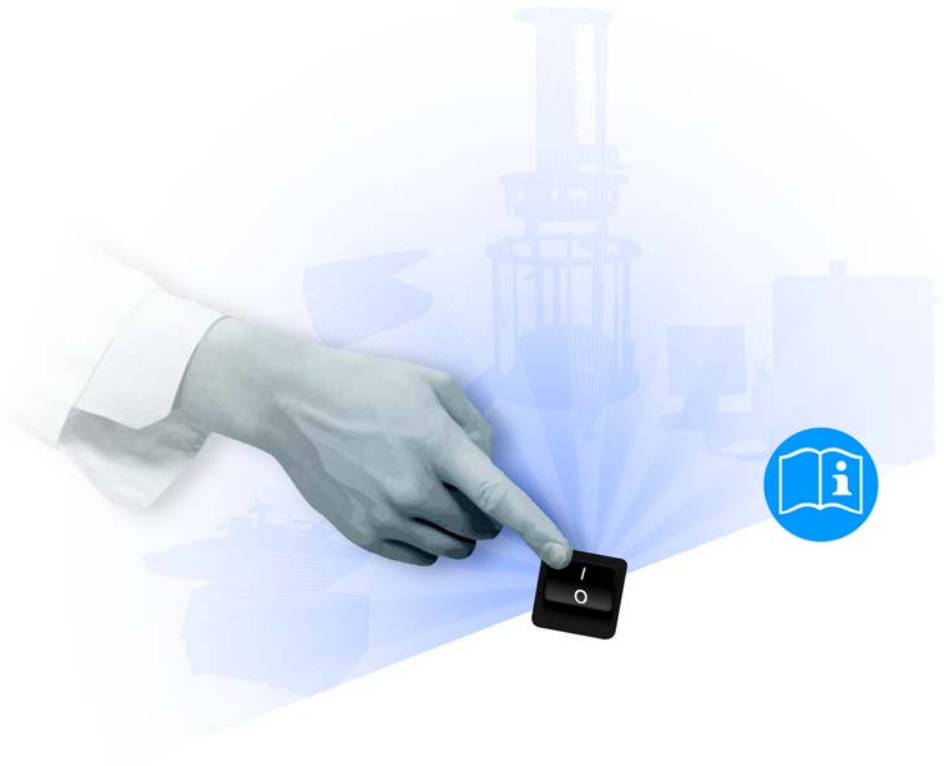
GE Healthcare
Life Sciences

Stand-alone instruments for WAVE Bioreactor™ 2/10 and 20/50

Operating instructions



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1 Introduction

Purpose of the manual

This manual provide you with the instructions needed to handle WAVE Bioreactor system optional stand-alone instruments. For further information regarding the use of WAVE Bioreactor 2/10, WAVE Bioreactor 20/50 and WAVEPOD™ II[1], refer to WAVE Bioreactor System BASE 2/10 EH Operating Instructions and WAVE Bioreactor 20/50 and WAVEPOD II User Manual, respectively.

Prerequisites

In order to operate WAVE Bioreactor stand-alone instruments safely and according to the intended purpose the following prerequisites must be met:

- You should be acquainted with the use of general laboratory equipment and with handling of biological materials.
- You must read the Safety Instructions in *Chapter 2*.
- The system should be installed according to the Installation sections in *Chapter 4* to *Chapter 9*.

In this chapter

This chapter contains important user information and a general description of WAVE Bioreactor stand-alone instruments, and their intended use.

[1] The optional WAVEPOD II controller integrates instrumentation associated with WAVE Bioreactor units. This includes pH, dissolved oxygen, and CO2/O2 gas mixing controls.

1.1 Important user information

Read this before using a WAVE™ stand-alone instrument

All users must read the Safety Instructions in *Chapter 2* of these Operating Instructions before installing, using or maintaining WAVE Bioreactor stand-alone instruments.

Do not operate WAVE Bioreactor stand-alone instruments in any other way than described in the user and product documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The WAVE Bioreactor system is intended to be used as development and manufacturing equipment for expansion of cells. Cultivation is performed in a single use, gamma irradiated bag, known as a Cellbag™, which is placed on the WAVE Bioreactor instrument.

Note: *When used for cell therapy applications outside the European Union and Australia, the WAVE Bioreactor system shall be used for research only.*

Control of various parameters can be made in different ways depending on WAVE Bioreactor system configuration. Optional stand-alone instruments can be used to control, for example, O_2 concentration and CO_2 concentration.

Safety notices

These Operating Instructions contain WARNINGS, CAUTIONS and NOTICES concerning the use of the product, with meanings as defined below.

WARNING
WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION
CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE
NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: *A Note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A Tip contains useful information that can improve or optimize your procedures.*

Typographical conventions

Software texts and commands are identified by ***bold italic*** text. A colon is used to separate menu levels (e.g., ***File:Open*** refers to the ***Open*** option in the ***File*** menu).

1.2 Regulatory information

This section lists the directives and standards that are fulfilled by WAVE Bioreactor stand-alone instruments.

Manufacturing information

Requirement	Content
Name and address of manufacturer	GE Healthcare Bio-Sciences AB, Björkgatan 30, SE 751 84 Uppsala Sweden
Name and ID of notified body	INTERTEK
Place and date of declaration	Uppsala, Sweden, Nov 2009
Identity of person authorized to sign DoC	See EC Declaration of Conformity.

CE Conformity

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive

International standards

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 no. 61010-1	Safety requirements for electrical equipment for measurement, control and laboratory use	
EN 61326-1	EMC emissions and immunity requirements for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN-ISO 12100-1, 12100-2	Safety of machinery – Basic concepts, general principles and design	Harmonized with 2006/42/EC
EN-ISO 14121-1, 14121-2	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC

CE marking

CE

The CE marking and the corresponding Declaration of Conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation or explicitly authorized by GE Healthcare.

Regulatory compliance of connected equipment

Any equipment connected to WAVE Bioreactor stand-alone instruments should meet the safety requirements of EN 61010-1/IEC61010-1 or relevant harmonized standards. Within the European Union, connected equipment must be CE-marked.

1.3 WAVE system configurations

There are several different sizes of WAVE systems available that provide scalable cell culturing possibilities and several different configurations available for control of key culture parameters:

- WAVE Bioreactor instrument options with different internal control modules.
- WAVEPOD II options with different internal control modules (available for WAVE Bioreactor 20/50 instrument).
- Stand-alone instruments for control of specific parameters (described in this manual).

For examples of how control of different parameters can be achieved, see *Table 1-1*. Note that more than one stand-alone instrument can be used with a WAVE Bioreactor instrument to get access to more control functions.

For some parameters, several control options are available. For example, pH in the culture medium can be controlled by:

- On-line control of pH by pH measurement and pH adjustment by acid and base additions. This requires a WAVEPOD II with pH control module and PUMP20.
- Indirect control of pH by changing the CO_2 concentration setpoint. This requires:
    - Integrated CO_2 control module (as in BASE20/50EHT-CO2), or
    - WAVEPOD II with CO_2 control module, or
    - the stand-alone instrument CO2MIX20 or CO2MIX20-R.

Table 1-1 shows example instrument and system configurations. The stand-alone instrument PERFCONT2E and all WAVEPOD II units are included, but not described further in this manual. See the respective product manuals for more information.

Table 1-1. Examples of WAVE Bioreactor 2/10 and 20/50 system configurations using WAVE Bioreactor instruments alone or in combination with stand-alone instruments. Several other options are possible. Some configurations of WAVE Bioreactor 20/50 system are available as Dual system configuration (WAVE Bioreactor instrument 20/50 EHTD), which enables operation of two Cellbag bioreactors in parallel.

System configuration		Control parameters						
Bioreactor base unit	Stand-alone instrument	Rocking	Temperature	Dissolved O_2	pH	Inlet gas CO_2 concentration	Inlet gas O_2 concentration	Perfusion culture
WAVE Bioreactor 2/10 system								
BASE2/10EH		+	+					
BASE2/10EH	DOOPT20	+	+	+				
BASE2/10EH	CO2MIX20	+	+			+		
BASE2/10EH	O2MIX20	+	+				+	
BASE2/10EH	PERFCONT2E	+	+					+
WAVE Bioreactor 20/50 system								
BASE20/50EHT		+	+					
BASE20/50EHT-CO2		+	+			+		
BASE20/50EHT-O2		+	+				+	
BASE20/50EHT-L	PUMP20	+	+					+
BASE20/50EHT	DOOPT20	+	+	+				
BASE20/50EHT	CO2MIX20	+	+			+		
BASE20/50EHT	O2MIX20	+	+				+	
BASE20/50EHT	WAVEPOD II PHOPT CO2	+	+		+	+		
BASE20/50EHT	WAVEPOD II DOOPT O2	+	+	+			+	
BASE20/50EHT	WAVEPOD II PHOPT DOOPT CO2 O2	+	+	+	+	+	+	
BASE20/50EHT-L	WAVEPOD II PHOPT DOOPT CO2 O2 PUMP20	+	+	+	+	+	+	+

1.4 Stand-alone instruments

Overview

Several stand-alone instruments for the WAVE Bioreactor 2/10 and 20/50 systems are available. The stand-alone instruments are used connected to the WAVE Bioreactor instrument and designed to fit in racks (SRACK) for single instruments. Racks include a power supply and a RS485 dataport communicating via the MODBUS RTU protocol for data acquisition.

The available stand-alone instruments are listed in the table below. See respective chapter for a description and instruction for use.

Name	Function	Chapter
DOOPT20	Dissolved oxygen monitor	*Chapter 4*
CO2MIX20	CO_2/air controller	*Chapter 5*
CO2MIX20-R	CO_2/air controller (rotameter model)	*Chapter 6*
O2MIX20	O_2/air controller	*Chapter 7*
O2MIX20-R	O_2/air controller (rotameter model)	*Chapter 8*
PUMP20	Peristaltic feed/harvest or acid/base pump	*Chapter 9*

1.5 Control software

The WAVE system stand-alone instruments are equipped with embedded software for control and supervision. See *Chapter 3* for instruction for use of common process controllers.

1.6 Literature

For further information regarding the use of WAVE Bioreactor 2/10, WAVE Bioreactor 20/50 and WAVEPOD II refer to the following:

- WAVE Bioreactor System BASE 2/10 EH Operating Instructions, article no. 87-4500-23.
- WAVE Bioreactor System 20/50 and WAVEPOD II User Manual, article no. 28-9846-80.

2 Safety instructions

This chapter describes safety compliance, safety labels, general safety precautions, emergency procedures, power failure and recycling of WAVE stand-alone instruments.

2.1 Safety precautions

Introduction

Before installing, operating or maintaining stand-alone instruments, you must be aware of the hazards described in WAVE Bioreactor System BASE 2/10 EH Operating Instructions and WAVE Bioreactor System 20/50EHT Operating Instructions, as well as hazards specifically related to WAVE stand-alone instruments, which are described in this manual. Follow the instructions provided to avoid personal injury or damage to the equipment.

The safety precautions in this section are grouped into the following categories:

- General precautions
- Personal protection
- Installing
- System operation
- Maintenance

General precautions

WARNING
Do not use any accessories not supplied or recommended by GE Healthcare.

Personal protection

WARNING
Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system.

Installing instrument

WARNING
Place CO2MIX20 and CO2MIX20-R instruments in a well ventilated environment. Failure to do so can lead to heightened CO_2 concentrations.

Care should be taken to remove pressurized CO_2 supply when not in use.

WARNING
Place O2MIX20 and O2MIX20-R instruments in a well ventilated environment. Failure to do so can lead to heightened O_2 concentrations.

Care should be taken to remove pressurized O_2 supply when not in use.

WARNING
Protective ground. The WAVE stand-alone instruments must always be connected to grounded power outlets.

WARNING
Supply voltage. Ensure that the supply voltage at the wall outlet corresponds to the marking on the instrument before connecting the power cord.

CAUTION
Ensure that all tubing, hoses and cables are placed so that the risk or tripping accidents is minimized.

System operation

CAUTION
Remove any spillage on the floor immediately to minimize the risk for slipping accidents.

Maintenance

WARNING
Electrical shock hazard. All repairs should be done by service personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in the user documentation.

WARNING
Disconnect power. Always disconnect power from the instrument before performing any maintenance task.

WARNING
Only spare parts that are approved or supplied by GE Healthcare may be used for maintaining or servicing the system.

2.2 Labels

This section describes safety labels and labels concerning hazardous substances that are attached to the WAVE instruments.

Labels on the instrument

The instruments are labeled on the rear panel. The example below is the label for the DOOPT20 instrument.

Symbols used in safety labels

Label	Description
	**Warning!** Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	The system complies with applicable European directives.

Labels concerning hazardous substances

Label	Description
	This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/ T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronics.

