GE Healthcare

WAVE Bioreactor™ 2/10

Operator Manual

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Content

1 Introduction

Purpose of the Operator Manual

The Operator Manual provides you with the instructions needed to handle and run the WAVE Bioreactor 2/10 system.

Prerequisites

In order to operate the WAVE Bioreactor 2/10 system 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 instructions in Chapter 3.

In this chapter

This chapter contains important user information and a general description of WAVE Bioreactor 2/10 and its intended use.

1.1 Important user information

Read this before using WAVE Bioreactor 2/10

All users must read the Safety Instructions before installing, using or maintaining the system.

Do not operate WAVE Bioreactor 2/10 in any other way than described in the user 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

WAVE Bioreactor 2/10 is intended to be used as manufacturing process equipment for expansion of cells.

WAVE Bioreactor 2/10 shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

This Operator Manual contains 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.

Typographical conventions

Software items are identified in the text by ***bold italic*** text. Hardware texts are identified in the text by **bold** text.

1.2 Regulatory information

This section lists the directives and standards that are fulfilled by WAVE Bioreactor 2/10.

Manufacturing information

Requirement	Content
Name and address of manufacturer	GE Healthcare Bio-Sciences AB, Björkgatan 30, SE 751 84 Uppsala Sweden

Requirement	Content
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, UL 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 CISPR 11 Group 1, Class A
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 2/10 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 Bioreactor introduction

The WAVE Bioreactor instrument family has been designed for cell culture, enabling scale-up from 0.2 liter to over 500 liters of culture volume, using a disposable bioreactor chamber. The large scale-up interval is achieved because WAVE Bioreactor systems do not use gas permeable membranes for oxygen transfer. Instead, oxygen transfer and mixing are accomplished by the novel principle of wave-induced agitation.

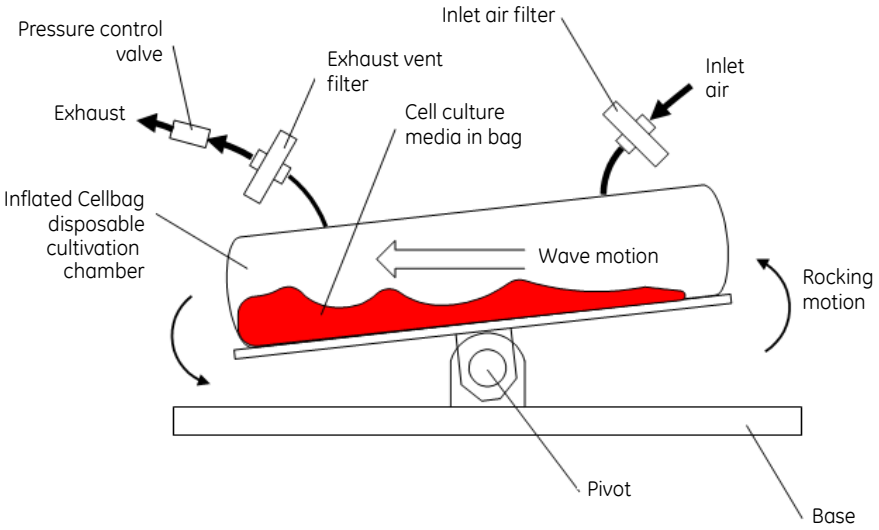
Description

WAVE Bioreactor systems consist of two main components: 1) the specially designed rocking unit (WAVE Bioreactor) and 2) a disposable bioreactor chamber (Cellbag™). A wave motion is induced in the culture fluid contained in the Cellbag bioreactor by a special rocking mechanism. This wave action generates free surface for bubble-free oxygen transfer from the headspace of the Cellbag bioreactor. The wave action also mixes the fluid in the Cellbag and suspends any cells and particles. The wave-induced agitation does not require an invasive mechanical mixer or gas sparging. This allows the design of a completely disposable presterilized bioreactor chamber.

WAVE Bioreactor systems are ideal for mammalian, insect and plant cell culture.

Fig 1-1. WAVE Bioreactor 2/10.

Fig 1-2. Cellbag bioreactor components.



Operation

The bioreactor chamber is provided presterilized by gamma radiation and is intended for single use. Cellbag is partially filled with culture media, inoculated, and placed on the rocking unit. The rocking motion may be adjusted to provide the desired degree of mixing and oxygen transfer. Air is continuously purged through the bioreactor headspace in order to supply oxygen and remove metabolic gases.

1.4 WAVE Bioreactor 2/10 Features

Disposable bioreactor chamber
Cells contact a plastic disposable cultivation chamber only. No cross-contamination, cleaning, sterilization, or other validation procedures.

Scalable
WAVE Bioreactor 2/10 can be used with Cellbag-500mL, Cellbag-1L, Cellbag-2L or Cellbag-10L disposable bioreactor chambers. Operating volume is 50 mL to 5 liters depending on Cellbag used.

Completely closed system
WAVE Bioreactor system is ideal for human T cell expansion, virus or vaccine production, high containment applications, and cGMP operations. No biosafety cabinet is needed, even for additions and sampling.

No cleaning or sterilization
Bioreactor chambers are made of USP Class VI plastics, typically used for biological fluid handling. They are delivered gamma sterilized and discarded after single use. Cellbags have integral inlet and outlet filters and fill/sample fittings.

Benchtop or incubator operation
The instrument has built-in temperature control for bench top operation. Alternatively, it may be placed inside an incubator.

Oxywell dissolved oxygen sensors
Dissolved oxygen can be measured by inserting our flexible micro DO polarographic electrode into Oxywell™ sheaths. Probes can be removed, readjusted and reinserted without compromising sterility.

Perfusion option
A perfusion module (PERFCONT2E) is available for WAVE Bioreactor 2/10. It provides weight-based feed and harvest control. It is designed for use with perfusion filter equipped Cellbags.

Easy to operate
WAVE Bioreactor system has no complex piping or tricky sterilization sequences. The bioreactor chamber is presterilized and is almost impossible to contaminate. Simply place a disposable cell cultivation bag on the rocker, fill with media, and add your cells.

2 Safety instructions

This chapter describes safety compliance, safety labels, general safety precautions, emergency procedures, power failure and recycling of WAVE Bioreactor 2/10 instrument.

2.1 Safety precautions

Introduction

Before installing, operating or maintaining the system, you must be aware of the hazards described in the user documentation. Follow the instructions provided to avoid personal injury or damage to the equipment.

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

- General precautions
- Personal protections
- Installing and moving the instrument
- System operation
- Maintenance

General precautions

WARNING
Do not operate WAVE Bioreactor 2/10 in any other way than described in the user documentation.

WARNING
Operation and user maintenance of the system should be performed by properly trained personnel only.

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

Using flammable liquids

WARNING
The WAVE Bioreactor systems are not designed to handle flammable fluids. The WAVE Bioreactor systems are not approved for work in a potentially explosive atmosphere.

Personal protection

WARNING
To avoid hazardous situations when working with the WAVE Bioreactor systems, take the following measures for personal protection.

WARNING
Always use appropriate personal protective equipment during operation and maintenance of WAVE systems.

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.

WARNING
Spread of biological agents. The operator has to take all necessary actions to avoid spreading hazardous biological agents in the vicinity of the instrument. The facility should comply with the national code of practice for biosafety.

Installing and moving the instrument

WARNING
Emergency stop. Position the WAVE Bioreactor 2/10 system so that the power switch is easily accessible for power shut off. The power switch is located at the rear side of the instrument.

WARNING
Protective ground. The WAVE Bioreactor 2/10 instruments must always be connected to a grounded power outlet.

WARNING
Heavy object. Filled Cellbags have a considerable weight and heavy lifts must be done with care. Use 1 person per multiple of 15 kg weight, for example 3 persons for 30 to 45 kg. All lifting and moving must be performed in accordance with local regulations.

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

CAUTION
Make sure that there is enough free space around the instrument for the rocking motion.

CAUTION
The safety switches of the base unit must be functionally tested after the installation of the equipment or after the instrument has been transported and every 6 months from then on. See Chapter 6 for proper method of testing.

System operation

WARNING
Biohazard. Make sure that the Cellbag is sealed before and during the fermentation process.

WARNING
Electrical shock hazard after spillage. If there is a risk that large volumes of spilled liquid may penetrate the casing of the WAVE Bioreactor 2/10 instrument, immediately switch off the instrument, disconnect the power cord, and contact an authorized service engineer.

CAUTION
Overheating is possible if the unit is operated without a liquid-containing bag. Do not touch.

CAUTION
Leakage risk of biological substances. Before every use, check all hoses for signs of cracking or tears. None of the air hoses should contain liquids of any kind.

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

CAUTION
Pinch hazard when using PERFCONT. Do not run the feed and harvest pump with the door open

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
Always clean the equipment in a well ventilated area. Never douse or immerse any part of the unit with any liquid. When cleaning is required, use only water and alcohol.

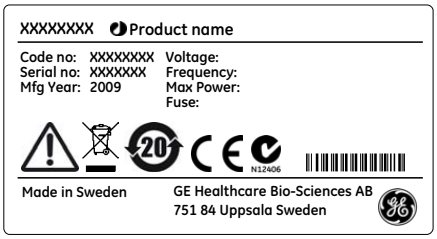
WARNING
Only spare parts that are approved or supplied by GE Healthcare may be used for maintaining or servicing the system.

2.2 Labels

This section describes safety labels and labels concerning hazardous substances that are attached to the WAVE Bioreactor 2/10 instruments.

Labels on the instrument

The illustration below shows an generic example of the identification label that is attached to the WAVE instruments.



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 Bioreactor 2/10 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	If required, disconnect the power cord from the power outlet.

Power failure

In the event of power failure, the run is immediately interrupted. If the ***AUTOSTART*** option is set to ***ON***, the operation is automatically resumed on power up. For further information, please refer to the user documentation.

