocell and plug it in again. Alternatively, switch off at the wall, wait 15 seconds and than switch on again.

10 Power supply

Caution – use only the 5 volt power unit provided. This can operate a number of Mitocells, each one powered from its neighbour with a link cable supplied by Strathkelvin Instruments. If your Mitocell was purchased with a Model 782 oxygen meter, a link cable was supplied instead of a power unit. This should be plugged into the '5V dc out' socket on the rear of the 782 meter.

Company/manuals/mitocellmanual.doc

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