2.3 Emergency procedures

This section describes how to do an emergency shutdown of a WAVE instrument. The section also describes the result in the event of power failure

Emergency procedures

In an emergency situation, do as follows to stop the run:

Step	Action
1	Switch off the power to the instrument by pressing the power switch to the **O** position.
2	Disconnect the power cord from the power outlet.

2.4 Recycling procedures

The equipment shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking WAVE instruments out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

WAVE instruments contain hazardous substances. Detailed information is available from your local GE Healthcare representative.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

3 Common process controllers

3.1 Functions

Most WAVE Bioreactor stand-alone instruments are controlled by use of integrated Process controllers. In the stand-alone instrument, the Process controller (large or small type) is integrated in the front of the instrument, see the example figure below.

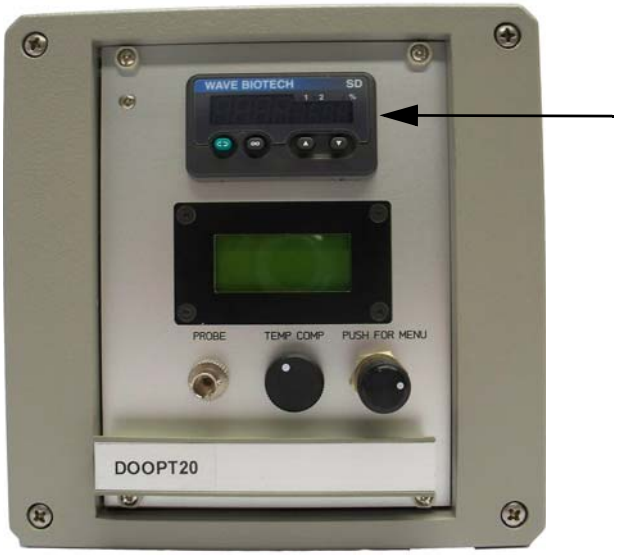


Figure 3-1. Example of stand-alone instrument (DOOPT20) with a small Process controller, see arrow.

The Process controllers are used in similar way in different stand-alone instruments and the main functionality is described in this chapter. Actual and setpoint values can be viewed and setpoint values can be changed.

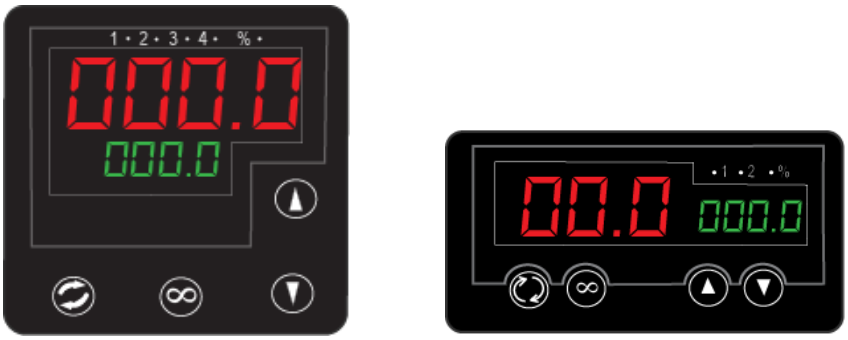


Figure 3-2. Large Process controller (left) and small Process controller (right).

Controller item	Function
• 1, 2, 3, 4 (Large controller) • 1, 2 (Small controller)	Output indicators light when corresponding output is activated.
%	Auto/manual indicator lights when corresponding output is activated
Upper display: red digits	Shows process value or parameter value
Lower display: green digits	Shows setpoint or parameter name.
Advance button	Press to display through menu items or parameters
Up arrow button	Press to increase setpoint or parameter
Down arrow button	Press to decrease setpoint or parameter
Home button	Press to return to normal operation

3.2 Process controller on stand-alone instruments

Table 3-1. Stand-alonestand-alone instruments with large and small Process controller.

Large Process controller	Small Process controller
O2MIX20, O_2/air controller	DOOPT20, dissolved oxygen monitor
O2MIX20-R, O_2/air controller	O2MIX20, O_2/air controller
CO2MIX20, CO_2/air controller	CO2MIX20, CO_2/air controller
O2MIX20-R, O_2/air controller	
CO2MIX20-R, CO_2/air controller	

3.3 Changing Process controller parameters

CAUTION
The Process controllers are factory set for optimum performance. Changes to any parameters should only be attempted by qualified personnel. Please consult GE Healthcare for assistance.

Changing the setpoint value

Change the setpoint by following the procedure below. This procedure is the same for all controller sizes.

Step	Action
1	**Increase** the setpoint value as desired by pressing the ▲ button.
2	**Decrease** the setpoint value as desired by pressing the ▼ button.

3.3.1 Large Process controller

Unlocking the controller

To avoid accidental changes, all Process controller parameters are locked. Unlock the Process controller by following the procedure.

Step	Action
1	Press and hold down the ↻ and ∞ buttons for 10 seconds to enter the ***CUST*** (customize) menu. This puts the controller into the ***Fcty*** (factory) display (shown in green in the lower display).  ***Note:*** *Displays may appear slightly different*.
2	Press the ▲ button to get the ***LOC*** menu (in red in the upper display).
3	While in the display ***Fcty*** and the menu ***LOC***, set the following parameters to ***chng***: • ***sp*** • ***cust*** • ***ope*** • ***set*** • ***cal*** For each parameter, set the value to ***chng*** by pressing the ▼ button. If the value ***chng*** is unavailable, set the value ***READ*** instead. Select a new parameter by pressing the ↻ button.
4	The Process Controller is now unlocked. Press the ∞ button to exit.

Changing addresses for stand-alone instruments

To change the MODBUS network address on the CO2MIX20 and the O2MIX20 instruments, follow the procedure below..

Step	Action
1	Press the ▲ and ▼ buttons together for 10 seconds until ***INPUT set*** is displayed.
2	Press the ▲ button until ***Out 4 Set*** is displayed.
3	Press the ↻ button until ***Addr*** is shown in green on the lower display. The upper display indicated the address number.
4	Press either the ▲ or ▼ buttons to change the address.
5	Press the ∞ button to save and exit.

Changing the baud rate for stand-alone instruments

To change the baud rate on the CO2MIX20 and the O2MIX20 instruments.

Step	Action
1	Press the ▲ and ▼ buttons together for 10 seconds until ***INPUT set*** is displayed.
2	Press the ▲ button until Out 4 Set is displayed.
3	Press the ↻ button until ***baud*** is shown in green on the lower display. The upper display indicated the address number.
4	Press either the ▲ or ▼ buttons to change the baud rate. Available baud rates are: 1200, 2400, 4800, 9600 and 19.2k. (The default baud rate is 9600)
5	Press the ∞ button to save and exit.

3.3.2 Small Process controller

Changing addresses for stand-alone instruments

To change the MODBUS network address on the DOOPT20 and Air Controllers.

Step	Action
1	Press and hold down the ▲ and ▼ buttons for 3 seconds. ***SET page*** is displayed.
2	Press the ↻ button repeatedly until ***ADDR*** is shown in green.
3	Press either the ▲ or ▼ buttons to change the address.
4	Press the ∞ button to save.

Changing the baud rate for stand-alone instruments

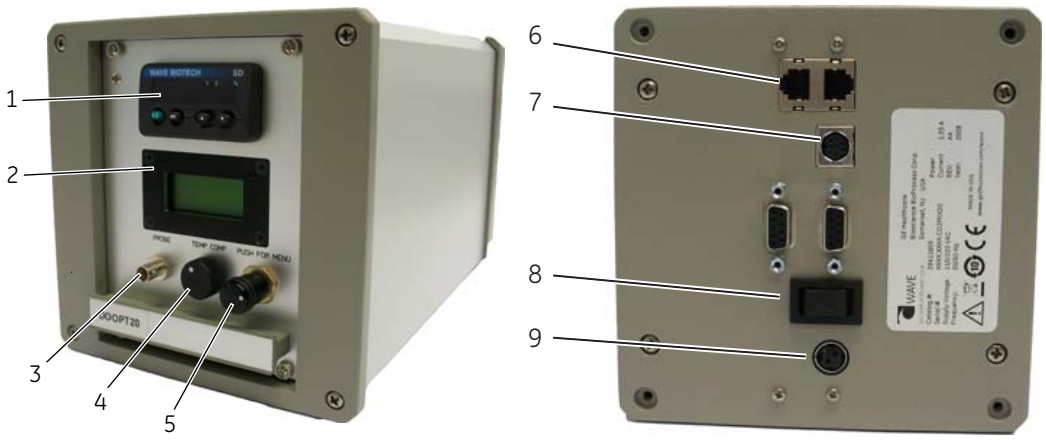
To change the baud rate on the DOOPT20 and Air Controllers.

Step	Action
1	Press and hold down the ▲ and ▼ buttons for 3 seconds. ***SET page*** is displayed.
2	Press the ↻ button repeatedly until ***BAUD*** is shown in green.
3	Press either the ▲ or ▼ buttons to change the baud rate. Baud rates of 9600, 19.2 and 38.4 are available. (The default baud rate is 9600)
4	Press the ∞ button to save.

4 DOOPT20 dissolved oxygen monitor

4.1 Introduction/view

The DOOPT20 stand-alone instrument provides amplification, display, and data transmission of the dissolved oxygen concentration. It allows the real-time measurement of dissolved oxygen concentration inside the Cellbag bioreactors. The illustration below shows the location of the main parts of DOOPT20.



Part	Description
1	Process controller
2	LCD display
3	Probe connector

Part	Description
4	**TEMP COMP** control
5	**PUSH FOR MENU** button
6	Dataport Connectors

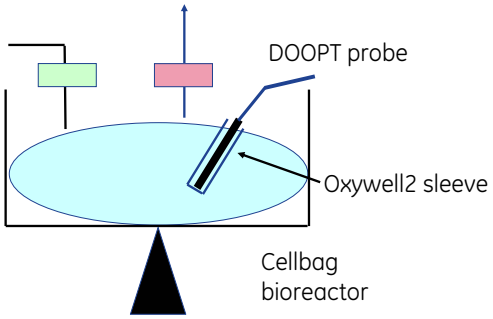
Part	Description
7	Analog output connector
8	Power switch, on (**I**), off (**O**)
9	Power Receptacle

DOOPT probes

The DOOPT20 stand-alone instrument can be connected to a DOOPT probe, which is used to monitor dissolved oxygen (DO) levels in cell cultures. A special fiber optic DO microsensor is used inside a sealed silicone Oxywell™2 sleeve. This allows the probe to

be inserted and removed without compromising sterility. DOOPT-PROBEs may be reused for many months until photo bleaching causes low signal amplitude and the probe becomes progressively too noisy for accurate measurements.

The silicone sleeve is made of FDA approved Class VI plastic and is the only contact surface. This eliminates any concern about toxicity of the sensing dye or leaching of the dye into the culture fluid.



For more information, see *Appendix B DOOPT20 measurement theory, on page 85*.

4.2 Installation

4.2.1 Site requirements

Parameter	Requirement
Electrical power	24 V DC through adapter (110 to 120 V~ or 220 to 240 V~, 50/60 Hz)
Placement	Stable laboratory bench
Operating temperature	4ºC to 40ºC
Humidity	<95%, non-condensing

4.2.2 Unpacking

Unpack the equipment.

Please check the packing list to see that all items on the list are included. If any parts are missing, contact your GE Healthcare representative.

Place the equipment on a stable surface.

Check the equipment for any apparent damage before starting installation. Document any damage carefully and contact your GE Healthcare representative.

4.2.3 Installation

Step	Action
1	Make sure that the DOOPT monitor is powered off.
2	Connect the transformer to a grounded power outlet.
3	Connect the transformer to the power inlet of monitor. The power connector is located on the rear of the instrument.
4	Remove the DOOPT probe from its packaging. The tip can be left in its protective tube until a calibration of the probe is needed.
5	Connect the DOOPT probe to the **PROBE** connector located on the front panel of the monitor. Make sure that the small ridge on the plug is lined up with the notch on the connector. Push the plug in and twist to lock it in position.

4.2.4 Spare parts and accessories

For correct up to date information on spare parts and accessories, see the web address on the back cover or contact your local GE Healthcare representative.

4.3 Operation

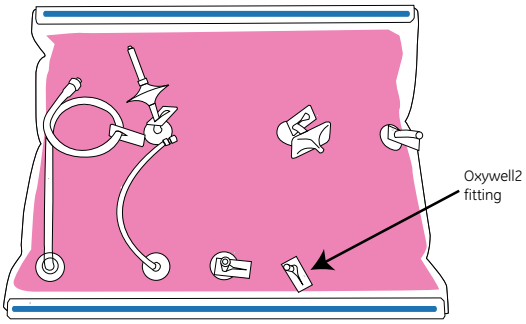
Control of DOOPT20 dissolved oxygen monitor

See *Section 4.1* for location of buttons and *Section 3* for description of Process controller.