Safety switches

Safety switches are located on top of the bioreactor base units. If any of the safety switches is hit, the rocking motion stops and rocking unit moves to a level position. If a safety switch has been hit, power must be switched off and then on again to reset the safety switch.

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 Bioreactor 2/10 systems out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

WAVE Bioreactor 2/10 systems 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 Installation

3.1 Before installation

Before you get started, please check to see that your WAVE Bioreactor 2/10 package includes all the items and options you ordered. If any parts are missing, contact GE Healthcare supplier immediately.

3.2 WAVE Bioreactor 2/10 rear panel

Fig 3-1. Rear panel connectors, see Table 3-1 for descriptions.

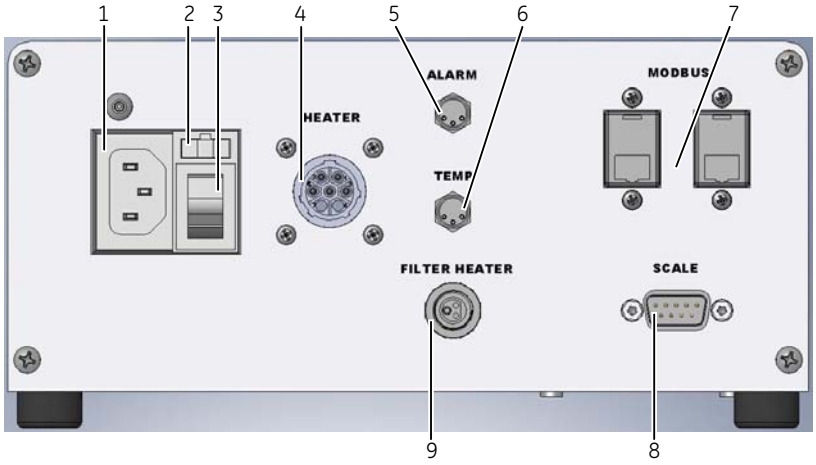


Table 3-1. Rear panel connectors

Part	Description	Function
1	Power connector	Used to connect a power cord to mains power.
2	Voltage selector	Used to select an operating voltage of either 115 VAC or 230VAC.
3	Power switch	Turns power on/off to the instrument.
4	**HEATER** connector	Used to connect to an exhaust filter heater to reduce condensation on exhaust line.
5	**ALARM** connector	Provides a set of contacts for external alarms. Operates with every alarm that occurs in the unit.

Part	Description	Function
6	**TEMP** connector	Used to connect to temperature sensor mounted in the KIT2EH.
7	**MODBUS** connectors	Two MODBUS connectors used to connect to other units in a daisy chain fashion.
8	**SCALE** connector	Used to connect to the optional perfusion module for control and monitoring purposes.
9	**FILTER HEATER** connector	Used to connect power to heater in KIT2EH on top of the instrument.

3.3 Installation of the Optional Perfusion Module (PERFCONT2E)

An optional perfusion module is available for WAVE Bioreactor 2/10. This module provides weight-based control of feed and harvest.

To install the module:

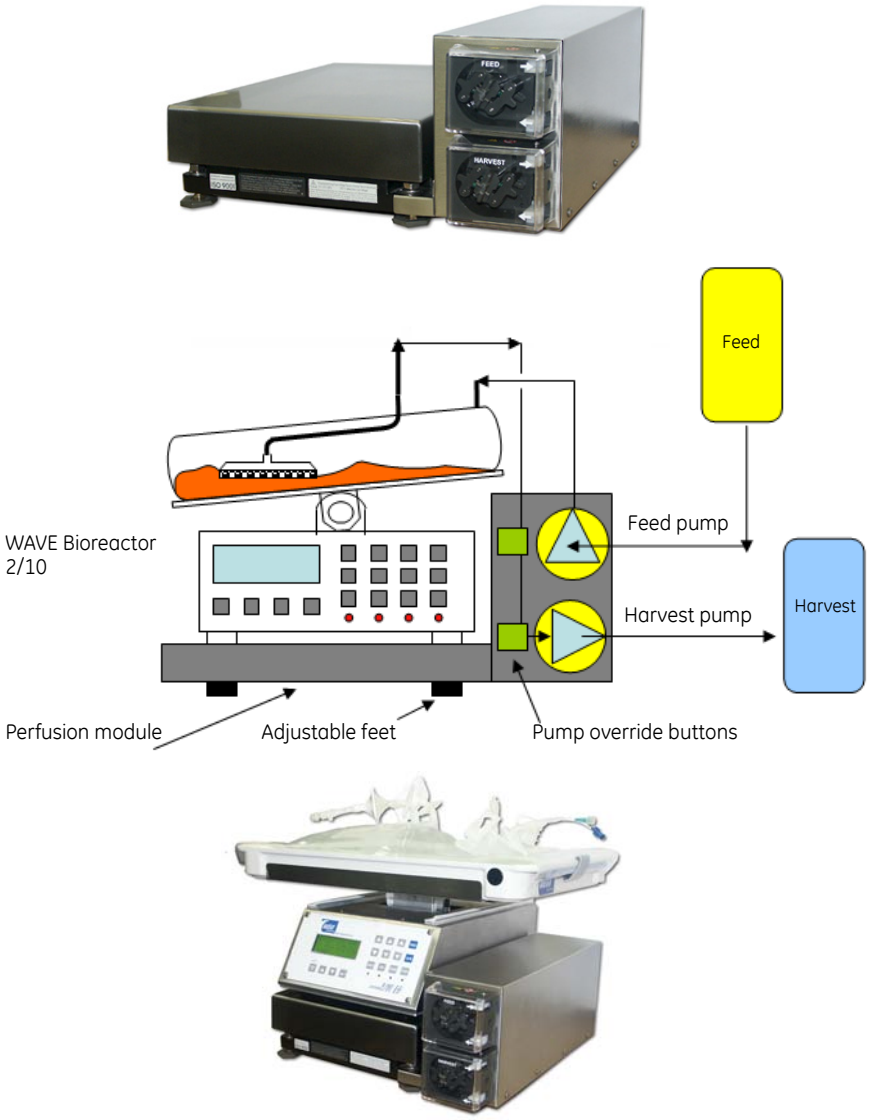
1 Unpack the perfusion module. Place on a stable surface. Adjust the leveling feet. Use a level to accurately level the unit.

2 Place WAVE Bioreactor 2/10 on top of the perfusion module. Make sure all four feet are properly placed on the stainless-steel platform.

3 Using the supplied PERFcable (DB9-F), connect the cable to the instrument's rear panel connector marked **SCALE**. Connect the other end of the cable to the connector at the rear of the perfusion module. Secure the cable at both ends using the thumbscrews.

Note: Chapter 6.3 describes how to connect the feed and harvest containers to Cellbag.

Fig 3-2. Perfusion module with installation setup.



3.4 Setting up the instrument

WAVE Bioreactor 2/10 is designed to hold Cellbag-500mL, Celbag-1L Cellbag-2L or Cellbag-10L bioreactor chamber. The instrument provides a reliable way of imparting a smooth wave motion to the bioreactor contents.

Follow the instructions below to set up WAVE Bioreactor 2/10. See Fig. 3-1 and Fig. 3-3 for illustrations of the rocking unit.

1 Unpack the instrument. Place it on a stable surface. Using the supplied cord set, connect AC power to the connector located on the back of the unit.

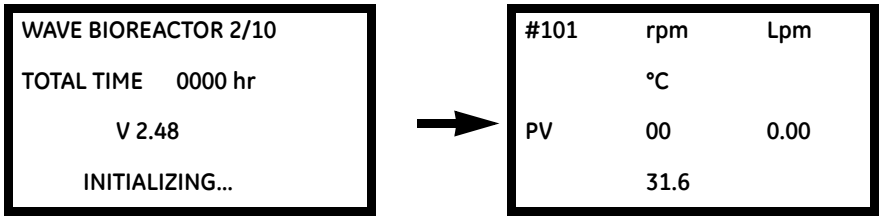
2 Examine the rear of the instrument and locate the power inlet. Check that the voltage selector is set to the correct voltage. Default voltage is 230VAC. If not, pry out the jumper using a screwdriver and reinsert with the arrow pointing to the desired line voltage. Remove the caution seal. Plug the supplied electrical cord into the power inlet and connect the other end to a suitable power outlet.

3 Place the tray KIT2EH on top of the stainless-steel rocker. The holder should fit snugly with the heater cable towards the rear.

4 Plug the heater cable plug into the jack labeled **HEATER** on the rear panel. Twist the lock nut to secure.

5 Plug the metal end of the yellow temperature cable into the jack on the back panel labeled **TEMPERATURE PROBE**. Twist the metal collar to lock the plug. Plug the other end to the temperature sensor attached to the KIT2EH. DO NOT TWIST THIS PLUG.

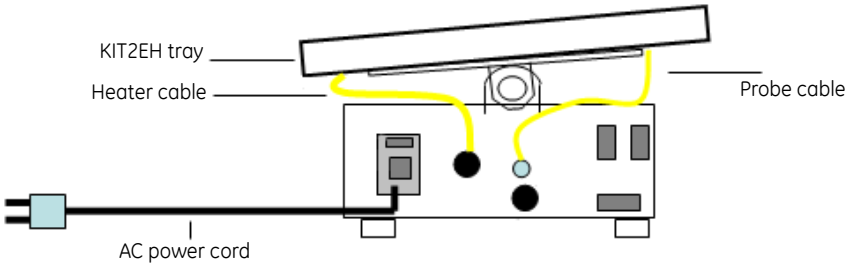
6 Use the power switch located on the rear to turn power on. The LCD screen will light up after a few seconds and the instrument will start to initialize. The machine will tilt in the proper direction to find its home position. Then it will stop in the sample position and the LCD will show the main display.



