User Manual UM-048

PhO₂xBox

Cell-Culture Chamber System

Affix Serial Number Sticker Here
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INTRODUCTION

Please read this manual carefully before using the PhO₂xBox and familiarise yourself with all aspects of using the workstation. The Baker Company (Baker) or Ruskinn Technology Ltd (Ruskinn) does not accept responsibility for accidents to personnel or damage to the PhO₂xBox workstation resulting from incorrect use.

The PhO₂xBox comprises of a Gas Controller and a Cell-Culture Chamber has been specifically designed and developed to meet the requirements of all laboratories.

The system has built in gas control with Oxygen and Carbon Dioxide sensors to enable constant monitoring of the atmosphere. Hypoxic conditions are created using Nitrogen (N₂), Carbon Dioxide (CO₂) and Compressed Air (O₂) (or 25% Oxygen in Nitrogen) to achieve the desired atmosphere. The user can select the Oxygen concentration from 0.1% to 20.9%, and Carbon Dioxide concentrations from 0.1% to 30.0%.

Many unique features of the PhO₂xBox workstation are covered in detail in this manual. It is recommended that the user be fully conversant with the instruction and procedures, and that the operator familiarises themselves with all aspects and functions of the system before it is commissions to maintain optimum performance.
SAFETY INSTRUCTIONS

Please read this manual carefully and familiarize yourself with all aspects of the Cell-Culture Chamber System.
The System comprises a Gas Controller and a Cell-Culture Chamber.
Baker and/or Ruskin does not accept responsibility for accidents to personnel or damage to the Cell-Culture Chamber System resulting from incorrect use.
The mains appliance coupler and plug are the AC mains supply isolation device and must be easily accessible when installed.
In case of emergency disconnect the Gas Controller from the AC Mains Outlet.
Ensure that the connecting cable is not squeezed or bent when the System is being installed or moved.
All installation work and adjustments to the System must be carried out by qualified personnel.
Work performed by persons with insufficient technical knowledge may adversely affect the performance of the system or cause physical injury or damage to the equipment.
All servicing and repairs must be carried out by a qualified customer service engineer.
Only genuine spare parts must be used.
All gas cylinders must be adequately secured before connection to the System.

CAUTION: Asphyxiation Risk

The PhO₂xBox uses Nitrogen (N₂) and Carbon Dioxide (CO₂) as part of normal use with the volume released externally is inconsequential. In the event of a leak or malfunction this gas release may become excessive. DO NOT OPERATE this unit in a SMALL ENCLOSURE such as a small room or walk-in closet. An accidental release of Nitrogen or Carbon Dioxide could create an asphyxiating atmosphere in a small space.

If the equipment is not use in a manor specified by the manufacture, the protection provided by the equipment may be impaired.

Failure to adhere to these safety instructions could cause serious injury and will invalidate the workstation warranty. Ruskin technology limited accepts no responsibility for any accident, injury or loss caused by unsafe operation of the workstation.
REGULATORY COMPLIANCE

WEEE:

This equipment must be disposed of in accordance with the Waste from Electrical and Electronic Equipment (WEEE) Directive.

This product must not be treated as household waste. Instead, it shall be handed over to an appropriate collection point for the recycling of electrical and electronic equipment.

If in doubt, please return this equipment to Ruskinn Technology Ltd who will correctly dispose of it for you. We strongly recommend that this product is returned to RTL at the end of its useful life.
## Symbols