NOTICE
When inserted into the Oxywell2 sheath, the probe has a longer response time due to diffusion through the silicone rubber membrane. The response time in the Oxywell2 is greatly improved by filling the tip with liquid such as distilled water or saline.

NOTICE
Sensitive DOOPT probe. Do not touch the tip of the probe with your finger or any object. Do not attempt to clean or dry the tip with a cloth. Gently wave the DOOPT probe back and forth to air-dry it.

Step	Action
1	Connect the probe to the connector on the front panel. Make sure that the small ridge on the plug is lined up with the notch on the connector. Push the plug in and twist to lock it in position, *do not force the plug*.
2	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
3	The displays should light up and a version number is displayed on the LCD display.
4	Within a few seconds, the LCD display shows T= temperature compensation value and A = amplitude. ***Note:*** *When the A -value is less than 5000, the DOOPT Probe has approximately 3 to 4 weeks of use left. Replace the probe immediately if A -value is under 2000.*
5	After a period of 15 seconds, the display indicates the temperature in °C and P = phase angle of the DOOPT probe. The phase angle and amplitude displays alternate every 15 seconds.
6	The Process control shows the dissolved oxygen value (red). ***Note:*** *Please wait while the unit scans the probe,*
7	Calibrate the DOOPT probe before use, see *Section 4.4.3* for instructions.

Step	Action
8	Insert the DOOPT probe in the Cellbag bioreactor. ***Notes:*** *The DOOPT probe can be removed and reinserted repeatedly without any danger of contamination.* • Locate the Oxywell2 fitting on the Cellbag bioreactor.  • Remove the luer cap from the fitting. • Fill the Oxywells with liquid using the fill kit provided with the DOOPT probe. Instructions are included with the probe. The water is critical to provide a response time (designated ***t90*** in control software) of about 3 to 5 minutes. Using the DOOPT in air will prolong the time considerably. • Insert the DOOPT probe carefully into the Oxywell2. • Secure the DOOPT probe by gently tightening the luer connector.
9	For some applications, calibration of the probe after insertion into the bag is desired (this is possible if the Cellbag bioreactor has not yet been inoculated). With aeration and rocking this should correspond to the 100% saturation point.
10	Let the Cellbag bioreactor equilibrate so that the medium is at the correct temperature and at 100% air saturation.
11	Set the manual temperature compensation to the temperature of the Cellbag bioreactor and recalibrate the 100% point, as described in *Section 4.4.3*.

4.4 Maintenance

4.4.1 Maintenance frequency

Regular maintenance of the WAVE system instruments is essential for reliable results. Perform maintenance at recommended frequencies.

Frequency	Action	Section
Before and after every use	Keep instrument clean and dry	*4.4.2*
Before every cultivation	Dissolved oxygen (DO) calibration	*4.4.3*
If desired	Extend DOOPT-probe lifetime by reducing the sampling frequency	*4.4.4*
When required	Repair broken instrument. ***Note:*** All repairs should be done by service personnel authorized by GE Healthcare.	*4.4.5*

4.4.2 Cleaning

Keep the instruments dry and clean. The instruments must be turned off and unplugged before cleaning begins. Clean the exterior of the instruments with a damp cloth with water and, if required, alcohol. Do not use abrasive cleaners. Water should not be applied directly to the instruments. Make sure that the instruments are completely dry before plugging them in.

4.4.3 Calibration

The DOOPT probe should be calibrated before starting cultivation.

DO calibration

Calibration of the DOOPT probe is performed using a two-point calibration method. Set the zero oxygen and 100% air saturation level, respectively, according to the instructions below.

Note: *The DOOPT instrument compensates for sensor temperature variations and solubility effects. However, probe calibrations should be performed at the same temperature as the process measurement when possible.*

Preparations

Step	Actions
1	Power up the instrument.
2	Connect the DOOPT probe to the **PROBE** connector on DOOPT20.
3	Open a new packet of zero oxygen calibration solution (ZERO OXYSOLN). Immerse the probe tip in the solution and wait for the reading to stabilize. ***Note:*** *Nitrogen gas (N_2) may be used instead of ZERO OXYSOLN for the zero oxygen calibration.*
4	Check the temperature compensation and adjust as necessary.
5	Set the zero oxygen and 100% air saturation level, respectively, according to the instructions below.

Zero oxygen calibration using DOOPT20

Follow the instruction below to perform a zero oxygen calibration using DOOPT20.
Note: *Replace the probe If the calibration error is 10% or larger.*

Step	Actions
1	Press the **PUSH FOR MENU** button. *Result:* The display shows ***--MENU-- 1.CALIB***.
2	Press the **PUSH FOR MENU** button. *Result:* The display shows ***SET ZERO -> NO***.
3	Turn the **PUSH FOR MENU** button to change ***NO*** to ***YES***.
4	Press the **PUSH FOR MENU** button. *Result:* The display shows ***PUSH2SET P=54.95°***. (***54.95°*** is an example of the phase angle. The numerical value shown can be slightly different).
5	When the phase angle reading is stable (within ±0.25°), press the **PUSH FOR MENU** button. *Result:* The display shows ***TRY0 0*** (no of scans), ***000.00%*** (actual reading). If the display shows ***NEED 0% CAL***, re-calibration is needed.

100% oxygen calibration using DOOPT20

Follow the instruction below to perform a 100% air saturation calibration using DOOPT20.

Step	Actions
1	Check the temperature compensation and adjust as necessary.
2	Remove the probe from the zero oxygen solution. Rinse the probe in water and shake dry. Allow the probe to equilibrate in air. Do not touch the probe tip or attempt to dry it with a cloth.
3	When the reading is stable (within ±2%) in 1 to 2 minutes, press the **PUSH FOR MENU** button. *Result:* The display shows ***--MENU-- 1.CALIB***.
4	Press the **PUSH FOR MENU** button. twice *Result:* The display shows ***SET 100% -> NO***.
5	Turn the **PUSH FOR MENU** button to change ***NO*** to ***YES***.
6	Press the **PUSH FOR MENU** button. *Result:* The display shows ***PUSH2SET P=25.94°***. (***25.94°*** is an example of the phase angle. The numerical value shown can be slightly different).
7	When the phase angle reading is stable (within ±0.25°), press the **PUSH FOR MENU** button. *Result:* The display shows ***TRY0 0*** (no of scans), ***100.00%*** (actual reading). If the display shows ***NEED 100% CAL***, re-calibration is needed.

If required, 100% DO level may be determined directly in the Bioreactor. Set the manual temperature compensation to the temperature of the Cellbag bioreactor and recalibrate the 100% point, as described in *Section 4.4.3*.

4.4.4 DOOPT probe maintenance and storage

General

The DOOPT-PROBE can be reused for many months. However, due to photo bleaching, the amplitude of the luminescence reading will gradually decrease and ultimately become too low for accurate measurement. Decreasing the sampling frequency will prolong the probe life.

The DOOPT20 instrument monitors the signal amplitude automatically and will warn the user when the probe should be replaced. The DOOPT- PROBE performance decays very slowly and the instrument provides ample warning before the probe becomes unusable. No polarization or warm-up time is necessary.

The DOOPT-PROBE fiber tip and coating can be easily damaged. Avoid hitting the probe on any hard surface or prying or scraping the fiber tip.

Handle the white fiber optic cable with care. Excessive force or a severe bend can snap the cable.

Amplitude values and lifetime

When the amplitude value is below 5000, the remaining lifetime of the probe is typically 3 to 4 weeks. Change the probe when the amplitude value goes below 2000.

Extending probe life

Probe life depends on the number of cumulative oxygen readings that are taken. Reducing the frequency of measurement will increase life, conversely increasing it will shorten probe life. The sampling frequency is normally set to once every 10 seconds but can be changed from 1 to 60 seconds.

Step	Actions
1	Push the ***MENU*** button. The display should show ***MENU 1.CALIB***. 
2	Turn the ***MENU*** knob to display ***MENU 2. SETUP***. 
3	Press the ***MENU*** button to enter the ***SETUP*** submenu. 
4	Turn the ***MENU*** knob to change the sampling frequency (1 to 60s).
5	Push the ***MENU*** button to accept the new value. The instrument will return to the main display screen and operate at the new sampling frequency.

4.4.5 Instrument repair

Instrument repair should only be done by service personnel authorized by GE Healthcare.

WARNING
Electrical shock hazard. Do not open any covers or replace parts.

4.5 Troubleshooting

Error symptom	Possible cause	Corrective action
Not getting 0%	Probe not properly connected	Check that the DOOPT probe is connected and that the temperature probe is connected (red LED is not lit).
Not getting 100%	Faulty DOOPT probe	• Check that the temperature probe is connected (red LED is not lit). • Try to achieve a 0% reading using the **SPAN** knob. If this is not possible, replace the DOOPT probe.
Reading precision is poor	Some fluctuation (±5%), especially at high DO levels is normal	The fluctuation can be damped by adjusting the filter time.
Low amplitude alarm	The probe is not properly connected	Check that the probe is connected correctly and the plug secure. Cycle power to the instrument to reset.
	The probe amplitude is low	If the error persists and the amplitude is below 2000, replace the probe.

Error symptom	Possible cause	Corrective action
No response from display	The internal PC board is defective	To check operation, turn off power to DOOPT20 and disconnect the probe. Turn on power to DOOPT20 and look directly into the probe connector. A pulsing light about once a second should be flashing. If no pulsing light appears, the unit internal PC board requires replacement. Contact GE Healthcare Technical support.
NEED 0% CAL or ***NEED 100% CAL*** is displayed	The probe requires calibration	Calibrate the probe. If the problem persists, replace the probe.
Unable to select menus	If you are unable to use the **PUSH FOR MENU** button, the switch is defective	Contact GE Healthcare Technical support.

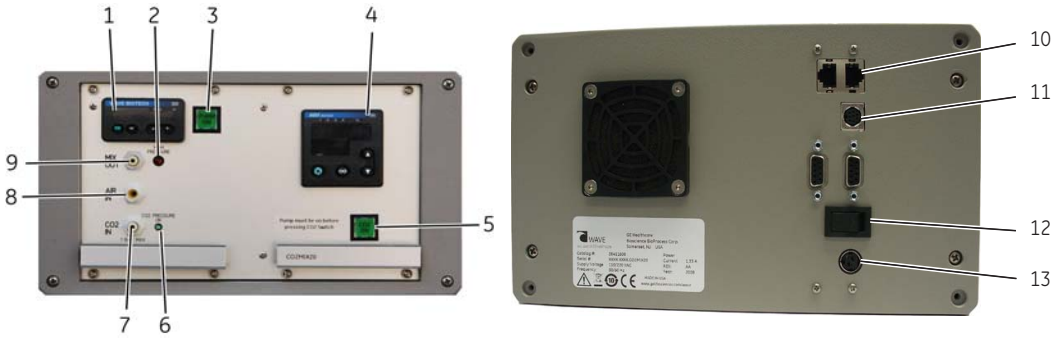
5 CO2MIX20 CO_2/air controller

5.1 Introduction/view

CO2MIX20 is used to inflate and aerate the Cellbag bioreactors with air containing a variable carbon dioxide (CO_2) concentration. CO_2 from a pipeline or cylinder is connected to the **CO2** port. Air is drawn from the **AIR IN** port and mixed with the CO_2. An internal CO_2 sensor measures the CO_2 concentration and controls the CO_2 concentration in the outlet gas to any user-specified value from 0 up to 15%. The CO_2 conditioned air is pumped from the **MIX OUT** port into the headspace of the Cellbag bioreactor to maintain bag pressure, and to provide air and carbon dioxide for aeration and pH control.

Note: CO2MIX20 is equipped with an internal bleeder valve. If the CO_2 gas supply is not turned off at the source, the CO2MIX20 will consume CO_2 gas even if the CO2MIX20 instrument is turned off and in a non-working state.

The illustration below shows the location of the main parts of CO2MIX20.



Part	Description
1	Airflow controller
2	**HIGH PRESSURE** LED
3	**PUMP ON** switch
4	Process controller
5	**CO2 ON** switch

Part	Description
6	**CO2 PRESSURE OK** LED
7	**CO2 IN** port
8	**AIR IN** port
9	**MIX OUT** port
10	Dataport Connectors

Part	Description
11	Analog output connector
12	Power switch, on (**I**), off (**O**)
13	Power connector

5.1.1 CO_2 leakage

The WAVE CO2MIX20 instrument utilizes an exhaust bleeder orifice to avoid pressure spikes during instrument operation. The CO_2 exhaust levels will remain until the pressurized CO_2 supply is removed, regardless if the instrument is powered on or off.