Initializing display Main display

Note: If the instrument fails to move or power-up, or the main screen is not displayed, please refer to 7 for help.

Fig 3-3. Rear view of WAVE Bioreactor 2/10 showing connections.

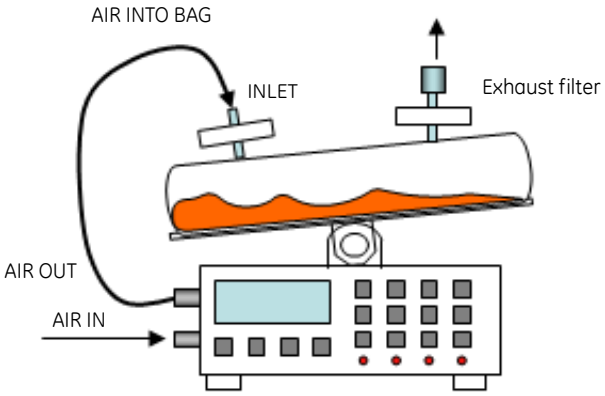


CAUTION! Check that the voltage selector is properly set to the correct operating voltage.

3.5 Setting up the aeration system

Cellbag disposable bioreactor chambers require aeration to keep them inflated and to provide ventilation. The air provides oxygen for cell culture and sweeps out products of respiration. The ventilation air can be room air, incubator air, or a special gas mixture depending on the cell line and buffer system in use.

Fig 3-4. Aeration system setup



The internal air pump is used to aerate Cellbag bioreactors.

1 Connect the supplied air tubing to the **AIR OUT** port located on the left side of the WAVE Bioreactor 2/10 instrument. Connect the other end to the inlet filter on Cellbag.

2 Leave the **AIR IN** port unconnected if ambient air is to be used for aeration. Otherwise connect the desired CO_2/air mixture. CO2MIX20 controllers from GE Healthcare can be used to produce any required CO_2 gas mixture.

> **CAUTION!** Pressure at the **AIR IN** port MUST NOT EXCEED 1 psig. Exceeding this limit may cause rupture of the Bioreactor bag. Provide a suitable safety pressure relief device.

3.6 Verification of check valve operation (syringe test)

1 Obtain syringe with luer lock end fitting.

2 Pull plunger back to fill syringe barrel with air.

3 Remove check valve from exhaust filter and attach to luer end of syringe.

4 Slowly depress syringe plunger to expel air completely from barrel through check valve.

If air flow passes, reattach check valve to Cellbag. If air does NOT pass, repeat syringe test with a replacement check valve.

> **CAUTION!** Do not inflate Cellbag with any device other than the built-in air pump. This pump is designed not to exceed a discharge pressure of 3 inches H_2O. This ensures that Cellbag cannot be over pressured. Direct connection to a gas source or use of any other pump to Cellbag will void the warranty and may result in rupture of Cellbag.

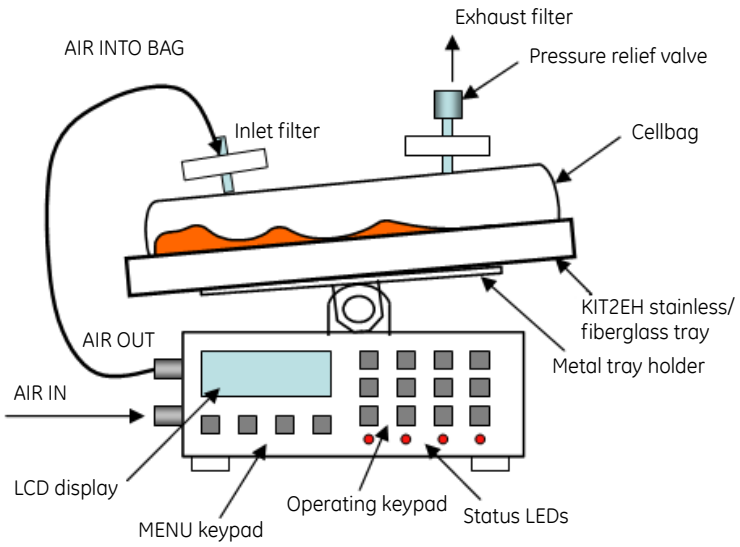
4 WAVE Bioreactor 2/10 system

4.1 WAVE Bioreactor 2/10 system components

4.1.1 WAVE Bioreactor 2/10 system overview

A complete WAVE Bioreactor system consists of two components: 1) the WAVE Bioreactor, which is a rocker unit that induces a wave motion in the bioreactor chamber, and 2) a disposable bioreactor chamber (Cellbag).

Fig 4-1. Overview of the WAVE Bioreactor 2/10 system



4.1.2 WAVE Bioreactor 2/10

The rocker unit is of very durable construction and is designed for continuous reliable operation. A display and keypad allows the user to view and set operating parameters.

Temperature is controlled by a heater and surface temperature probe in the KIT2EH Cellbag tray. pH control is achieved by using bicarbonate buffered media and controlling the CO_2 concentration of the air pumped into Cellbag. Air is drawn in by the internal pump from the inlet port located on the left side and pumped into the headspace of Cellbag. For normal operation, leave this port open and

unconnected. Alternatively, CO_2 or O_2 enriched air can be connected to this port. Please refer to cell culture protocols for more detailed aeration information

The instrument can be controlled by an external PC using the built-in serial communications port. WAVE Bioreactor 2/10 is operated electrically using 110/220 VAC.

CAUTION
Inlet pressure. Inlet pressure at the AIR IN port MUST NOT EXCEED 0.07 bar (1 psi).

4.1.3 The Cellbag bioreactor

Cell cultivation is performed inside Cellbag disposable bioreactor chambers. These chambers are delivered presterilized and ready for single use.

Please take a few moments to familiarize yourself with the features of Cellbags. Cellbag disposable chambers are packed in a protective outer plastic bag. The entire contents of this bag have been sterilized by gamma radiation.

These bioreactor chambers are provided sterile by gamma radiation and are to be discarded after single use. No sterilization or cleaning is required. The cell contact surface is an FDA approved ethylene vinyl acetate (EVA) / low density polyethylene copolymer of the type routinely used for blood collection and handling of biological fluids. Outer layers are made of proprietary composites that provide exceptional strength and extremely low gas permeability.

Each Cellbag bioreactor chamber has the following presterilized components:

Needle-less Sampling/Inoculation Port

This port is equipped with a self-sealing luer fitting for easy sampling using a NEEDLELESS conventional syringe.

Inlet Filter

The hydrophobic inlet filter sterilizes the air entering Cellbag. The filters used are rated to remove air-borne particulate of 0.2 micron or larger. Air is introduced to inflate the bag and to provide continuous ventilation during cultivation.

Outlet Filter

The hydrophobic outlet filter ensures that air vented from the bioreactor is sterile. This filter is also rated to remove air-borne particulates of 0.2 micron or larger, allowing for the complete containment of the bioreactor contents. The special filter media passes wet gases and condensate without clogging.

Pressure relief valve

The pressure relief valve is attached to the outlet vent filter. The purpose of the pressure relief valve is to maintain constant pressure in the bioreactor regardless of inlet airflow.

Tubing Connection

A length of C-Flex™ tubing is provided for easy sterile connections using standard tube fusing devices. This tubing is terminated with a standard luer connector so that connections can be made inside a laminar flow hood.

Luer Connections

Additional luer connections may be present on Cellbag for addition and withdrawal of media.

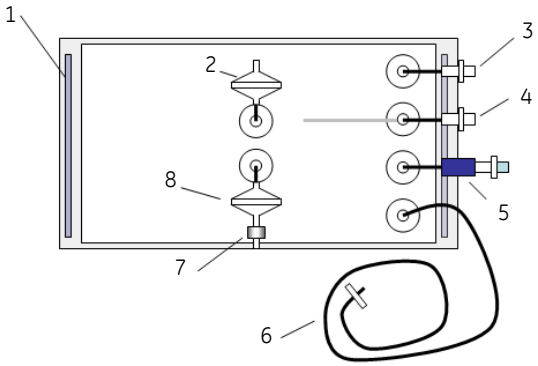
Oxywell ports

All Cellbags have ports for dissolved oxygen probes.

Options

Internal perfusion filters and screw caps are available. Custom Cellbags can also be manufactured for specific applications. A typical configuration is shown in Figure 4-2.

Fig 4-2. Cellbag configuration example.



Part	Description
1	Cellbag rod
2	Inlet air filter
3	Spare Luer port
4	Oxywell2™ probe port

Part	Description
5	Needleless sampling port
6	Inoculation/harvest lines
7	Pressure relief valve
8	Outlet air filter

Convenient harvesting

Cellbag bioreactor is in itself a convenient harvest container. There is no need to pump out the Cellbag contents into another tank.

The system has minimal turnaround time. Simply remove the completed batch and place a new disposable chamber on the rocker base. In the event of product changeover or contamination, conventional bioreactors require complex validated cleaning and decontamination. This can take several weeks during which the bioreactor cannot be used. WAVE Bioreactor system has no downtime. It also offers complete assurance against cross-contamination or product carryover.

WAVE Bioreactor holders provide an easy way to store and securely transport batches. Additional holders can be purchased to streamline operations.