Before using the PhO₂xBox, please ensure that you are familiar with the symbols.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Book" /></td>
<td>Refer to user manual.</td>
</tr>
<tr>
<td>~</td>
<td>Alternating current</td>
</tr>
<tr>
<td>O</td>
<td>Off</td>
</tr>
<tr>
<td>I</td>
<td>On</td>
</tr>
<tr>
<td><img src="image" alt="Functional Earth Connection" /></td>
<td>Functional Earth Connection</td>
</tr>
<tr>
<td><img src="image" alt="Protective Earth Connection" /></td>
<td>Protective Earth Connection</td>
</tr>
<tr>
<td><img src="image" alt="Warning" /></td>
<td>Caution, do not remove covers. No end user serviceable parts behind covers. Please refer to this manual in all cases where this symbol appears, in order to find out the nature of the Potential Hazard and actions to be taken in order to avoid the Hazard.</td>
</tr>
<tr>
<td><img src="image" alt="Warning" /></td>
<td>Warning, this equipment contains high voltage circuitry.</td>
</tr>
<tr>
<td><img src="image" alt="Warning" /></td>
<td>Contains material or substances that may be hazardous to human health. Please refer to your local biohazardous material handling procedure for further advice on the handling and disposal of these items.</td>
</tr>
<tr>
<td><img src="image" alt="PhO₂xBox" /></td>
<td>PhO₂xBox contains hazardous components and must not be disposed of at a household waste site. Instead it should be taken to the appropriate collection point for the recycling of electrical and electronic equipment.</td>
</tr>
<tr>
<td><img src="image" alt="USB socket" /></td>
<td>USB socket</td>
</tr>
<tr>
<td><img src="image" alt="Date of manufacture" /></td>
<td>Date of manufacture in format YYYY MM</td>
</tr>
</tbody>
</table>

*Table 1: List of Symbols*
TRANSPORT AND STORAGE

When not in use, the PhO₂xBox controller box & cell culture chamber must only be stored within a temperature of between 0°C and 30°C.

Storage outside of this range may damage the gas controller.

LOCATION AND HANDLING OF THE PHO₂XBOX

The PhO₂xBox should only be installed or relocated by a qualified engineer. To arrange installation or relocation please contact your local distributor.

The mains appliance coupler and plug are the AC mains supply isolation device and must be easily accessible.

ENVIRONMENTAL OPERATING CONDITIONS

The PhO₂xBox should only be operated under the following environmental conditions:

- Temperature – Between 15°C and 30°C
- Humidity – Between ambient and 90% RH, Non-Condensing

The workstation must be located in a well-ventilated area.
SERVICE REQUIREMENTS

Electrical Supply Requirements

The Gas Controller must be connected to a mains power supply. A power cord is supplied to connect the workstation to the mains supply. If an alternative power cord is used it must be rated appropriately for the power requirements of the Gas Controller, refer to Table 2. The Gas Controller must be connected to a protective earth.

To ensure safe operation of the system, it must be connected to a supply of the correct voltage and frequency as stated on the rating label shown.

The mains supply voltage fluctuations must not exceed +/- 10% of the nominal mains voltage.

<table>
<thead>
<tr>
<th>Voltage Range</th>
<th>Frequency</th>
<th>Rated Current</th>
<th>Rated Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>220±20V</td>
<td>50Hz</td>
<td>0.1A</td>
<td>35W</td>
</tr>
<tr>
<td>110±10V</td>
<td>60Hz</td>
<td>0.1A</td>
<td>25W</td>
</tr>
</tbody>
</table>

*Table 2: Electrical Service Requirements*

Gas Supply Requirements

The gas regulator should provide a minimum supply pressure of 2 bar gauge. The maximum supply pressure permissible is 3 bar gauge. See Table 4 below. A supply pressure greater than this will damage internal components of the gas mixer and will invalidate the warranty.

<table>
<thead>
<tr>
<th>Gas</th>
<th>Symbol</th>
<th>Specification</th>
<th>Regulator Output Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>N₂</td>
<td>Oxygen Free (Industrial or medical)</td>
<td>2 to 3 bar</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>CO₂</td>
<td>100% (Industrial or medical)</td>
<td>2 to 3 bar</td>
</tr>
</tbody>
</table>

*Table 3: Standard Gases*

For the gas supply we recommend direct connections of the gas cylinder regulator to ensure minimal pressure drops and flow rate restrictions. Only the above stated gases are to be used with the system. Failure to comply with this may cause the product to become hazardous.

Only the above stated gases are to be used with the workstation. Failure to comply with this may cause the product to become hazardous.
PHO\textsubscript{2}XBOX OVERVIEW

Background

The System comprises a Cell Culture Chamber, which comes in two sizes (Small and Large) and different colours (Black & Transparent), and a Gas Controller. The Gas Controller is connected to the Cell Culture Chamber. The Cell Culture Chamber can hold cell culture flasks and 6, 12, 24 or 96 well plates. The gas concentration of the Cell Culture Chamber can be changed automatically by using the touchscreen on the Gas Controller.