5.2 Installation

WARNING
Place CO2MIX20 instruments in a well ventilated environment.
Failure to do so can lead to heightened CO_2 concentrations.

Care should be taken to remove pressurized CO_2 supply when not in use.

5.2.1 Site requirements

Parameter	Requirement
Electrical power	24 V DC through an adapter (110 to 120 V~ or 220 to 240 V~, 50/60 Hz)
Placement	Stable laboratory bench
Operating temperature	4ºC to 40ºC
Humidity	<95%, non-condensing

5.2.2 Unpacking

Unpack the equipment.

Please check the packing list to see that all items on the list are included. If any parts are missing, contact your GE Healthcare representative.

Place the equipment on a stable surface.

Check the equipment for any apparent damage before starting installation. Document any damage carefully and contact your GE Healthcare representative.

5.2.3 Installation

Connection overview

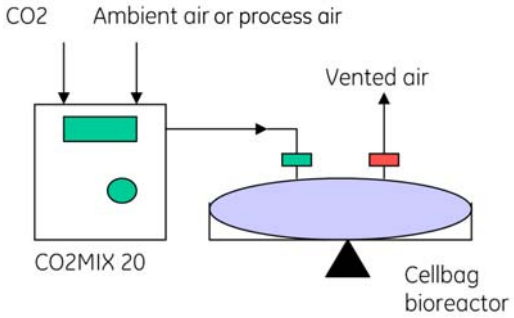


Figure 5-1. Diagram of CO2MIX20 connected to the base unit.

Step	Action
1	Make sure that the CO2MIX20 and the Cellbag bioreactor are turned off.
2	Connect the transformer to a grounded power outlet.
3	Connect the transformer to the power connector located on the rear of the instrument.
4	Press the power switch to the on (**I**) position.
5	Set the external CO_2 source between 0.7 and 1 bar (10 and 15 psig). Connect the external CO_2 source to the **CO2 IN** port on the front panel.
6	If ambient air is to be mixed with the CO_2 stream, leave the **AIR IN** port unconnected. Otherwise, connect the desired process air mixture to the **AIR IN** port. External gas pressure must be regulated to between 0.1 and 0.2 bar (1 and 3 psig).
7	Connect the **MIX OUT** port to the inlet air filter on the Cellbag bioreactor using the tubing provided.

5.2.4 Spare parts and accessories

For correct up to date information on spare parts and accessories, please refer to the web address on the back cover or contact your local GE Healthcare representative.

5.3 Operation

See *Section 5.1* for location of buttons and *Section 3* for description of Process controller.

Use as aeration pump only

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Set the air flow setpoint by the up/down arrow buttons on the airflow controller. The setpoint is shown in green. The actual airflow is shown in the red. Control range is up to 0.5 liters/minute.
4	Air is drawn from the **AIR IN** inlet connector located on the front of the instrument. Any special gas mixture (process air) can be connected here in place of room air. However, the external gas pressure *must* be regulated to between 0.1 and 0.2 bar (1 to 3 psig).

Use as CO_2/air mix controller

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Set the air flow setpoint by the up/down arrow buttons on the airflow controller. The setpoint is shown in green. The actual airflow is shown in the red. Control range is up to 0.5 liters/minute.
4	Press the **CO2 ON** switch to switch on the CO_2 controller. The switch lights up indicating that CO_2 control is active.

Step	Action
5	Select the %CO_2 desired by adjusting the setpoint on the Process controller. The setpoint is displayed in green. The actual CO_2 reading is displayed in red. The setpoint can be changed by the up/down arrow buttons.

High pressure alarm and shutoff

If the pressure at the **AIR OUT** port exceeds 0.010 bar (0.14 psig) due to blockage or obstruction of the air flow to the Cellbag bioreactor, the red **HIGH PRESSURE** LED starts blinking.

If the overpressure condition continues for longer than 1 to 2 minutes, the airpump shuts down preventing the pressure from rising higher. When the overpressure condition clears, the airpump resumes operation.

Note: CO2MIX20 is equipped with a bleeder valve. If the CO_2 gas source is not turned off the CO2MIX20 will consume CO_2 gas, even if the CO2MIX20 is turned off.

WARNING
Carbon dioxide can cause asphyxiation. Make sure that the ventilation is sufficient. Avoiding installing the unit in closed spaces.

5.4 Maintenance

5.4.1 Maintenance frequency

Regular maintenance of the WAVE system instruments is essential for reliable results. Perform maintenance at recommended frequencies.

Frequency	Action	Section
Before and after every use	Keep instrument clean and dry	*5.4.2*
Every six months	CO_2 sensor calibration	*5.4.3*
When required	Repair broken instrument. ***Note:*** All repairs should be done by service personnel authorized by GE Healthcare.	*5.4.4*

5.4.2 Cleaning

Keep the instruments dry and clean. The instruments must be turned off and unplugged before cleaning begins. Clean the exterior of the instruments with a cloth, damp with water and – if required – alcohol. Do not use abrasive cleaners. Water should not be applied directly to the instruments. Make sure that the instruments are completely dry before plugging them in.

5.4.3 Calibration

The CO_2 sensor needs to be recalibrated every six months to ensure accuracy. This can be done by returning the unit to GE Healthcare. Contact your GE Healthcare representative for more information and help.

5.4.4 Instrument repair

Instrument repair should only be done by service personnel authorized by GE Healthcare.

WARNING
Electrical shock hazard. Do not open any covers or replace parts.

5.5 Troubleshooting

Error symptom	Possible cause	Corrective action
The display shows a CO_2 concentration in air which deviates from the expected value (0.0 to 0.1%).	A minor deviation from the expected value is not unusual. The tolerance of the CO_2 sensor is ±0.38% in the range 0% to 7.5% CO_2.	Contact a service representative.
CO_2 reading keeps drifting down	The CO_2 gas supply pressure is too low	Check that the green CO_2 inlet pressure light is lit. The CO_2 supply pressure must between 0.7 and 1.0 bar (10 and 15 psig).

Error symptom	Possible cause	Corrective action
The CO_2 consumption is larger than expected.	The internal bleeder valve leaks CO_2 during system shut down.	Turn off CO_2 at the source.
Faulty CO_2 control	The CO_2 supply is not properly connected	Verify that CO_2 is connected to the correct inlet port. Check that the air pump is on and that the flow rate is between 0.1 and 0.5 liters/minute. Check that the CO_2 switch is on.
	Wrong setpoint set	Check the setpoint on the controller.
The Cellbag bioreactor does not stay inflated	No air flow	Verify that there is airflow to the Cellbag bioreactor.
	Faulty check valve	Check that the check valve on the Cellbag bioreactor is correctly installed. Close off the Cellbag bioreactor outlet using the pinch clamp and see if the Cellbag bioreactor inflates. Then confirm that the check valve is working by connecting a short length of tubing to the check valve outlet and dip it into water. Tubing should be immersed to a depth of 5 to 10 mm. Opening the outlet pinch valve should cause bubbles to appear and bubbles should continue to flow as the flow and pressure stabilizes.

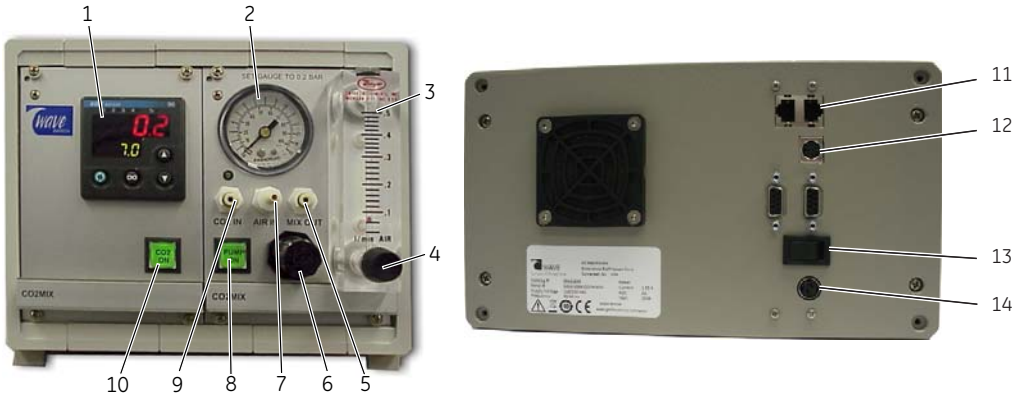
6 CO2MIX20-R CO_2/air controller

6.1 Introduction/view

CO2MIX20-R (rotameter model) is used to inflate and aerate the Cellbag bioreactors with air containing a variable carbon dioxide (CO_2) concentration. CO_2 from a pipeline or cylinder is connected to the CO2MIX20-R, which has a precision regulator to adjust the gas pressure. Air is drawn from the **AIR IN** and mixed with the CO_2 (connected to **CO2 IN**). An internal CO_2 sensor measures the CO_2 concentration and controls it to any user-specified value up to 15%. The CO_2 conditioned air is pumped from the **MIX OUT** port into the headspace of the Cellbag bioreactor to maintain bag pressure, and to provide air and carbon dioxide for aeration and pH control.

Note: CO2MIX20-R is equipped with a bleeder valve. If the CO_2 gas source is not turned off, the CO2MIX20-R will consume CO_2 gas even if the CO2MIX20-R is turned off.

The illustration below shows the location of the main parts of CO2MIX20-R.



Part	Description
1	Process controller
2	CO_2 gauge
3	Airflow rotameter
4	Flow rate adjust
5	**MIX OUT** port

Part	Description
6	CO_2 pressure regulator
7	**AIR IN** port
8	**PUMP ON** switch
9	**CO2 IN** port
10	**CO2 ON** switch

Part	Description
11	Dataport Connectors
12	Analog output connector
13	Power switch, on (**I**), off (**O**)
14	Power connector

6.1.1 CO_2 leakage

The WAVE CO2MIX20 instrument utilizes an exhaust bleeder orifice to avoid pressure spikes during instrument operation. The CO_2 exhaust levels will remain until the pressurized CO_2 supply is removed, regardless if the instrument is powered on or off.

6.2 Installation

WARNING
Place CO2MIX20-R instruments in a well ventilated environment.
Failure to do so can lead to heightened CO_2 concentrations.

Care should be taken to remove pressurized CO_2 supply when not in use.

6.2.1 Site requirements

Parameter	Requirement
Electrical power	24 V DC through an adapter (110 to 120 V~ or 220 to 240 V~, 50/60 Hz)
Placement	Stable laboratory bench
Operating temperature	4ºC to 40ºC
Humidity	<95%, non-condensing

6.2.2 Unpacking

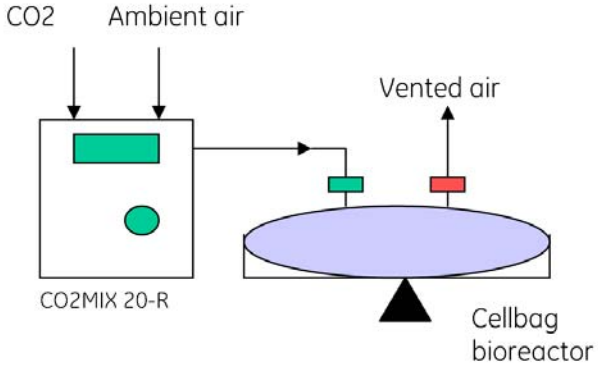
Unpack the equipment.

Please check the packing list to see that all items on the list are included. If any parts are missing, contact your GE Healthcare representative.

Place the equipment on a stable surface.

Check the equipment for any apparent damage before starting installation. Document any damage carefully and contact your GE Healthcare representative.

Connection overview



6.2.3 Installation

Step	Action
1	Make sure that the Cellbag bioreactor and the CO2MIX20-R are turned off.
2	Connect the transformer to the power connector, located on the rear of the instrument.
3	Connect the transformer to a grounded power outlet.
4	Press the power switch to the on position. Allow five minutes for the instrument to warm up and the reading to stabilize.
5	Set the external CO_2 source between 0.7 and 1.0 bar (10 and 15 psig). Connect the external CO_2 source to the **CO2 IN** port on the front panel.
6	Adjust the CO_2 pressure to 0.4 bar using the regulator knob and the and the gauge located on the front panel of the instrument. The regulator knob must be pulled to out to unlock.
7	If ambient air is to be mixed with the CO_2 stream, leave the **AIR IN** port unconnected. Otherwise, connect the desired air mixture to the **AIR IN** port. External gas pressure must be regulated to between 0.1 and 0.2 bar (1 and 3 psig).
8	Connect the **MIX OUT** port to the inlet filter on the Cellbag bioreactor using the tubing provided. The CO_2 /air mixture is pumped out from this port.

6.2.4 Spare parts and accessories

For correct up to date information on spare parts and accessories, see the web address on the back cover or contact your local GE Healthcare representative.