4.2 Optional perfusion module PERFCONT2E

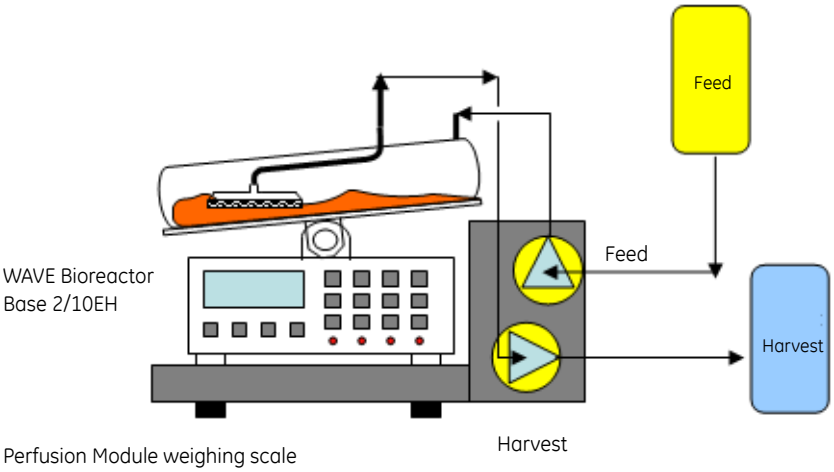
Perfusion is enabled if an external instrument module for perfusion culturing, PERFCONT2E, is installed. Perfusion may increase the cell concentration and viability.

The optional PERFCONT2E perfusion module is a convenient device for running perfusion culture. In this operation, feed is added periodically and cell-free culture media removed. The unit is designed for use with GE Healthcare patented perfusion cell culture bags. These bags have an internal filter that only allows cell-free harvest to be removed without the need for any external recycle loop or device.

The PERFCONT2E consists of a digital weighing scale and peristaltic feed and harvest pumps. The WAVE Bioreactor 2/10 instrument is placed on the PERFCONT2E weigh platform and this provides real-time weight data that is used to control the feed and harvest pumps. The PERFCONT2E is connected to WAVE Bioreactor 2/10 by a DB9 cable for control signals and power.

The PERFCONT2E unit calculates the time between each harvest-feed. Each scheduled perfusion starts with a harvest, which is immediately followed by a feed. Both the harvest and the feed operation are divided in a number of mini shots in order to come as close to the set shot volume as possible.

Fig 4-3. Feed-Harvest operation using the perfusion module.



4.3 WAVE Bioreactor 2/10 control system

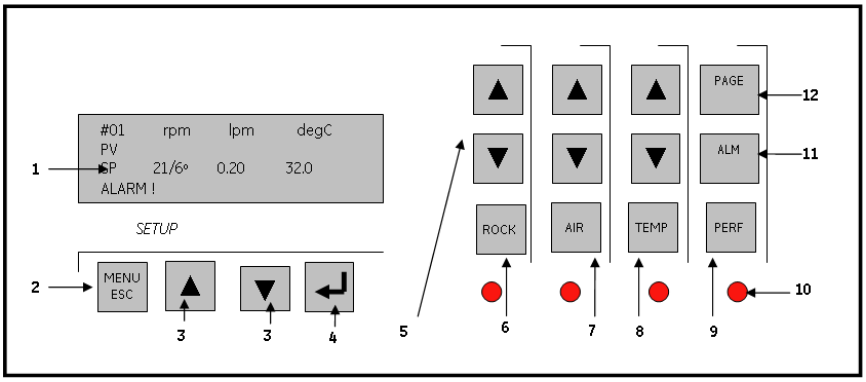
4.3.1 Switching on

Turn power on to the rocker using the switch located on the back panel. On power up, the rocker will tilt in the proper direction until it is level. If the rocker is not level after initialization is complete, you can correct it by using the ***CAL LEVEL*** option from the ***SETUP*** menu. The LCD display on the front panel will show the unit address and the operating parameters.

4.3.2 Front panel controls

WAVE Bioreactor 2/10 is controlled from the keypad on the front panel.

Fig 4-4. Front panel controls..

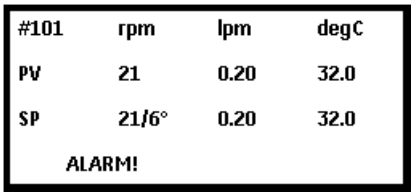


Part	Description	Part	Description
1	LCD display	7	**AIR** button
2	**SETUP MENU ESC** button	8	**TEMP** button
3	**SETUP** up and down arrow buttons	9	**PERF** button (perfusion)
4	**SETUP** Enter button	10	**ROCK**, **AIR**, **TEMP** and **PERF** LED indicators
5	**ROCK**, **AIR** and **TEMP** up and down arrow buttons	11	**ALM** button (alarm)
6	**ROCK** button	12	**PAGE** button

4.3.3 LCD display main screen

During normal operation the LCD displays the main screen shown below. For location of the LCD display, see Figure 4-4.

Fig 4-5. LCD display, main screen.



Item	Function
#101	Communications address
rpm	Rock speed (rocks per minute)
lpm	Air flow (Liters per minute)
degC	Temperature (°C)
PV	Process (actual) values In this example 21 (***rpm***), 0.20 (***lpm***) and 32.0 (***degC***). These values are not shown when the instrument is off.
SP	Setpoint values In this example 21/6° (***rpm***/rock angle), 0.20 (***lpm***) and 32.0 (***degC***)
ALARM!	***ALARM!*** • A flashing ***ALARM!*** indicates a new alarm not yet acknowledged. • A steady ***ALARM!*** indicates an alarm that has been acknowledged but not fixed. • No ***ALARM!*** visible indicates that there is no current ***ALARM!***

Note: *If another screen is selected that screen is displayed.*

4.3.4 Rocking controller

ROCK ON/OFF

Press the ***ROCK*** button to start or stop the rocking. When rocking is on, the LED under the ***ROCK*** button will be lit.

Speed adjustment

To change the rocking speed press the ***UP***/***DOWN*** buttons above the ***ROCK*** button. These will change the rocking speed setpoint by ±1 rpm. The selected rocking speed is shown on the ***SP*** row. The actual rocking speed is shown on the ***PV*** row. Range is 2 to 40 rpm.

#101	rpm	Lpm	degC
PV	11	0.00	30.6
SP	11/5°	0.01	35.6
ALARM!			

Angle

The rocking angle selected is shown next to the speed ***SP***. The angle is relative to the horizontal datum. The rocking angle may changed [2° to 9°] using the ***SETUP*** menu.

```
--- SET ANGLE -------

NEW ANGLE =  3

UP / DOWN to change

ESC [quit] ENT [accept]
```

Address

Each unit can be assigned a unique address. This enables multiple units to be daisy-chained on a single data acquisition cable. The current address is displayed on the upper left corner of the display. The address can be changed [101-110] using the ***SETUP*** menu. See Appendix D – Communications – for more information.

Note: In the event the rocking is obstructed by a hand or other object, the safety touch switches will stop the rocking instantaneously. Reset power to resume operation.

4.3.5 Aeration controller

Airpump ON/OFF

Press the ***AIR*** button to start or stop the internal air pump. When the air pump is ***ON***, the LED under the ***AIR*** button will be lit.

Airflow adjustment

To change the aeration flow rate, press the ***UP***/***DOWN*** buttons above the ***AIR*** button. These will change the airflow setpoint by ±0.01 Lpm. The selected air flow rate setpoint is shown on the ***SP*** row. The measured airflow is shown on the ***PV*** row. Range is 0.01 to 0.50 Lpm.

#101	rpm	Lpm	degC
PV	11	0.22	30.6
SP	11/5°	0.22	35.6
ALARM!			

Note: The air pump will automatically shutdown if the outlet pressure exceeds 3 inch H_2O for more than 10 seconds. This is to prevent over pressurizing the bag in the event of clogging. Air flow will resume when the pressure returns to normal.

4.3.6 Temperature controller

Heater ON/OFF

Press the ***TEMP*** button to start or stop the temperature control system. When the controller is enabled, the LED under the ***TEMP*** button will be lit.

Temperature adjustment

To change the temperature setpoint press the ***UP***/***DOWN*** buttons above the ***TEMP*** button. These will change the temperature setpoint by ±0.1°C. The selected temperature setpoint is shown on the ***SP*** row. The measured temperature is shown on the ***PV*** row. Range is 10°C to 50°C.

#101	rpm	Lpm	degC
PV	11	0.22	35.6
SP	11/5°	0.22	35.6

Note: The temperature system will automatically shutdown if the unit is not rocking. This is to prevent hotspots due to stagnant liquid motion. Control will resume once the unit is rocking again.

Note: When heating up it is normal for the temperature to overshoot by 1°C. Control should be within ±0.5°C.

4.3.7 Perfusion controller

Perfusion requires that an external instrument module for perfusion culturing, PERFCONT2E, is installed.

Perfusion Module ON/OFF

Press the ***PERF*** button to start or stop the weight-based perfusion control system. When the controller is enabled, the LED under the ***PERF*** button will be lit.

Parameters in SETUP

The desired feed rate in grams per day, the weight set point and the volume of each feed shot are specified in the ***SETUP*** menu.

Using the PAGE Button

Pressing the ***PAGE*** button will toggle the display to show perfusion data and set points. Pressing ***PAGE*** again will return to the ***MAIN*** screen. The perfusion screen shows the following information:

PERF wt	shot	Σg
PV 01000	000	F000000
SP 01000	030	H000000
	PERF ON	

#101	rpm	Lpm	degC
PV	00	0.00	31.6
SP	11/5°	0.01	30.6

PV Process value is the current net weight displayed in grams. The system can be tared from ***SETUP*** menu.

SP Set point is the desired weight of the media in the Cellbag, displayed in grams.

shot The ***PV*** under the shot column shows the current net weight loss (or gain) in grams during each feed or harvest operation. The ***SP*** row shows the total shot volume that the controller will add or remove during each cycle.

Σg The cumulative amount fed or harvested is shown in the third column. The F prefix corresponds to the total amount fed and H corresponds to the total amount harvested. The cumulative amounts can be cleared to zero from the ***SETUP*** menu.

Perfusion time and volume

The perfusion screen will display ***FEED DELAY*** when the unit is waiting for the weight to stabilize before continuing the feed shot. ***FEED ON*** will be displayed when the feed pump is on. The perfusion screen will display ***WAIT xx between feed shots***. The time shown is the minutes to go until the next shot.