Hypoxic conditions are created inside the Culture Chamber by using Nitrogen (N\textsubscript{2}), Carbon Dioxide (CO\textsubscript{2}) and laboratory air (which is sucked into the Gas Controller via an Air pump). The user can select the Oxygen concentration from 0.3% to 20.0%, and the Carbon Dioxide concentration from 0.3% to 20.0%. The Cell Culture Chamber is well-sealed so it can reach the desired culture conditions quickly. Built in Oxygen and Carbon Dioxide sensors enable constant monitoring of the atmosphere inside the Cell Culture Chamber.

The Cell Culture Chamber can be placed into a CO\textsubscript{2} incubator, CO\textsubscript{2}/O\textsubscript{2} incubator or Physoxia/Hypoxia workstation.

CONTENTS (QTK-AO1/AO2)

1 mains lead
1 signal lead
1 blue tube (1m)
1 black gas hose (CO\textsubscript{2}) (3m)
1 blue gas hose (N\textsubscript{2}) (3m)
1 Cell Culture Chamber (Large QTK-AO2 or Small QTK-AO1)

Large: W x D x H (mm)= 360 x 300 x 230
Small: W x D x H (mm)= 340 x 300 x 160

1 Gas Controller (with air pump built in)
Figure 1: Blue Tube

Figure 2: Mains Lead

Figure 3: Signal Lead

Figure 4: Gas Controller (With air pump)
Figure 5: N₂ Gas Hose

Figure 6: CO₂ Gas Hose

Figure 7: Cell Culture Chamber (Small QTK-A01)

Figure 8: Cell Culture Chamber (Large QTK-A02)
Figure 9: Cell Culture Chamber (Large QTK-AB02)
SETTING UP THE GAS CONTROLLER AND CULTURE CHAMBER

Setting up the cell culture chamber on a laboratory bench

The Cell Culture Chamber can be placed adjacent to or on top of the Gas Controller. Connect the blue tube to InA on the Cell Culture Chamber and OutA on the Gas Controller. Connect the signal lead to InB on the Cell Culture Chamber and OutB on the Gas Controller.

Figure 10: Rear of Cell culture chamber

Figure 11: Rear of Cell culture multi chamber
Use the blue hose to connect N\textsubscript{2} on the Gas Controller to the N\textsubscript{2} gas cylinder and the black hose to connect CO\textsubscript{2} on the Gas Controller to the CO\textsubscript{2} cylinder.

Connect the mains lead to the right port shown on Gas Controller.

Open gas cylinders and turn the gas controller power on.

**For installation inside a workstation (SCI-tive or InvivO\textsubscript{2}) or CO\textsubscript{2} Incubator**

Place Cell Culture Chamber in desired Workstation or Incubator.

Place the Gas Controller adjacent to the Workstation or Incubator.

Locate Universal Multi-Cable Gland on left side panel of Workstation or Incubator.
Unscrew inside black frame of the Universal Multi-Cable Gland and remove the blue plug. The Plug can contain up to 6 pre-drilled holes depending on how it was ordered. Additional holes can also be drilled by user.

Connect the blue tube to InA on the Cell Culture Chamber and OutA on the Gas Controller. The blue tube has to be put through the Universal Multi-Cable gland.

Connect the signal lead to InB on the Cell Culture Chamber and OutB on the Gas Controller. The lead has to be put through the Universal Multi-Cable gland.

Ensure that the blue tube and signal cable goes through the Universal Multi-Cable Gland into the plug from the inside.
Use the blue hose to connect $N_2$ on the Gas Controller to the $N_2$ gas cylinder and the black hose to connect $CO_2$ on the Gas Controller to the $CO_2$ cylinder.

Connect the mains lead to the right port shown on Gas Controller.

Open gas cylinders and turn the Gas Controller power on.
USING THE GAS CONTROLLER

Gas Controller

The Gas Controller is shown as follows.

Figure 18: Front View

Figure 19: Left View

Figure 20: Right View

Figure 21: Rear View
Figure 22: Rear View (Multi version)

Figure 23: Top View
THE TOUCH SCREENS

Start up screens

When the unit is first powered a Logo Screen (Screen 1) will appear and then will be replaced by the Main Screen (Screen 2).

![Logo Screen](image)

*Figure 24: Screen 1 – Logo screen*

Main screens

From this menu you will be able to access the Main screen below.