6.3 Operation

See *Section 6.1* for location of buttons and *Section 3* for description of Process controller.

Use as aeration pump only

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Adjust the flow rate by turning the flow rate adjust knob.
4	Air is drawn from the **AIR IN** inlet connector located on the front of the instrument. Any special gas mixture can be connected here in place of room air. External gas pressure *must* be regulated to between 0.1 and 0.2 bar (1 to 3 psig). ***Note:*** *The CO2 pressure regulator (part 6 in Section 6.1) can not be used for controlling the external gas pressure.*

Use as CO_2/air mix controller

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Press the **CO2** button to switch on the CO_2 controller. The button lights up indicating that CO_2 control is active.
4	Select the %CO_2 desired by adjusting the setpoint on the Process controller. The setpoint is displayed in green. The actual CO_2 reading is displayed in red. The setpoint can be changed by the up/down arrow buttons.

6.4 Maintenance

6.4.1 Maintenance frequency

Regular maintenance of the WAVE system instruments is essential for reliable results. Perform maintenance at recommended frequencies.

Frequency	Action	Section
Before and after every use	Keep instrument clean and dry	*6.4.2*
Every six months	CO_2 sensor calibration	*6.4.3*
When required	Repair broken instrument. ***Note:*** All repairs should be done by service personnel authorized by GE Healthcare.	*6.4.4*

6.4.2 Cleaning

Keep the instruments dry and clean. The instruments must be turned off and unplugged before cleaning begins. Clean the exterior of the instruments with a damp cloth with water and, if required, alcohol. Do not use abrasive cleaners. Water should not be applied directly to the instruments. Make sure that the instruments are completely dry before plugging them in.

6.4.3 Calibration

The CO_2 sensor needs to be recalibrated every six months to ensure accuracy. This may be done by returning the unit to the GE Healthcare. Contact GE Healthcare for more information and help.

6.4.4 Instrument repair

Instrument repair should only be done by service personnel authorized by GE Healthcare.

WARNING
Electrical shock hazard. Do not open any covers or replace parts.

6.5 Troubleshooting

Error symptom	Possible cause	Corrective action
The display shows a CO_2 concentration in air which deviates from the expected value (0.0 to 0.1%).	A minor deviation from the expected value is not unusual. The tolerance of the CO_2 sensor is ±0.38% in the range 0% to 7.5% CO_2.	Contact a service representative.
CO_2 reading keeps drifting down	The CO_2 gas supply pressure is too low	Check that the green CO_2 inlet pressure light is lit. The CO_2 supply pressure must between 0.7 and 1.0 bar (10 and 15 psig).
Faulty CO_2 control	The CO_2 supply is not properly connected	Verify that CO_2 is connected to the correct inlet port. Check that the air pump is on and that the flow rate is between 0.1 and 0.5 liters/minute. Check that the CO_2 switch is on.
	Wrong setpoint set	Check the setpoint on the controller.
The CO_2 consumption is larger than expected.	The internal bleeder valve leaks CO_2 during system shut down.	Turn off CO_2 at the source.
No reading on rotameter	Obstructed airflow	Check that airflow to the Cellbag bioreactor is not obstructed. Verify that the pump is working by disconnecting the **MIX OUT** coupling on the instrument and the Cellbag.

Error symptom	Possible cause	Corrective action
The Cellbag bioreactor does not stay inflated	No air flow	Verify that there is airflow to the Cellbag bioreactor.
	Faulty check valve	Check that the check valve on the Cellbag bioreactor is correctly installed. Close off the Cellbag bioreactor outlet using the pinch clamp and see if the Cellbag bioreactor inflates. Then confirm that the check valve is working by connecting a short length of tubing to the check valve outlet and dip it into water. Tubing should be immersed to a depth of 5 to 10 mm. Opening the outlet pinch valve should cause bubbles to appear and bubbles should continue to flow as the flow and pressure stabilizes.

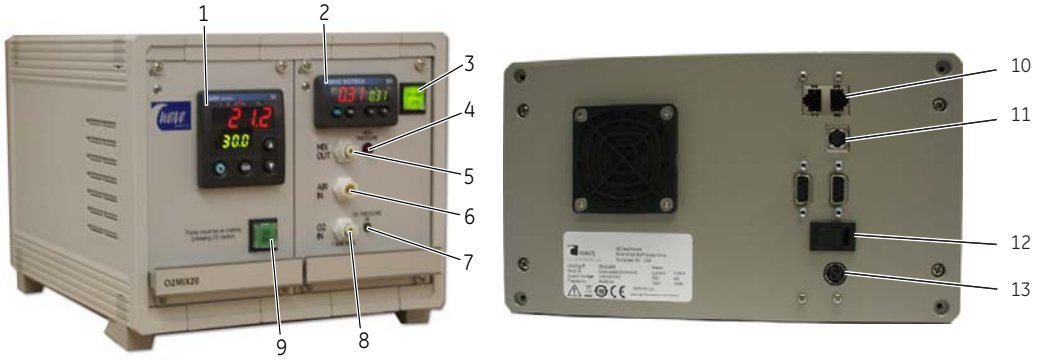
7 O2MIX20 O_2/air controller

7.1 Introduction/view

O2MIX20 is used to inflate and aerate the Cellbag bioreactors with air containing a variable O_2 concentration. O_2 from a pipeline or cylinder is connected to O2MIX20. Air is drawn from the **AIR IN** port and mixed with the O_2 (connected to **O2 IN**). An O_2 sensor measures the O_2 concentration and controls it to any user-specified value from ambient O_2 up to 50%. The O_2 conditioned air is pumped from the **MIX OUT** port into the headspace of the Cellbag bioreactor to maintain bag pressure and provide oxygen to the culture.

Note: O2MIX20 is equipped with a bleeder valve. If the O_2 gas source is not turned off at the source, the O2MIX20 will consume O_2 gas even when the instrument is turned off.

The illustration below shows the location of the main parts of O2MIX20.



Part	Description
1	Process controller
2	Airflow controller
3	**PUMP ON** switch
4	**HIGH PRESSURE** LED
5	**MIX OUT** port

Part	Description
6	**AIR IN** port
7	**O2 PRESSURE OK** LED
8	**O2 IN** port
9	**O2 ON** switch
10	Dataport Connectors

Part	Description
11	Analog output connector
12	Power switch, on (**I**), off (**O**)
13	Power connector

7.2 Installation

7.2.1 Site requirements

Parameter	Requirement
Electrical power	24 V DC through an adapter (110 to 120 V~ or 220 to 240 V~, 50/60 Hz)
Placement	Stable laboratory bench
Operating temperature	4ºC to 40ºC
Humidity	<95%, non-condensing

7.2.2 Unpacking

Unpack the equipment.

Please check the packing list to see that all items on the list are included. If any parts are missing, contact your GE Healthcare representative.

Place the equipment on a stable surface.

Check the equipment for any apparent damage before starting installation. Document any damage carefully and contact your GE Healthcare representative.

7.2.3 Installation

WARNING
Fire hazard. Oxygen can leak out and cause fire. Do not open any covers or replace parts. Shut off the O_2 source when not in use.

Step	Action
1	Make sure that the O2MIX20 and the Cellbag bioreactor are powered off.
2	Connect the transformer to a grounded power outlet.
3	Connect the transformer to the power connector located on the rear of the instrument.

Step	Action
4	Press the power switch to the on position. Allow five minutes for the instrument to warm up and the reading to stabilize.
5	Set the external O_2 source between 0.7 and 1.0 bar (10 and 15 psig). Connect the external O_2 source to the **O2 IN** port on the front panel.
6	If ambient air is to be mixed with O_2, leave the **AIR IN** port unconnected. If process air is desired, connect this air to the **AIR IN** port. External gas pressure must be controlled to between 0.1 and 0.2 bar (1 and 3 psig).
7	Connect the **MIX OUT** port to the inlet filter on the Cellbag bioreactor using the tubing provided with the O2MIX instrument.

7.2.4 Spare parts and accessories

For correct up to date information on spare parts and accessories, please refer to the web address on the back cover or contact your local GE Healthcare representative.

7.3 Operation

See *Section 7.1* for location of buttons and *Chapter 3* for description of Process controller.

Use as aeration pump only

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Set the air flow setpoint by the up/down arrow buttons on the airflow controller. The setpoint is shown in green. The actual airflow is shown in the red. Control range is up to 0.5 liters/minute.
4	Air is drawn from the **AIR IN** inlet connector located on the front of the instrument. Any special gas mixture, such as process air, can be connected here in place of ambient air. External gas pressure *must* be controlled to between 0.1 and 0.2 bar (1 to 3 psig).

Use as O_2/air mix controller

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Set the air flow setpoint by the up/down arrow buttons on the airflow controller. The setpoint is shown in green. The actual airflow is shown in the red. Control range is up to 0.5 liters/minute.
4	Press the **O2 ON** switch to switch on the O_2 controller. The switch lights up indicating that O_2 control is active.
5	Select the %O_2 desired by adjusting the setpoint on the Process controller. The setpoint is displayed in green. The actual O_2 reading is displayed in red. The setpoint can be changed by the up/down arrow buttons.

High pressure alarm and shutoff

If the pressure at the **AIR OUT** port exceeds 7.5 mbar (0.11 psig) due to blockage or obstruction of the air flow to the Cellbag bioreactor, the red **HIGH PRESSURE** LED starts blinking. If the overpressure condition continues for longer than 1 to 2 minutes, the internal air flow valve shuts down preventing the pressure from rising higher. When the overpressure condition clears, the airpump resumes operation.

Note: O2MIX20 is equipped with a bleeder valve. If the O_2 gas source is not turned off the O2MIX20 will consume O_2 gas even if the O2MIX20 is turned off.

7.4 Maintenance

7.4.1 Maintenance frequency

Regular maintenance of the WAVE system instruments is essential for reliable results. Perform maintenance at recommended frequencies.

Frequency	Action	Section
Before and after every use	Keep instrument clean and dry	*7.4.2*
Every six months	O_2 sensor calibration	*7.4.3*

Frequency	Action	Section
When required	Repair broken instrument. ***Note:*** All repairs should be done by service personnel authorized by GE Healthcare.	7.4.4

7.4.2 Cleaning

Keep the instruments dry and clean. The instruments must be turned off and unplugged before cleaning begins. Clean the exterior of the instruments with a damp cloth with water and, if required, alcohol. Do not use abrasive cleaners. Water should not be applied directly to the instruments. Make sure that the instruments are completely dry before plugging them in.

7.4.3 Calibration

The O_2 sensor does not need to be calibrated. Over time (10 years typical), the sensor will decay and will need to be replaced. Calibration can easily be checked by turning the control function off. Turn the pump on so that air is introduced to the sensor. The display should show 21% oxygen. (+/-0.6%).

For recalibration or sensor replacement please contact GE Healthcare for return of the unit for service.

7.4.4 Instrument repair

Instrument repair should only be done by service personnel authorized by GE Healthcare.

WARNING
Electrical shock hazard. Do not open any covers or replace parts.

7.5 Troubleshooting

Error symptom	Possible cause	Corrective action
The display shows a O_2 concentration in air which deviates from the expected value (21.0%).	A minor deviation from the expected value is not unusual. The tolerance of the O_2 sensor is ±0.6% in the range 21% to 40% O_2.	If the reading is not within the tolerance, the O_2 offset can be adjusted.
O_2 reading keeps drifting down	The O_2 gas supply pressure is too low	Check that the green O_2 inlet pressure light is lit. The O_2 supply pressure must between 0.7 and 1.0 bar (10 and 15 psig).
The O_2 consumption is larger than expected.	The internal bleeder valve leaks O_2 during system shut down.	Turn off O_2 at the source when the O2MIX20 instrument is not used.
Faulty O_2 control	The O_2 supply is not properly connected	Verify that O_2 is connected to the correct inlet port. Check that the O_2 supply pressure is set to 0.7 bar minimum. Check that the air pump is on and that the flow rate is between 0.1 and 0.5 liters/ minute. Check that the O_2 switch is on.
	Wrong setpoint set	Check the setpoint on the controller.