The minimum shot size is 20 mL and the maximum is 15% of the weight setpoint. The system can maintain a long-term perfusion accuracy of ± 5%.

The time in minutes between each harvest-feed operation (here called cycle time) is calculated as:

$$\frac{\text{minutes per day} \times \text{shot volume}}{\text{feed rate per day}}$$

Example:

Shot volume	50 mL
Feed rate per day	2000 mL
Cycle time	1440*50/2000 = 36 minutes

4.3.8 Setup

The ***SETUP*** menu contains additional operation parameters, for example stop position, temperature calibration and perfusion options. The ***SETUP*** buttons are found in the lower left part of the front panel, see Figure 4-4, and function according to the table below.

Button	Function
MENU ESC	Display the ***SETUP*** menu screen/ return to the ***MAIN*** screen.
▲	Increment parameter value (***UP***)
▼	Decrement parameter value (***DOWN***)
↵	Enter button (***ENT***). Accepts changes and displays next parameter

Note: To reach a certain parameter, press ENT repeatedly to advance in the menu.

The following parameters can be changed from the ***SETUP*** menu:

Changing the rocking angle

```
--- SET ANGLE -------
NEW ANGLE = 3
UP / DOWN to change
ESC [quit] ENT [accept]
```

Enable/disable perfusion option

```
---SET PERFUSION-------
PERFUSION ENABLE ? Y
UP/DOWN to change
ESC [quit] ENT [accept]
```

Tare the perfusion load cell

```
---SET TARE---------------
TARE NOW ?              N
GROSS WT =00000    gm
ESC [quit] ENT [accept]
```

Clear stored cumulative feed/harvest amounts

```
---CLEAR MEMORY-------
CLEAR CUM VALUES? N
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the weight control setpoint

```
----SET WEIGHT   SP----
WEIGHT SP g=      1000
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the desired daily perfusion rate

```
---SET PERF RATE----
FEED ml/DAY=     1000
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the desired feed shot volume

```
---SET FEED SHOT----
FEED   ml/SHOT       50
UP/DOWN to change
ESC [quit] ENT [accept]
```

Calibrate the temperature sensor

The temperature can be offset to compensate for probe-to-probe variations.

```
---CALIB TEMPERATURE
OFFSET °C/10=      0
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the sample stop position

The sample stop position is the angle of the rocking tray when rocking is stopped. The default is 9 degrees.

```
----STOP POSITION----
STOP@deg/10=       0
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the Unit Address

Unit address can be set from 101 to 110.

```
-----UNIT ADDRESS----
101 TO 110 =110
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the AUTOSTART option

If the ***AUTOSTART*** option is set (=1) then, if the unit is shutdown (or power lost) with rocking, air, temperature, or perfusion ***ON***, the respective control will automatically be restored to the ON state on power up. This option is useful if the unit is to recover automatically from power failure. The default is ***OFF***.

```
--AUTOSTART----------
O=OFF   1=ON          0
UP/DOWN to change
ESC [quit] ENT [accept]
```

Set the calibration level

This allows adjusting the level when the unit is stopped. Level = 0.0 deg (+/- 0.5). You can only enter a whole number.

LEVEL@deg/10=1 means 1 divide by ten which will give you 0.1 offset.

```
--CAL LEVEL----------
LEVEL@deg/10=    -1
UP/DOWN to change
ESC [quit] ENT [accept]
```

Pressing ***MENU/ESC*** button after ***CAL LEVEL*** will return to the ***SETUP*** screen.

4.3.9 Handling alarms

An alarm is displayed in the lower left corner of the LCD display. .

```
#101      rpm      Lpm      degC
```

PV	00	0.00	30.6
SP	11/5°	0.01	35.6
ALARM!			

- A flashing ***ALARM!*** indicates a new alarm not yet acknowledged.
- A steady ***ALARM!*** indicates an alarm that has been acknowledged but the reason for the alarm still exists.
- No ***ALARM!*** visible indicates that there is no current ***ALARM!***

Handle alarm

- Press ***ALM*** button to acknowledge the alarm and display the alarm screen. In this example a temperature alarm (temperature deviation) is displayed.

ALARMS
TMPDEV

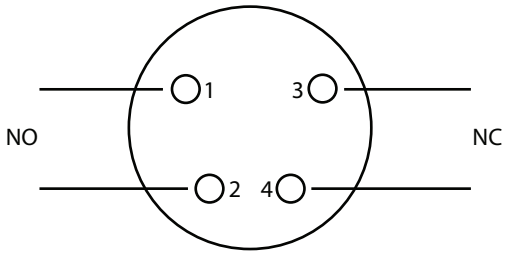
- Press ***ALM*** button again to return to the ***MAIN*** screen. The alarm is now steady.

Note: A new alarm will cause the alarm message to begin flashing again.

Alarm connector

A 4-Pin connector located on the rear of the unit provides NO and NC contacts that close and open respectively When an alarm occurs. Pin 1 and 2 is a NO contact and Pin 3 and 4 is a NC contact. The pin description is shown in Figure 4-6 below.

Fig 4-6. Alarm contact connector, front view.



5 Tips for operation

This chapter provides information that may be helpful for a successful cell culture in the WAVE Bioreactor system.

5.1 Tips for setting operating conditions

Each cell line and medium requires some optimization of operating conditions. The following table provides some typical parameters. Helpful example protocols for specific cell types are provided with the unit.

liquid vol (liter)/ Cellbag vol.	Rocking rate (rpm)	Aeration rate (Lpm)
0.2 liter/2L	6 to 15	0.1 to 0.2
1 liter/2L	10 to 25	0.1 to 0.2
0.5 liter/10L	6 to 15	0.1 to 0.2
5 liter/10L	10 to 25	0.2 to 0.3

Rocking

The rocking rate is set at the minimum rate that provides mixing and oxygen transfer without excessive foaming. In general, any rocking rate higher than 6 rocks per minute will be sufficient for particle suspension and bulk mixing. Cell population and metabolism will determine the necessary rocking rate for oxygen transfer. Typically, a rocking rate of 20 to 25 rocks per minute will satisfy oxygen demands for a cell density up to 5×10^6 cells/mL.

Typical rock angle is 6 to 8 degrees. See also Foaming and Chapter 5.3 below.

Aeration rate

Aeration rate has little effect on oxygen transfer. Aeration rates should be kept to a minimum to reduce evaporation. Aeration rate may be adjusted to change the pCO_2 or pH. Typically, aeration should be set at 0.2 to 0.4 liters/minute.

Foaming

It is critical that the rocking rate is sufficient to generate a visible surface wave. Some foam is typical, but reduce the rocking rate if there is excessive foaming, for example if more than 50% of the surface is covered with foam.

It is usual practice to add 0.01 mg/liter of pluronic F-68 to the culture media to minimize foam damage. Most commercial cell culture media already contains pluronic.

Foaming can also be reduced by lowering the rocking angle. Typical rock angle is 6 to 8 degrees. For very foamy media the rock angle should be reduced to 4 to 5 degrees. For cells with high oxygen demand, such as insect cells, the rock angle can be increased to 9 degrees.

Excessive foaming will also occur if the bioreactor chamber is not rigidly inflated. Check that you have sufficient airflow and that the pressure relief valve is functioning.

5.2 Sampling the Bioreactor

Sterility

The bioreactor may be sampled by attaching a standard luer type syringe onto the special needleless connector. The needleless connector was designed for hospital use. A silicone septum automatically seals the device whenever it is disconnected. It is not necessary to perform this procedure in a laminar flow cabinet. A standard syringe or luer connector may be used without a needle.

Sample may be taken at least 50 times using the same sampling connector without any danger of compromising sterility. The sample is also completely contained as no aerosols are vented.

Representative samples

At low rocking rates (<15 rpm) it is sometimes difficult to get a representative samples due to settling. In this situation, the recommended technique is to raise the rocking rate to 15 rpm, 5 to 10 minutes prior to sampling. As always, the rocking is stopped during sampling.

Some air may need to be released from Cellbag for sampling at low operating volumes (less than 500 mLs). Remember to re-inflate Cellbag to the original tension after sampling is complete.

5.3 pH and dissolved oxygen control

WAVE Bioreactor 2/10 is designed to provide an excess of oxygen for most culture systems. Appendix A provides data on the oxygen transfer capability of the system. Typically, the system is not limited by oxygen transfer and will support a cell density in excess of 7×10^6 cells/mL.

Dissolved oxygen (DO) measurements are the best way to determine the required rocking rate. This may be done off-line by taking samples by syringe and quickly determining the pO_2 concentration using a blood-gas analyzer. This technique requires that the sample has minimal contact with air and analysis must be performed within 5 minutes of sampling. An alternative is on-line measurement using a fiber optic monitor and probe (DOOPT) system available from GE Healthcare. This probe fits into the optional Oxywell sheath built into each Cellbag and can be inserted and removed repeatedly for calibration without compromising sterility.

Increasing the rocking speed will increase the oxygen transfer capability and the dissolved oxygen concentration will rise. Decreasing the rocking will have the opposite effect. Dissolved CO_2 concentrations are influenced in the inverse manner.

pH is typically controlled by the use of appropriate buffers. WAVE Bioreactor 2/10 allows the use of bicarbonate buffers by purging CO_2 gas over the liquid surface.

5.4 Scaling up culture volume

One of the big advantages of the WAVE Bioreactor system is the large range in operating volume. This makes it very suitable for inoculum scale up and eliminates the tedious sequential transfers that are necessary with other bioreactor systems, such as spinners, that have a narrow volume range.

In WAVE Bioreactor system you can start at low volume and simply continue to add fresh media to the bioreactor as the cells grow. Up to a 10 × expansion is possible in a single chamber. A typical inoculum sequence is:

1. Start with 200 mL media in Cellbag-2L. Add inoculum.
2. When cells reach 2×10^6 cells/mL, add 300 mL media to the Cellbag.
3. When cells again reach 2×10^6 cells/mL, add more media to bring the volume to 1 liter.