![Main Screen](image)

*Figure 25: Screen 2 – Main screen*

The multi chamber version chamber selection must first be made.
Once chamber has been selected the main menu is then displayed with the currently active chamber displayed in the top left corner.

Atmosphere Control Screen

Pressing the “Atmosphere Control” bar on Screen 2 will open up the settings screen shown in Screen 3.
O₂ and CO₂ set point can be changed by pressing O2 SET POINT or CO2 SET POINT. A pop-up will appear for the new setting to be selected, as shown in Screen 4. Press Enter once the desired value of O₂ is selected.

![Screen 4](image)

*Figure 29: Screen 4 – Level SET POINT Screen*

Repeat for CO₂.

Once O₂ and CO₂ set-points have been selected press the START button in the bottom left of the screen to start the gas control.
Re-setting the O₂ and CO₂ value during operation

To re-set the value during operation, press the “stop” button, change the value, and press the “start” button. The new value will now become the Set Point.

![Image](ATMOSPHERE_CONTROL.png)

Figure 30: Screen 5 – Atmosphere Control Screen

Hypoxic Cycle Menu Settings

The Hypoxic Cycle menu is accessed from Screen 2 to allow for dynamic changing of the Oxygen and Carbon Dioxide levels over fixed time periods. Screen 6 shows the initial screen display for continuous cycling for up to 4 set-point.

![Image](Hypoxic_Cycle_Menu_Settings.png)

Figure 31: Screen 6 – Hypoxic Cycle Menu Settings

The user can set hypoxic cycle on the left side of screen 6. The timing values define how long the Oxygen and Carbon Dioxide levels are maintained for once they have been achieved. This means that there is a time period between the steps that cannot be set as it is dependent on the difference between the set-points within the gas control software.

Pressing on any of the values will bring up a dialogue box to enter the required setting. If only 2 values are required for the cycling then set step 1 and 2 as required, then set step 3 times period to 0 minutes with the O₂ and CO₂ set-points the same as step 2.

Once the setting is correct then press the start button to begin the cycling. At this point the start button will change to a Stop button. The continuous cycling will stop by pressing the Stop button. The left side of the screen is the current status of hypoxic cycle.
Data Trend Log

The Data Trend Log screen can be accessed from the LOG button on screen 6.

Screen 7 shows the Trend Log display. The earliest recorded data will be displayed. The window can be scrolled through on the control screen. To view previous data, the USB is to be removed and connected to a computer. The USB can capture approximately 6 months of data.

Delete: Press delete button and the data will disappear from the system.

USB Save: Press USB Save button and the data log will downloaded to a USB.

![Trend Log](image)

**Figure 32: Screen 7 – Trend Log**

Engineer Mode Screen

To set the control parameters, enter the Engineer Mode screen. Entry to the Engineer Mode screen is available when the time shows 29s-30s or 59s-00s. Double click on the corner of the top right of the atmosphere control screen, and then you will enter the engineer screen. Press the value, and a pop-up will appear for the new setting to be selected, then press “save” button and “back” button, and you will return to the running mode.

![Engineer Mode](image)

**Figure 33: Screen 8 – Engineer Mode Screen**
Parameter Ranges and Meanings

Offset: range is 0.1-1.0%, when the set point and current value difference is less than offset the unit enters the intermittent adjustment mode.

Deadband setting: range is 0-0.5%, for example when the deadband setting is 0.2% and O₂ set point is 5.0%, when the current value is 4.8%-5.2%, no gases will be injected to adjust the concentration.

Switch on: range 1-20s, means injecting time when the unit enters the intermittent adjustment mode.

Switch off: range 1-20s, means rest time when the unit enters the intermittent adjustment mode, for example if set switch on at 2s and switch off at 10s, the Gas Controller will inject gas at 2s and rest at 10s.

Recording time: Range 1-10s, means record the set time interval between 1 and 10 seconds. For example, set recording time 2s, means it will record once every 2 seconds.

Date and time: to change the date and time, press “setting time” button, and then enter a windows screen, choose the date and time, press back to return to the running mode.
OTHER INFORMATION

The material of the Gas Controller, the Cell Culture Chamber and the shelf is made of acrylic, The maximum temperature that the chamber can withstand up to 60°C.