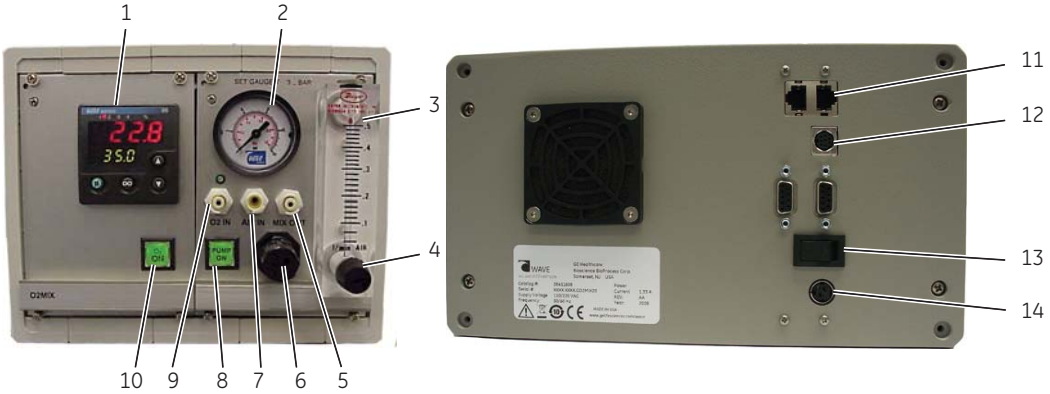
8 O2MIX20-R O_2/air controller

8.1 Introduction/view

O2MIX20-R (rotameter model) is used to inflate and aerate Cellbag bioreactors with air containing a variable O_2 concentration. O_2 from a pipeline or cylinder is connected to O2MIX20-R which has a precision regulator to adjust the gas pressure. Air is drawn from the **AIR IN** port and mixed with the O_2 (connected to **O2 IN**). An O_2 sensor measures the O_2 concentration and controls it to any user-specified value from 21% to 50%. The O_2 conditioned air is pumped from the **MIX OUT** port into the headspace of the Cellbag bioreactor to maintain bag pressure, and to provide oxygen to the culture.

Note: O2MIX20-R is equipped with a bleeder valve. If the O_2 gas source is not turned off the O2MIX20-R will consume O_2 gas even if the O2MIX20-R instrument is turned off.

The illustration below shows the location of the main parts of O2MIX20-R.



Part	Description
1	Process controller
2	O_2 gauge
3	Airflow rotameter
4	Flow rate adjust
5	**MIX OUT** port

Part	Description
6	O_2 pressure regulator
7	**AIR IN** port
8	**PUMP ON** switch
9	**O2 IN** port
10	**O2 ON** switch

Part	Description
11	Dataport Connectors
12	Analog output connector
13	Power switch, on (**I**), off (**O**)
14	Power connector

8.2 Installation

8.2.1 Site requirements

Parameter	Requirement
Electrical power	24 V DC through an adapter (110 to 120 V~ or 220 to 240 V~, 50/60 Hz)
Placement	Stable laboratory bench
Operating temperature	4ºC to 40ºC
Humidity	<95%, non-condensing

8.2.2 Unpacking

Unpack the equipment.

Please check the packing list to see that all items on the list are included. If any parts are missing, contact your GE Healthcare representative.

Place the equipment on a stable surface.

Check the equipment for any apparent damage before starting installation. Document any damage carefully and contact your GE Healthcare representative.

8.2.3 Installation

WARNING
Fire hazard. Oxygen can leak out and cause fire. Do not open any covers or replace parts. Shut off the O_2 source when not in use.

.

Step	Action
1	Make sure that the O2MIX20-R and the Cellbag bioreactor are powered off.
2	Connect the transformer to a grounded power outlet.
3	Connect the transformer to the power connector located on the rear of the instrument.

Step	Action
4	Press the power switch to the on position. Allow five minutes for the instrument to warm up and the reading to stabilize.
5	Set the external O_2 source between 0.7 and 1.0 bar (10 and 15 psig). Connect the external O_2 source to the **O2 IN** port on the front panel.
6	Adjust the O_2 pressure to 0.4 bar using the regulator knob and the and the gauge located on the front panel of the instrument. The regulator knob must be pulled to out to unlock.
7	If ambient air is to be mixed with the O_2 stream, leave the **AIR IN** port unconnected. Otherwise, connect the desired air mixture to the **AIR IN** port. External gas pressure must be regulated to between 0.1 and 0.2 bar (1 and 3 psig).
8	Connect the **MIX OUT** port to the inlet filter on the Cellbag bioreactor using the tubing provided. The O_2 /air mixture is pumped out from this port.

8.2.4 Spare parts and accessories

For correct up to date information on spare parts and accessories, see the web address on the back cover or contact your local GE Healthcare representative.

8.3 Operation

See *Section 8.1* for location of buttons and *Section 3* for description of Process controller.

Use as aeration pump only

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Adjust the flow rate by turning the flow rate adjust knob.

Step	Action
4	Air is drawn from the **AIR IN** inlet connector located on the front of the instrument. Any special gas mixture can be connected here in place of room air. External gas pressure *must* be regulated to between 0.1 and 0.2 bar (1 to 3 psig).

Use as O2/air mix controller

Step	Action
1	Turn the power switch on (**I**). The power switch is located on the back of the stand-alone instrument.
2	Press the **PUMP ON** switch to start the air pump.
3	Adjust the flow rate by turning the flow rate adjust knob.
4	Press the **O2** button to switch on the O_2 controller. The button lights up indicating that O_2 control is active.
5	Select the %O_2 desired by adjusting the setpoint on the Process controller. The setpoint is displayed in green. The actual O_2 reading is displayed in red. The setpoint can be changed by the up/down arrow buttons.

8.4 Maintenance

8.4.1 Maintenance frequency

Regular maintenance of the WAVE system instruments is essential for reliable results. Perform maintenance at recommended frequencies.

Frequency	Action	Section
Before and after every use	Keep instrument clean and dry	*8.4.2*
Every six months	O2 sensor calibration	*8.4.3*

Frequency	Action	Section
When required	Repair broken instrument. ***Note:*** All repairs should be done by service personnel authorized by GE Healthcare.	8.4.4

8.4.2 Cleaning

Keep the instruments dry and clean. The instruments must be turned off and unplugged before cleaning begins. Clean the exterior of the instruments with a damp cloth with water and, if required, alcohol. Do not use abrasive cleaners. Water should not be applied directly to the instruments. Make sure that the instruments are completely dry before plugging them in.

8.4.3 Calibration

The O_2 sensor does not need to be calibrated. Over time (10 years typical), the sensor will decay and will need to be replaced. Calibration can easily be checked by turning the control function off. Turn the pump on so that air is introduced to the sensor. The display should show approximately 21% oxygen. (+/-0.6%).

For recalibration or sensor replacement please contact GE Healthcare for return of the unit for service.

8.4.4 Instrument repair

Instrument repair should only be done by service personnel authorized by GE Healthcare.

WARNING
Electrical shock hazard. Do not open any covers or replace parts.

8.5 Troubleshooting

Error symptom	Possible cause	Corrective action
The display shows a O_2 concentration in air which deviates from the expected value (21.0%).	A minor deviation from the expected value is not unusual. The tolerance of the O_2 sensor is ±0.6% in the range 21% to 40% O_2.	If the reading is not within the tolerance, the O_2 offset can be adjusted.
O_2 reading keeps drifting down	The O_2 gas supply pressure is too low	Check that the green O_2 inlet pressure light is lit. The O_2 supply pressure must between 0.7 and 1.0 bar (10 and 15 psig).

Error symptom	Possible cause	Corrective action
Faulty O_2 control	The O_2 supply is not properly connected	Verify that O_2 is connected to the correct inlet port. Check that the O_2 supply pressure is set to 0.7 bar minimum. Check that the air pump is on and that the flow rate is between 0.1 and 0.5 liters/ minute. Check that the O_2 switch is on.
	Wrong setpoint set	Check the setpoint on the controller.
The O_2 consumption is larger than expected.	The internal bleeder valve leaks O_2 during system shut down.	Turn off O_2 at the source.
No reading on rotameter	Obstructed airflow	Check that airflow to the Cellbag bioreactor is not obstructed. Verify that pump is working by disconnecting the **MIX OUT** coupling on the instrument.

9 PUMP20 peristaltic feed/harvest or acid/base pump

9.1 Introduction/view

PUMP20 is a peristaltic pump. It is intended for use as a feed/harvest pump or for acid/base additions for the WAVE Bioreactor systems. PUMP20 can be placed on either side of a WAVE Bioreactor system and is available in two versions, PUMP20-L facing left and PUMP20-R facing right.

The illustration below shows the location of the main parts of PUMP20.

Part	Description
1	**PUMP ON** switch
2	**RUN** switch

Part	Description
3	Digital speed control buttons
4	24 V DC power connector

Part	Description
5	Dataport Connectors

9.2 Installation

9.2.1 Site requirements

Parameter	Requirement
Electrical power[1]	24 V DC through an adapter (110 to 120 V~ or 220 to 240 V~, 50/60 Hz)
Placement	Stable laboratory bench
Operating temperature	4ºC to 40ºC
Humidity	<95%, non-condensing

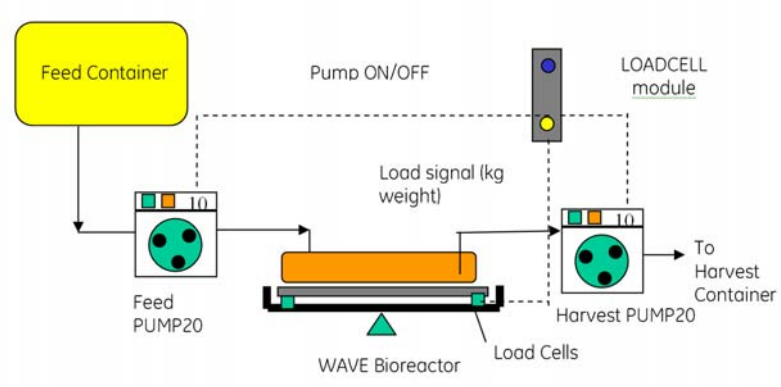
[1] The pump runs on 24 V and can be powered by WAVEPOD II without a transformer.

9.2.2 Unpacking

Step	Action
1	Unpack the equipment.
2	Please check the packing list to see that all items on the list are included. If any parts are missing, contact your GE Healthcare representative.
3	Place the equipment on a stable surface.
4	Check the equipment for any apparent damage before starting installation. Document any damage carefully and contact your GE Healthcare representative.

9.2.3 Installation

Overview diagram



When used with a LOADCELL equipped WAVE Bioreactor 20/50, PUMP20 can be automatically switched on and off to deliver a set feed rate or controlled harvest. When used with a shorting plug, PUMP20 can be used as a variable speed standalone pump.

Installation instruction

Follow any of the instructions below to install the peristaltic feed/harvest pump.

Use with WAVE Bioreactor 20/50

Step	Action
1	Make sure that the WAVE Bioreactor 20/50 and PUMP20 are powered off.
2	Connect the supplied DB9M-M cable to the connector on the rear of PUMP20.
3	Connect the other end of the cable to the **FEED PUMP** or **HARVEST PUMP** connector on the rear panel of the WAVE Bioreactor 20/50.

Standalone use

Step	Action
1	Make sure that the PUMP20 is powered off.
2	Connect a power cord from the power adapter to the power connector located on the rear panel of PUMP20.
3	Connect the local shorting plug to the DB9 connector on the rear panel of PUMP20. This will permit local operation.

Step	Action
4	Connect the mains power cord from the power adapter to an appropriate mains power outlet.

9.2.4 Spare parts and accessories

For correct up to date information on spare parts and accessories, see the web address on the back cover or contact your local GE Healthcare representative.

9.3 Operation

See *Section 9.1* for location of buttons.

CAUTION
Pinch Hazard Remain clear of all moving parts and do not open the cover during operation. Make sure that the pump is switched off when working on the moving parts.

Step	Action
1	• *Left-hand versions of PUMP20*: Feed the tubing so that the **inlet** side is located at the **lower** clip and the outlet in the upper clip. • *Right-hand versions of PUMP20*: Feed the tubing so that the **inlet** side is located at the **upper** clip and the outlet in the lower clip.
2	Rotate the pumphead and ensure the tubing is securely held with no slack. The roller tension should be adjusted. Adjust the clips so that the tubing is not pulled into the pump when the head rotates. ***Note:*** *The pump always runs in a clockwise direction.*
3	Press the **PUMP ON** switch to turn power on. The **PUMP ON** button is lit. • If connected to a BASE20/50EHT, the pump does not run unless this device sends a REMOTE ON signal through the DB9 connecting cable. The pump runs at the speed set on the panel digital indicator. • If the shorting plug is installed, then the pump runs at the speed set on the panel digital indicator.
4	Press the **RUN** button to run the pump at full speed as long as the button is held down. **PUMP ON** switch indicator light must be on (green). This function is useful for priming tubing.

Step	Action
5	The **RUN** button lights up whenever the pump is running.
6	The pump speed can be set 0% to 99% by pushing the +/- buttons on the digital speed control.

9.4 Maintenance

9.4.1 Maintenance frequency

Regular maintenance of the WAVE system instruments is essential for reliable results. Perform maintenance at recommended frequencies.