WAVE Bioreactor holders provide an easy way to store and securely transport batches. Additional holders can be purchased to streamline operations.

6 Operation

This chapter describes the operation of WAVE Bioreactor 2/10, including preparation of the system, sampling and harvesting. For more information about the control system, see Chapter 4.

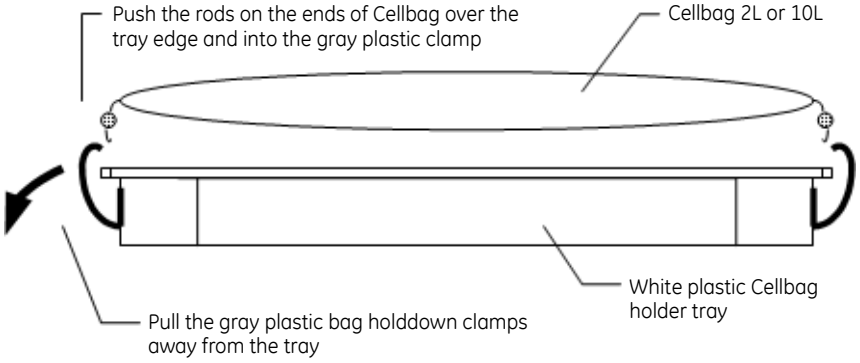
6.1 Switch on

Turn power on to the rocker using the switch located on the back panel. On power up, the rocker will tilt in the proper direction until it is level. If the rocker is not level after initialization is complete, you can correct it by using the ***CAL LEVEL*** option from the ***SETUP*** menu. The LCD display on the front panel will show the unit address and the operating parameters.

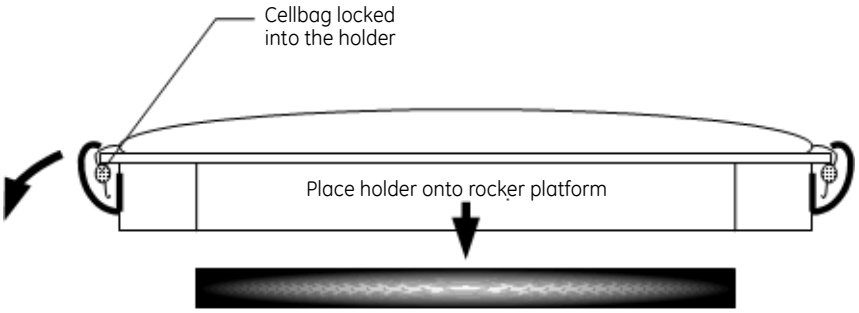
6.2 Prepare WAVE Bioreactor 2/10 for use

Due to the disposable design, WAVE Bioreactor 2/10 can be prepared for use within a few minutes:

1. Remove a disposable Cellbag bioreactor from the protective plastic bag.
2. Lay Cellbag on the KIT2EH tray and push the rod on each end into the gray plastic clips located on either side. See the figure below. The rods should snap into the clips and secure Cellbag firmly in the holder tray.



3 Place the holder on the stainless rocker platform. Ensure that is firmly seated in the platform and does not shift when rocked.



4 Connect the ventilation air line from the **AIR OUT** outlet located on the left side of the rocker to the inlet filter on Cellbag. The inlet filter is the filter without the pressure relief valve.

5 Attach the filter heater to the outlet filter and check that it is plugged into the **FILTER HEATER** jack on the back panel. Check that the heater is warm.

6 Press ***AIR*** on the front panel to switch the air pump on and introduce air into the bioreactor chamber. Press ***UP/DOWN*** to set the airflow setpoint to 0.2 to 0.5 Lpm. Do not increase airflow beyond 0.5 liter/minute.

7 Verify that the bioreactor chamber is firmly inflated and secured to the tray and rocker unit. The bioreactor chamber should not be creased. Verify that air is being released through the outlet pressure relief vent by pressing down gently on the chamber and observing a release of air through the outlet pressure relief valve.

8 Press ***ROCK*** on the front panel to turn the rocker unit on. Verify that the rocker unit is rocking and press ***UP/DOWN*** to set the speed to the desired rocks/minute. Consult the Cell Culture Protocols provided by GE Healthcare for specific recommendations.

9 If desired, change the rocking angle and the stop position in the ***SETUP*** menu.

10 Reduce the headspace airflow to 0.1 to 0.2 liters/minute. WAVE Bioreactor 2/10 is now ready for operation.

11 If you are planning to use the perfusion module, continue with the instructions in Section 6.3.

CAUTION! Do not inflate WAVE Bioreactor with any device other than the built-in air pump or the CO_2-mixer. This pump is designed not to exceed a discharge pressure of 3 inches H_2O. This ensures that WAVE Bioreactor cannot be over pressured. Direct connection to a gas source, or use of any other pump will void the warranty and may result in rupture of Cellbag.

6.3 Perfusion set-up operation

If the optional perfusion module PERFCONT2E is installed and will be used, follow this instruction after you have prepared Cellbag.

1 Connect tubing from the feed and harvest containers to the Cellbag. Make sure the flow direction corresponds to the arrows marked on the peristaltic pumps and that the tubing is properly pinched in the pump rollers.

2 Open any clamps on the feed and harvest lines. Swing the covers on the pump override buttons to the right and press the buttons to test and prime the pumps.

3 Enable perfusion option:

- Press the ***MENU/ESC*** button to enter the setup menu.
- Press ***ENT*** to continue from ***SET ANGLE*** to ***SET PERFUSION.***
- Press ***UP*** or ***DOWN*** to change to ***PERFUSION ENABLE? Y*** (YES), if necessary.
- Press ***ENT*** to accept and proceed to the next menu.

---SET PERFUSION-------

PERFUSION ENABLE ? Y

UP/DOWN to change

ESC [quit] ENT [accept]

4 Tare the scale of the perfusion module.

Note: Make sure all tubings and the filter heater are connected to the Cellbag before taring.

- Press ***UP*** or ***DOWN*** to change to ***TARE NOW? Y*** (YES), if necessary.
- Weight a few seconds until the weight is tared. Do not touch the unit!
- Press ***ENT*** to accept and proceed to the next menu.

```
---SET TARE----------
TARE NOW ?          Y
GROSS WT =00000   gm
ESC [quit] ENT [accept]
```

5 Clear stored cumulative feed/harvest amounts.

- Press ***UP*** or ***DOWN*** to change to ***CLEAR CUM VALUES? Y*** (YES), if necessary.
- Press ***ENT*** to accept and proceed to the next menu.

```
---CLEAR MEMORY-------
CLEAR CUM VALUES? Y
UP/DOWN to change
ESC [quit] ENT [accept]
```

6 Set the weight control setpoint.

- Press ***UP*** or ***DOWN*** to set the ***WEIGHT SP*** in grams.
- Press ***ENT*** to accept and proceed to the next menu.

```
----SET WEIGHT  SP----
WEIGHT SP g=     1000
UP/DOWN to change
ESC [quit] ENT [accept]
```

7 Set the desired daily perfusion rate.

- Press ***UP*** or ***DOWN*** to set the ***FEED*** in mL per day.
- Press ***ENT*** to accept and proceed to the next menu.

```
---SET PERF RATE----
```

```
FEED ml/DAY=     1000
UP/DOWN to change
ESC [quit] ENT [accept]
```

8 Set the desired feed shot volume.

- Press ***UP*** or ***DOWN*** to set the ***FEED*** in mL per shot.
- Press ***ENT*** to accept.
- Press ***ESC*** to leave the set-up menu.

```
---SET FEED SHOT----
FEED   ml/SHOT       50
UP/DOWN to change
ESC [quit] ENT [accept]
```

9 Press ***PAGE*** to view perfusion data.

Note: Make sure that the PV is set to zero.

10 Fill Cellbag with media, set the temperature control and inoculate Cellbag according to Chapter 6.4, 6.5, and 6.6.

Note: If the present value of the total weight differ from the set point at start up the system will start by doing a feed or harvest to reach the set point value.

11 Press ***PERF*** to start Perfusion.

6.4 Fill Cellbag with media

Once WAVE Bioreactor 2/10 is ready for use, Cellbag must be filled with culture media.

Note: The bioreactor should be inflated prior to filling with media, according to the instruction above. This will reduce foaming.

1 Press **ROCK** on the front panel to stop the rocking motion during filling. The unit stops according to the ***STOP POSITION*** parameter in the set-up menu. Continue airflow to the headspace to keep the bag rigidly inflated.

2 Connect, in a sterile manner, tubing from the media container to Cellbag using either the inlet tubing with a tube fusing device, or by connecting a male luer fitting to one of the luer ports.

Note: Use of the luer fitting may require moving the holder containing the bioreactor chamber into a biosafety cabinet. The sampling luer may also be used.

3 Pump the desired volume of media into the bioreactor.

Note: Media may also be added and removed in this manner during the cultivation.

4 Press ***ROCK*** to turn the rocker unit on. Adjust the speed to get a visible wave on the surface of the liquid. Typical initial rocking speed is 10 to 20 rocks/minute. Reduce the speed if you see excessive foaming.

6.5 Set the temperature control

1 Make sure that the unit is rocking and that Cellbag is fully inflated. Make sure that the temperature probe is located under Cellbag (gold side up) and that liquid is moving over the probe. Check that you have a temperature reading on the display. Make sure that the heater cable is plugged into the heater jack on the rear of the unit.

2 Press the **TEMP** button to turn heating on. The **TEMP** LED should light up. Press ***UP/DOWN*** to set the desired setpoint.

3 Allow the medium to equilibrate for two hours.

Note: It is normal for the temperature to overshoot the set point by up to 0.5°C especially at low volumes. The system will adjust and control within 0.5°C within two hours.