The Cell Culture Chamber is connected to Gas Controller, which contains O₂ and CO₂ sensors.

A dish of distilled water can be installed in the culture chamber to increase humidity

Cleaning: Use special cleaning kit supplied by Baker Ruskinn (code 262-345) or < 70% ethanol wiping, please do not use ultraviolet irradiation.
Ruskinn Technology Limited warrants for the applicable time period that the PhO$_2$xBox will substantially perform in accordance with the user documentation. The terms of this Agreement do not affect or prejudice the statutory rights of a consumer acquiring the Ruskinn Technology Limited PhO$_2$xBox otherwise than in the normal course of a business.

THIS WARRANTY DOES NOT APPLY IN THE FOLLOWING CIRCUMSTANCES:

(A) IF THE Ruskinn Technology Limited PhO$_2$xBox HAS BEEN REPAIRED BY PERSONS NOT AUTHORIZED BY Ruskinn Technology Limited; OR

(B) THE Ruskinn Technology Limited PhO$_2$xBox and associated accessories/peripherals HAVE BEEN ALTERED, MODIFIED, OR MISUSED; OR

(C) THE Ruskinn Technology Limited PhO$_2$xBox IS USED WITH NON- Ruskinn Technology Limited COMPONENTS; OR

(D) THE Ruskinn Technology Limited PhO$_2$xBox OR A COMPONENT IS USED FOR OTHER USES (FOR EXAMPLE USE WITH OTHER CIRCUIT BOARDS OR SOFTWARE) OR

(E) THE Ruskinn Technology Limited PhO$_2$xBox HAS NOT BEEN MAINTAINED OR USED IN ACCORDANCE WITH THE INSTALLATION AND USER GUIDE. UNLESS PROHIBITED BY LAW, THIS WARRANTY IS MADE IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO THE IMPLIED WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE, THE IMPLIED WARRANTY OF MERCHANTABILITY, OR ANY IMPLIED WARRANTY ARISING OUT OF A COURSE OF DEALING OR OF PERFORMANCE, CUSTOM OR USAGE OF TRADE. Ruskinn Technology Limited DOES NOT WARRANT THAT THE Ruskinn Technology Limited PhO$_2$xBox WILL FUNCTION ERROR FREE.

If within the Warranty Period, the Ruskinn Technology Limited PhO$_2$xBox does not conform to the express warranty set forth above, Ruskinn Technology Limited’s sole obligation and Users sole remedy shall be, at Ruskinn Technology Limited’s option: 1. to repair or replace the non-conforming component; or, 2. refund the purchase price.

LIMITATION OF LIABILITY.

UNLESS PROHIBITED BY LAW, Ruskinn Technology Limited WILL NOT BE LIABLE TO USER OR OTHERS FOR ANY OTHER DIRECT, INDIRECT, CONSEQUENTIAL, INCIDENTAL OR SPECIAL DAMAGES INCLUDING, FOR EXAMPLE, LOST PROFITS, BUSINESS, INVESTMENTS, OR OPPORTUNITIES EVEN IF Ruskinn Technology Limited HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

The parties agree that Ruskinn Technology Limited total cumulative liability to User for direct damages for all causes under this Agreement shall not exceed £5,000,000 (FIVE MILLION UK STERLING POUNDS), or the price paid for the Ruskinn Technology Limited PhO$_2$xBox whichever is higher. Some states or countries may have laws which require liability rights different from those stated above. In such states or countries, the minimum required liability terms shall apply.
DISPOSAL INFORMATION

PhO₂xBox contains hazardous components and must not be disposed of at a household waste site. Instead it should be taken to the appropriate collection point for the recycling of electrical and electronic equipment. Alternatively, please contact your local distributor for disposal instructions.

PhO₂xBox contains recyclable parts. Please contact your local distributor for more advice.
CONTACT DETAILS

Ruskinn Technology Limited

Address:
8 & 9 York Park,
Bridgend Industrial Estate
Bridgend CF31 3TB
United Kingdom

Phone: +44 (0)1656 645988
Fax: +44 (0)1656 667966

Email:
Sales: sales@bakerruskinn.com
Technical support: techsupport@bakerruskinn.com
General enquiries: ruskinnoffice@bakerruskinn.com
Website: www.bakerruskinn.com
YouTube channel http://www.youtube.com/ruskinntechnology

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