Frequency	Action	Section
Before and after every use	Keep instrument clean and dry	*9.4.2*
Before every use	Adjust pump roller tension	*9.4.3*
When required	Repair broken instrument. ***Note:*** All repairs should be done by service personnel authorized by GE Healthcare.	*9.4.4*

9.4.2 Cleaning

Keep the instruments dry and clean. The instruments must be turned off and unplugged before cleaning begins. Clean the exterior of the instruments with a damp cloth with water and, if required, alcohol. Do not use abrasive cleaners. Water should not be applied directly to the instruments. Make sure that the instruments are completely dry before plugging them in.

9.4.3 Pumphead tensioning

CAUTION
Pinch Hazard Make sure that the pump is switched off when working on the moving parts.

It is important that the pump roller tension be adjusted.

- If the tension is too low, then liquid will bypass the roller and the flow rate will be low.
- If the tension is too high then pump power will be wasted. There is also a risk of wearing down the tubing during log time usage which will lead to rupture of the tubing.

The roller tension can be adjusted using a screwdriver. Adjust the spring tension and rotate the pumphead by hand. The tubing should be completely squeezed as the pumphead rotates but must not require excessive effort to turn.

9.4.4 Instrument repair

Instrument repair should only be done by service personnel authorized by GE Healthcare.

9.5 Troubleshooting

Error symptom	Possible cause	Corrective action
The **PUMP ON** button on the front panel of PUMP20 is not lit	The pump is not powered	• Make sure that the PUMP20 is plugged in. • If using a WAVE Bioreactor 20/50 instrument for power, check that the WAVE Bioreactor 20/50 instrument is turned on. • For standalone use, make sure that PUMP20 is properly connected to an external power supply.

Error symptom	Possible cause	Corrective action
PUMP20 does not run	A shorting plug is not connected	For standalone use, make sure that a shorting plug is connected to the DB9 connector on the rear panel of PUMP20
The pump speed does not change	The speed settings are not correct	• Check the speed settings on the front panel of the PUMP20. • Press the **RUN** button to run the pump all full speed regardless of the speed setting.

10 Reference information

This chapter contains technical data for the stand-alone instruments in this manual.

10.1 Specifications

DOOPT20

Parameter	Value
Supply voltage	24 VDC through an adapter (100-120 V ~ or 220-240 V ~, 50 to 60 Hz)
Dimensions (h × w × d)	170 × 117 × 265 mm
Weight	1.5 kg
Operating temperature	4 to 40°C

CO2MIX20 and CO2MIX20-R

Parameter	Value
Supply voltage	24 VDC through an adapter (100-120 V ~ or 220-240 V ~, 50 to 60 Hz)
Dimensions (h × w × d)	200 × 320 × 170 mm
Weight	2.7 kg
Operating temperature	4 to 40°C
Max pressure CO_2	1 bar
Max air pressure	0.2 bar

O2MIX20 and O2MIX20-R

Parameter	Value
Supply voltage	24 VDC through an adapter (100-120 V ~ or 220-240 V ~, 50 to 60 Hz)
Dimensions (h × w × d)	200 × 320 × 170 mm
Weight	2.7 kg
Operating temperature	4 to 40°C
Max pressure O_2	1 bar
Max air pressure	0.2 bar

PUMP20

Parameter	Value
Supply voltage	24 VDC through an adapter (100-120 V ~ or 220-240 V ~, 50 to 60 Hz)
Dimensions (h × w × d)	108 × 103 × 193 mm
Weight	1.0 kg
Operating temperature	4 to 40°C

Power adapter

Parameter	Value
Supply voltage	100-240 V ~, 47 to 63 Hz
Supply current	Max 1.0 A
Output voltage	24 VDC +/- 5%
Output current	Max 1.66 A
Power	40 W
Outlet	Kycon 3 pin connector fir on 1.2 m shielded cable (Pin 1 +, Pin 2 - and house to shield)
Dimensions (h × w × d)	34.5 × 52 × 118 mm
Operating temperature	4 to 40°C

10.2 Ordering information

For ordering information, visit www.gelifesciences.com/wave.

Appendix A Communications and data acquisition

A.1 Overview

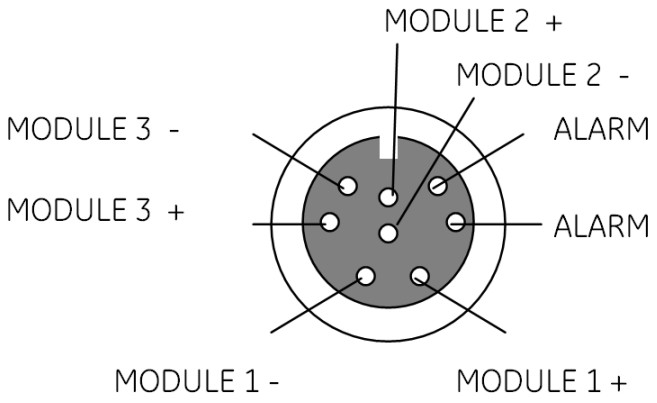
Two options for data communications are built into the stand-alone instruments:

1 **ANALOG**: The measured parameters are available on the back panel as voltages for driving chart recorders. These measurements are direct from adjusted sensors and simplify validation as they are not digitally processed. In addition, a unit alarm is available on the back panel jack. This is a normally closed contact that will open if any of the stand-alone instruments are in alarm. This is a dry contact output that can be used to interface to user alarm monitoring systems.

2 **DIGITAL**: Digital communication ports are provided on the back panel for RS-485 multidrop data acquisition. The digital interface provides seamless interfacing with GE Healthcare instrumentation and other WAVE Bioreactor instruments.

A.2 Analog/Alarm port

A 8pin female mini-DIN jack on the back panel provides analog signals and the alarm contact. Use a standard 8pin male mini-DIN cable to connect to this port.



A.3 Analog signals

Various signals are available as analog voltages depending on the modules installed. The signals and ranges are shown in the table below.

The default module shows typical wiring. Units may vary depending on user options chosen. Please refer to the pinout sticker on the back of the instrument. The ALARM connections are dry-contact.

PARAMETER	Voltage Range	Span	Default Module #
Weight	1 to 5 volts	0 to 40 kg	Module #1
CO_2	1 to 5 volts	0 to 15%	Module #2
O_2	1 to 5 volts	0 to 50%	Module #2
DO	1 to 5 volts	0 to 500%	Module #3

A.4 Alarm contact

A unit alarm is provided. This is a normally closed dry contact that will open if any of the following alarm conditions occur:

PARAMETER	Default value
Max current	30 V (RMS) / 70 mA (RMS)
DO High Limit	150%
DO Low Limit	40%
Weight High Deviation	+0.5 kg
Weight Low Deviation	- 0.5 kg
CO2 High Deviation	+2%
CO2 Low Deviation	- 2%
O2 High Deviation	+2%
O2 Low Deviation	- 2%

Deviation alarms are relative to the setpoint. If the setpoint is changed the alarms will be adjusted automatically to be relative to the new setpoint. Limit alarms are absolute values.

They can be changed by accessing the ALM menu on the controllers and editing the A3Lo and A3Hi settings.

A.5 Digital communication protocol

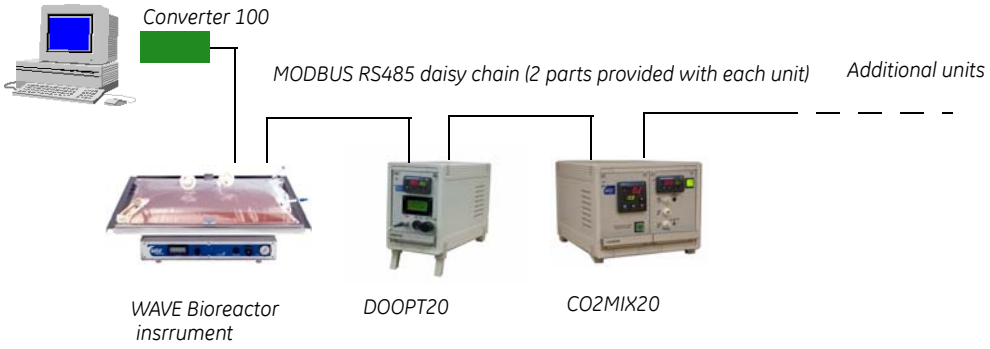
There are two digital ports on the back panel. These are RJ11-6 telephone style jacks. Both jacks are wired internally in parallel to facilitate daisy-chain wiring. The communications parameters are:

- 9600 baud
- No Parity
- 8 data bits
- 1 stop bit

The communication protocol used is standard MODBUS RTU.

A.6 Communication wiring

Multiple units and instruments may be daisy-chained together to form a RS-485 network. Each standalone unit has two 6-pin RJ12 connectors for MODBUS located on the rear panel. One cable is supplied with each standalone unit.



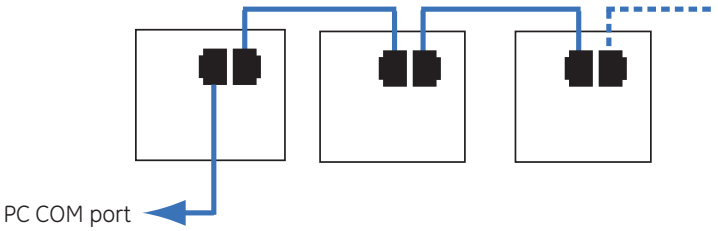
Connector labeled **Primary** or **Secondary** on the rear panel of Converter 100.

Note: *Standalone units can not be used when a WAVEPOD II unit is connected to the converter.*

Daisychaining standalone units

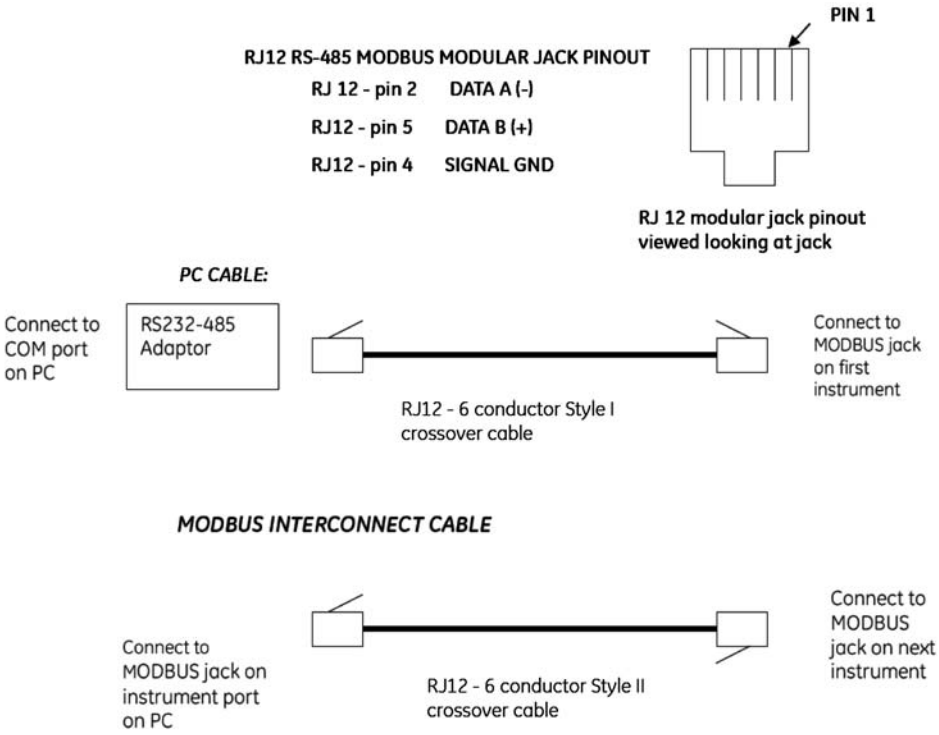
Add another standalone unit to the chain by connecting a MODBUS cable between an RJ12 connector of one unit to an RJ12 connector of the next unit.

Standalone units



MODBUS cable pinout

One cable is supplied with each standalone instrument..



A.7 Digital instrument addressing

Each instrument on the RS-485 MODBUS link must be configured with a unique address from 1 to 247. The default addresses are:

DOOPT20	1
CO2MIX20	3
O2MIX20	7
AIRPUMP	8*
BASE20/50EHT	10

* Only in CO2MIX20 and O2MIX20

Several instruments may be connected to a single PC COM port by daisychaining. All instruments in the chain must have a unique address. The default address of a unit is the same for all units of the same type.

- As long as there is only one instrument of a certain type in the chain, the default address need not be changed.
- If an instrument is to be added to the chain, and the chain already contains an instrument of the same type, the default address of one of the units must be changed.:

Tag	Address	Location
DOOPT20	1	First DO instrument
DOOPT20-02	11	Second DO instrument
CO2MIX-01	3	First CO2 controller
CO2MIX-02	13	Second CO2 controller
Base20/50EHT	10	Third unit

If certain instruments are not present in your configuration, their addresses can be left blank.