6.6 Inoculate Cellbag

1 Press **ROCK** on the front panel to stop the rocking motion during inoculation. The unit stops according to the ***STOP POSITION*** parameter in the set-up menu. Continue headspace aeration.

2 Connect in a sterile manner tubing from the inoculum container either to the inlet tubing using a tube fusing device, or to a male luer connector. Small inoculum volumes may also be added by syringe through the sampling port.

3 Pump the desired volume of inoculum into the bioreactor.

4 Press **ROCK** to start the rocking motion. Adjust the speed to get a visible wave on the surface of the liquid. Reduce the speed if you see excessive foaming.

6.7 Sampling

About sampling

The bioreactor may be sampled by attaching a standard luer type syringe onto the special needleless connector. No needle is used, see Figure 6-1. It is not necessary to perform this procedure in a laminar flow cabinet. A standard syringe or luer connector may be used without a needle,

NOTICE
The sampling device has a fairly small orifice. If you are working with large microcarriers or large cell aggregates do not use the sampling connector

Note: At low rocking rates (<15 rpm), raise the rocking rate to 15 rpm, 5 to 10 minutes prior to sampling in order to get a representative sample. As always, stop the rocking before sampling.

Note: Some air may need to be released from Cellbag for sampling at low operating volumes (less than 500 mL). Remember to re-inflate Cellbag to the original tension after sampling is complete.

Perform sampling

1 Press **ROCK** on the front panel to stop the rocking motion during sampling. The unit stops according to the ***STOP POSITION*** parameter in the set-up menu. The Cellbag will stop tilted towards the sample connector. This facilitates sampling.

2 Remove the dust cap from the sampling connector

3 Wipe the top of the sampling connector with 70% alcohol (or equivalent).

4 Using aseptic technique, attach a sterile disposable syringe onto the connector. Release the tubing clamp and withdraw a sample into the syringe.

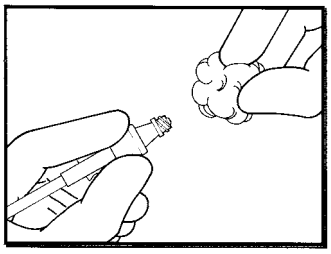
Note: You may need to push down on the bioreactor chamber to force the liquid up the sample tube.

5 Remove the syringe and wipe the top of the sampling connector again with 70% alcohol and replace the dust cap.

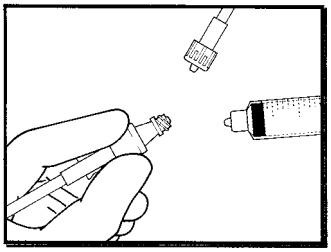
6 Pinch the sampling connector tubing a few times to ensure that any liquid in the tubing drains back into the bioreactor. Close the tubing clamp.

7 Press **ROCK** to start the rocking motion.

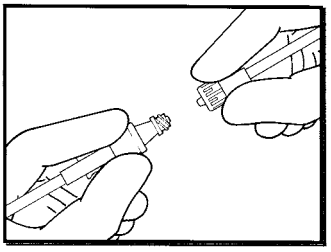
Fig 6-1. Attach a standard luer type syringe onto the special needleless connector.



1. Wipe connector tip with alcohol swab.



2. Aseptically connect tubing or syringe to the connector. Twist the luer to lock firmly.



3. After sampling, disconnect the syringe. Hold the connector so that it does not twist off the Cellbag. Then wipe tip of the connector with alcohol.

6.8 Media exchange

Media may also be easily exchanged in WAVE Bioreactor systems.

Note: To avoid possible oxygen depletion, this entire operation should be performed in less than one hour.

1 Press **AIR** on the front panel to stop the aeration.

2 Press **ROCK** on the front panel to stop the rocking.

3 Clamp off the inlet and outlet filters.

4 Remove the holder from the rocker platform and place it in the vertical position resting against a support.

5 Allow the cells or microcarriers 10 to 15 minutes to settle.

6 Connect tubing to the tubing on the bioreactor. The other end of this tubing should be connected to a sterile collection vessel.

7 Using a peristaltic pump, remove the desired amount of supernatant culture liquid by manually manipulating the flexible bioreactor wall.

8 Disconnect the tubing and reconnect to fresh media to refill the bioreactor.

9 Place the holder back on the rocker.

10 Open the inlet and outlet filter clamps.

11 Restart aeration and rocking.

6.9 Remove culture and harvesting

Cellbag bioreactor is in itself a convenient harvest container. There is no need to pump out the Cellbag contents into another tank.

Remove Cellbag

1 Press **AIR** on the front panel to stop the aeration.

2 Press **ROCK** on the front panel to stop the rocking.

3 Clamp off all connections.

4 Disconnect the air inlet tube.

5 Remove the holder containing Cellbag from the rocking unit and place it for processing at any suitable location.

7 Troubleshooting

7.1 General problems

Bag appears to be over inflated

The bioreactor should be inflated so that it requires force to squeeze it. However, it should not be so pressurized that creases form near the attachment points.

If the bioreactor is over inflated, check that the airflow to the bioreactor does not exceed 0.5 Lpm. Next, check that air is passing out of the pressure relief valve. This can be done be attaching a short length of tubing to the exhaust vent and immersing the tubing in water to a 1 cm depth. Bubbles should appear indicating flow. If no flow is observed remove the pressure relief valve. The outlet filter may be plugged and removing the pressure relief valve may allow continued operation. If the bioreactor continues to over inflate then transfer the contents to another Cellbag.

Bag appears to be under inflated

An under inflated bag will generate excessive foam and poor mixing. Check the display to make sure that there is sufficient air flow to the inlet filter. Check that you have connected the inlet air supply line to the inlet filter (does not have the pressure relief valve). Check that the pressure relief valve is present on the exhaust filter. Check that air inlet and out flow paths are unobstructed. If the exhaust filter is clogged then the contents can be transferred to another Cellbag.

Excessive foaming in the Bioreactor

Some amount of foam is typical. Foam should not cover more than 50% of the surface area. In the event of excessive foaming, first check that the bag is rigidly inflated. A poorly inflated bag will foam rapidly.

In many cases foam will subside after a few hours of operation. If foam is still excessive, reduce the rocking rate. Check that this rocking rate still provides a sufficient dissolved oxygen concentration.

If foam continues to be a problem, the rock angle should be reduced. The units are factory shipped set to ±6° from the horizontal. This angle has been found to be optimal for most cell lines.

7.2 Rocker

Power up

On power up, the rocker performs a self-test and zeros the angle sensor. The platform first tilts right against the safety stop and then moves to the left safety stop. Then it moves to the sampling position as specified in the ***SETUP*** menu. If the unit does not initialize properly it is possible that the safety switches have malfunctioned. Do not operate the unit in this condition. Please contact Service.

Wrong angle

The unit initializes the angle sensor on power-up. Normally, this assures correct operation. However, if the rocking is obstructed, it is possible for the angle datum to be affected. Ensure that nothing is obstructing the rocking and cycle power to the unit to reinitialize the angle measurement.

Airflow control problems

Air is switched on and off by the controller. Flow rate is measured by a thermal mass flow meter and controlled by an electronic control valve. Typical operating range is 0.1 to 0.5 Lpm. Flow fluctuation +/- 0.05 Lpm is normal and does not affect performance.

No heating

A HTROUT message will be generated if the heater is unplugged. Check that the heater cord is firmly connected. The HTROUT message will also be generated if the heater overheats (> 60°C).

WARNING! Overheating is possible if the unit is operated without a liquid-containing bag. The tray temperature may exceed 60°C. Do not touch.

Check that the sensor is correctly installed and a bag is present. In the event of an overheat condition, the system will reset automatically once the heater cools down sufficiently.

A RTDFAIL message indicates that the temperature probe is disconnected or giving invalid data. This will also cause the heater to shut off. Check that the sensor is properly connected to the rear panel jack. If the problem persists, replace the temperature sensor.

Check that unit is rocking. Remember the heating controller is switched off when the unit is not rocking in order to prevent local hot spots when the liquid is not in

motion. Starting the rocking will automatically switch the heaters on if TEMP control is enabled.

Poor temperature control

Under normal conditions the temperature PV (measured value) and the SP (setpoint) should be within 0.5°C. If larger deviations are observed, check that the surface temperature probe is correctly installed. The gold side should be facing up against the underside of the bag. Check that the bag is correctly placed so that the contents of the bag cover the temperature probe.

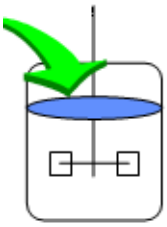
Appendix A Technical reference

A.1 Oxygen Transfer in WAVE Bioreactors

The rocking motion of liquid in Cellbag bioreactor generates a large free surface that results in much higher oxygen transfer than static culture. Conventional cell culture systems such as spinners are limited by the gas-liquid surface area.

Fig A-1. Wave motion for improved oxygen transfer.

Oxygen transfer is limited by air-liquid surface.



Spinner System
1 to 2 liter max

Wave motion induced by rocking generates large air-liquid surface for oxygen transfer.

WAVE Bioreactor has no volume limitation. Up to 500 liter working volume has been demonstrated.