Appendix B DOOPT20 measurement theory

B.1 Introduction

The DOOPT20 is used to monitor dissolved oxygen (DO) levels in Cellbag bioreactors.

The DOOPT20 instrument uses an optical oxygen measurement DOOPT probe. The principle of operation is based on the oxygen quenching of luminescence of dye molecules immobilized in the probe.

A quenching reaction occurs during excitation by sinusoidally modulated light. As a consequence, there is a phase angle shift which can be measured and be correlated to the amount of oxygen in the sample.

Figure 10-1. DOOPT20 Instrument Module

The major advantages of phase-angle based oxygen sensing over conventional Clark-type electrodes are:

1 No oxygen is consumed in the measurement

2 Fast response time (t_{90} < 10 s)

3 Can measure oxygen in gas or dissolved in liquids

4 Miniature size due to fiber optic technology

5 Long life with little drift

6 Not prone to drying out

In the WAVE Bioreactor application, the DOOPT-PROBE is used in the proven Oxywell configuration. The bare probe with its fast response time can be used for dynamic oxygen transfer measurements.

B.2 Measurement theory

The DOOPT20 instrument measures the luminescence decay time of the immobilized luminophore on the tip of the DOOPT-PROBE. The luminophore is typically a transition metal complex of Ruthenium. On excitation by incident light, the luminophore emits light. In the presence of a quencher, such as molecular oxygen, the luminophore becomes deactivated and does not emit light. A relationship exits between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime. This relationship is described in the Stern-Volmer equation:

$$\tau = f([O_2])$$

or

$$(\tau_0)/\tau = 1 + K_{sv}[O_2]$$

Where τ_0 is the luminescence decay time in the absence of oxygen

τ is the luminescence decay time in the presence of oxygen

K_{sv} is the Stern-Volmer constant and depends on the immobilized dye

$[O_2]$ is the oxygen concentration

While it is possible to estimate the oxygen concentration by measuring the luminescence intensity, the results are not very reliable. The intensity varies with time as the sensor photobleaches. It is also sensitive to ambient light. In addition, the slope of the intensity response becomes quite shallow with oxygen concentrations greater the 60% and this leads to very poor accuracy and reproducibility above this oxygen concentration.

For these reasons, the DOOPT20 instrument does not use luminescence intensity in order to estimate oxygen concentration. Instead, the luminescence decay time is measured using a phase-modulation technique. The luminophore is excited with sinusoidally modulated light. The decay time of the luminophore causes a delay in the emitted light signal. This delay is manifested in a shift in the phase angle between the excited and the emitted light signal. The amount of this phase shift is a function of the oxygen concentration as shown in the following equation:

$$\tau = \frac{\tan\Phi}{2\pi f_{mod}} \text{ or } \tan\Phi = 2\pi f_{mod}\tau$$

where Φ is the phase angle and f_{mod} the modulation frequency

Measurement of the phase shift has several advantages:

- Variations in the indicator dye luminescence or in the intensity of the light source do not effect the measurement.
- The decay time is not influenced by bending of the fiber or tip geometry.
- The measurement is not significantly influenced by the concentration of the dye. This reduces the impact of photobleaching and leaching of the dye.
- The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and color.

The oxygen measurement is reported at % of air saturation. Air is referenced to 20.95% oxygen, which corresponds to 100% saturation. Since the Stern-Volmer equation is highly non-linear, appropriate linearization calculations are performed by the microprocessor inside the DOOPT20 instrument.

Water vapor influences the oxygen partial pressure in air and for most accurate results, the probe calibration should be made in air-saturated water or water-saturated air.

B.3 Effect of temperature

Temperature affects the luminescence decay time as well as the intensity. The diffusion of oxygen is greater at higher temperatures and this also causes variations.

In addition, solubility of oxygen in water is temperature dependent and can be described by Henry's law.

B.4 Measurement range

The oxygen measurement range is from 0 to 250% air-saturation. The phase resolution is better than 0.05°. The accuracy of the measurement is as follows:

Parameter	Value
1 to 30% air-saturation	±0.2%
30 to > 100%	±0.5%
100 to > 250%	±2.0%
Temperature range	-10°C to +80°C

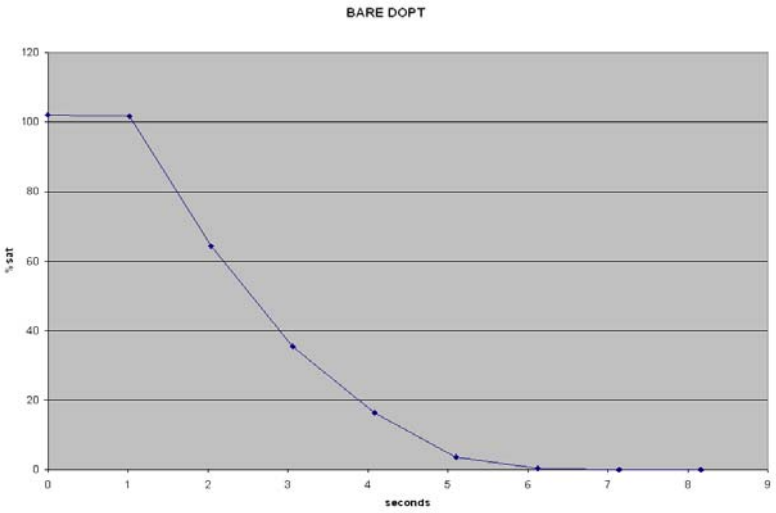
B.5 Cross-sensitivity

There is no cross sensitivity for CO_2. Increasing the salt (chloride) concentration decreases the solubility of oxygen. This is why it is best to readjust the 100% point in the actual culture fluid (in Oxywell2) once the system temperature has equilibrated. Physiological saline is used in the Oxywell2 to minimize any salt concentration artifacts.

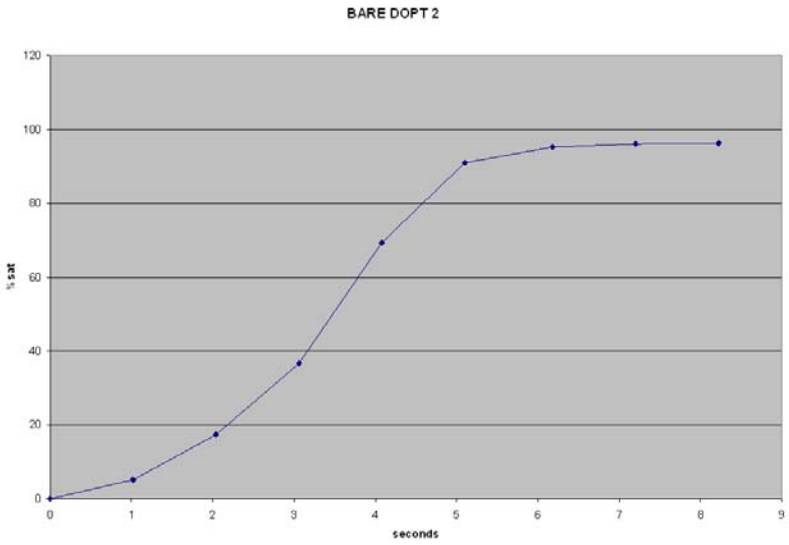
B.6 Response time

B.6.1 Bare probe

The bare probe has a response time (t_{90}) of about 4 to 5 seconds. The graph below shows the response going from air saturation to 0% oxygen solution.

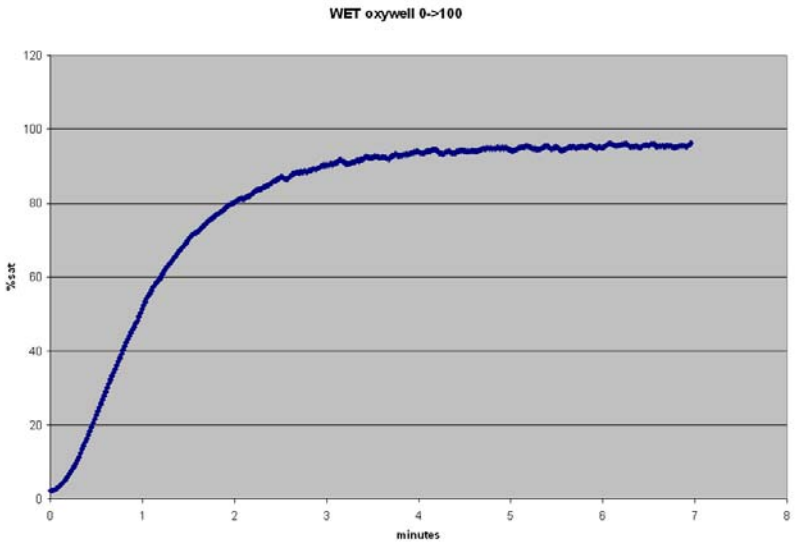


The graph below shows the response time going from 0% oxygen to air saturation. The response time (t_{90}) is about the same (4 to 5 seconds).

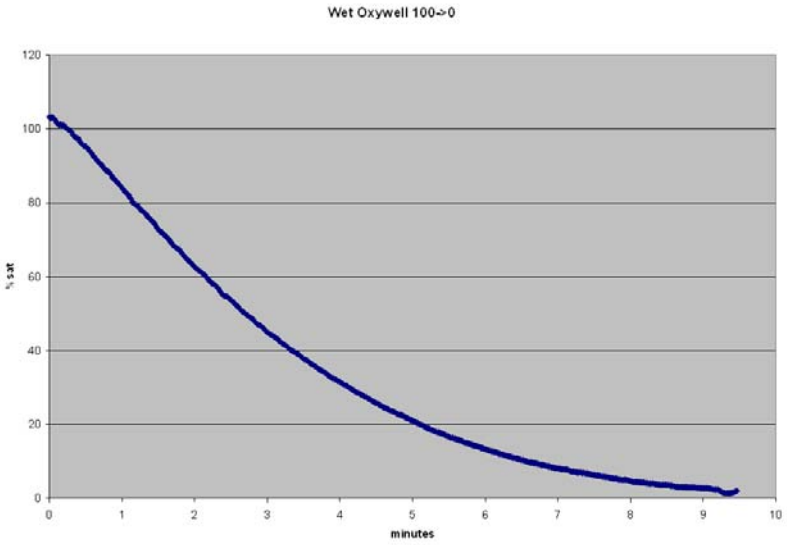


B.6.2 Wet Oxywell2

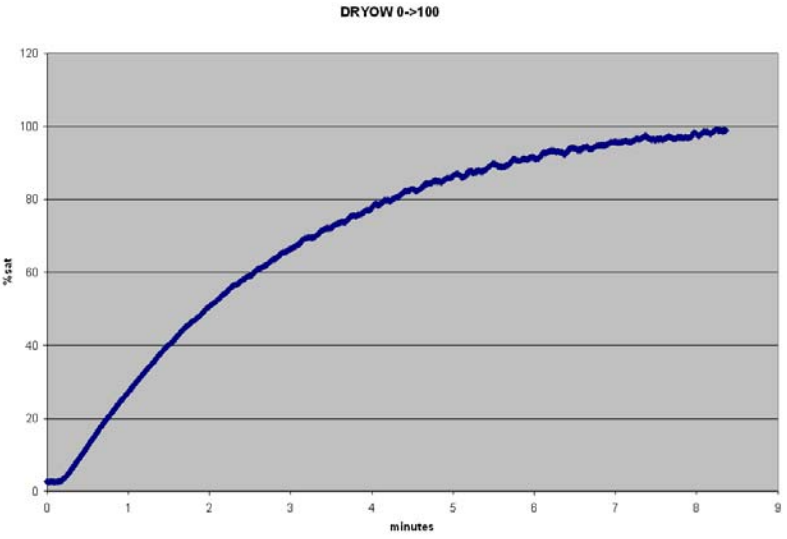
The response time (t_{90}) in the filled Oxywell2 is about 2 to 3 minutes as shown in the following graph:



B.6.3 Dry Oxywell2



In a dry Oxywell2 the response time is quite long due to the relatively large amount of stagnant air in the tip that must diffuse through the silicone. The response time (t_{90}) can range from 5 to 6 minutes as shown in the graph below.



Note: *Use of a dry Oxywell2 for oxygen monitoring in Cellbag bioreactors is not recommended.*

B.7 Stability

For long term stability, the probe is only illuminated intermittently. The default sampling frequency is every 10 seconds. The sampling frequency can be changed for dynamic measurements to as high as once per second.

The drift due to photo-bleaching is < 0.6% air-saturation per 100,000 measurements. At 10 second sampling this corresponds to 10 days. The Oxywell2 device permits recalibration of the probe without risk of contamination.

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87-4500-09 AC 02/2011