The oxygen transfer capabilities of the system were assessed by measuring the volumetric oxygen transfer coefficient k_La at various rocking rates, liquid volumes and aeration rates. The classic dynamic method was used for k_La measurement. Here, the liquid in the bioreactor chamber is deoxygenated by passing nitrogen through the headspace. Once the dissolved oxygen (DO) concentration was near zero, air was introduced in to the headspace and the rise in DO recorded. k_La was calculated from the slope of the following mass balance equation:

$$ln\frac{(C^* - C_1)}{(C^* - C_2)} = k_L a^*(t_2 - t_1)$$

where C* is the saturation DO
C_1 is the DO at time t_1
C_2 is the DO at time t_2

The dynamic method was used for the measurement of oxygen transfer. A fiber optic dissolved oxygen probe was used. For fast response, the probe was used without an Oxywell sheath. This configuration has response time of a few seconds and the probe dynamics can be ignored:

The experimental procedure is as follows:

1. Evacuate the headspace in the bag by pressing down on the bag to force out all the gas in the bag.
2. Fill the headspace with nitrogen (N_2). Set an aggressive rock rate. Evacuate the headspace once more and refill with N_2 gas. Continue aggressive rocking for 2 to 3 minutes, or until the O_2 monitor reads 0%.
3. Evacuate the headspace, refilling it with air. Set the desired rock rate and begin recording the data as you start the rocking.
4. Record the time and dissolved oxygen concentration.
5. The data is then fitted by plotting ln(C* - C) vs. time in seconds. The plot should be a straight line and the slope is equal to the oxygen transfer coefficient k_La:

$$K_L a(t) = -ln(C_L^* - C_L)$$

The slope value it gives you will be the negative K_La in units sec^{-1}; to get the results in $hour^{-1}$, multiply the slope by 3600 to get the K_La value.

Oxygen transfer data in WAVE Bioreactor system

The k_La in WAVE Bioreactor system is a function of rocking speed and rock angle. Aeration rate does not appear to have any significant effect. In general, aeration rates should be between 0.01 and 0.1 vvm to ensure that the headspace composition is homogenous. The amount of liquid in WAVE Bioreactor system has a significant influence on the k_La.

Oxygen transfer data - Cellbag-20L

Figure A-2 shows the increase in oxygen transfer capability as a function of rocking speed and angle. The liquid volume is 5 liters in a Cellbag-20L.

Fig A-2. 5 liter fill in Cellbag-20L.

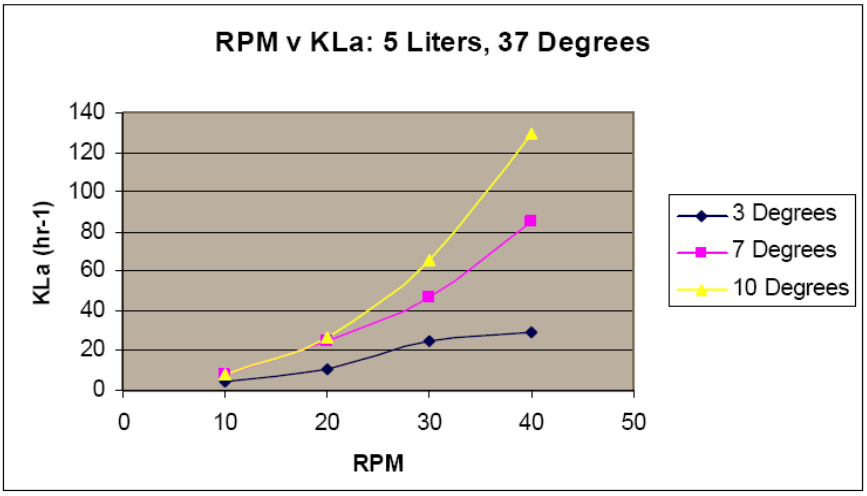
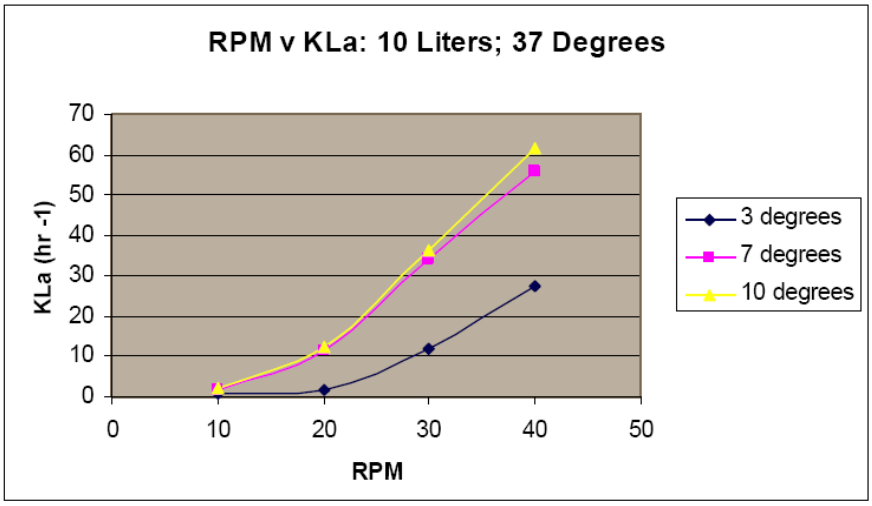


Figure A-3 shows that increasing the volume to the maximum (10 liters in Cellbag-20L) reduces the volumetric oxygen transfer rate.

Fig A-3. 10 liter fill in Cellbag-20L.



Typical operating conditions of 25 rpm and 7 degree angle will provide a $k_L a$ between 25 to 30 hr^{-1}.

Oxygen transfer data - Cellbag-50L

Figure A-4 and Figure A-5 show the oxygen transfer capabilities of 12.5 and 25 liters fill in a Cellbag-50L.

Fig A-4. 12.5 liter fill in Cellbag-50L.

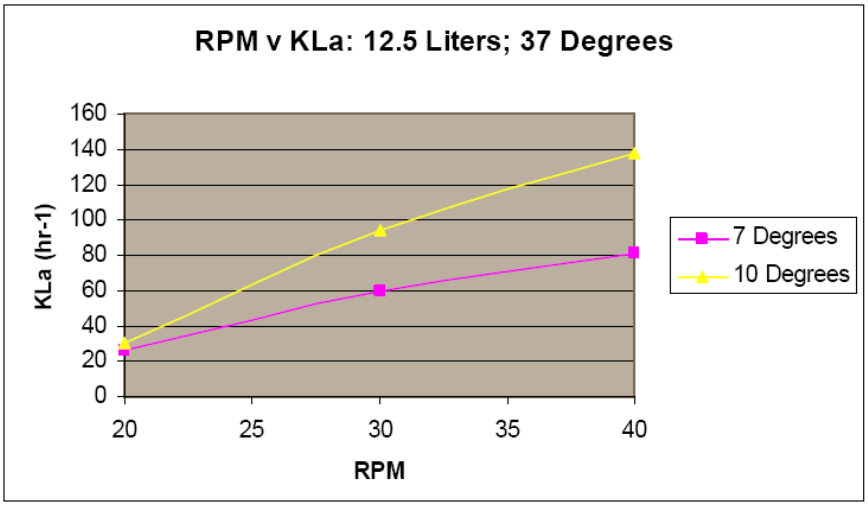
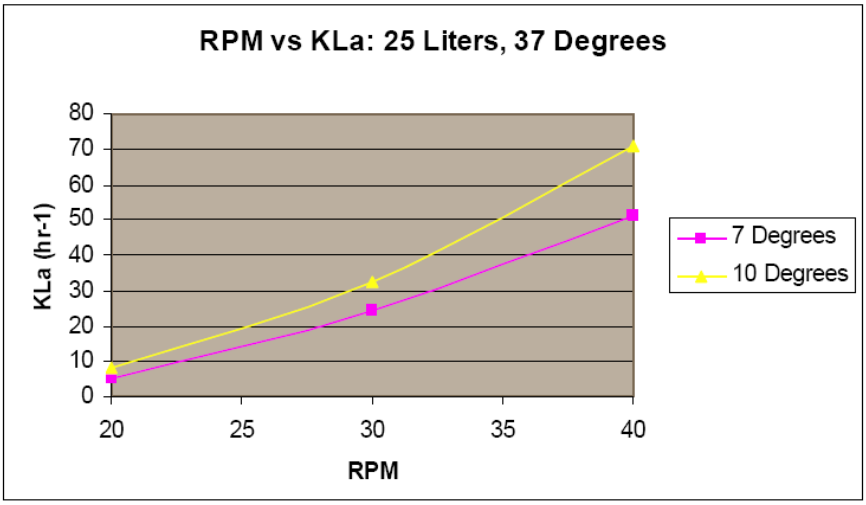


Fig A-5. 25 liter fill in Cellbag-50L.



Typical operating conditions of 25 rpm and 7 degree angle will provide a k_La between 15 to 40 hr^{-1}.

Oxygen transfer data - Cellbag-100L and Cellbag-200L

Figure A-6 and Figure A-7 show oxygen transfer data in Cellbag-100L and Cellbag-200L, respectively.

Fig A-6. 50 liter fill in Cellbag-100L.

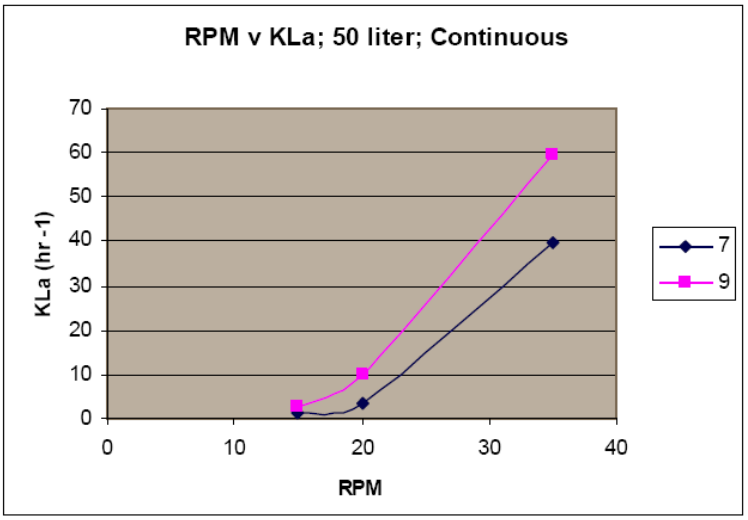


Fig A-7. 50 liter fill in Cellbag-200L.

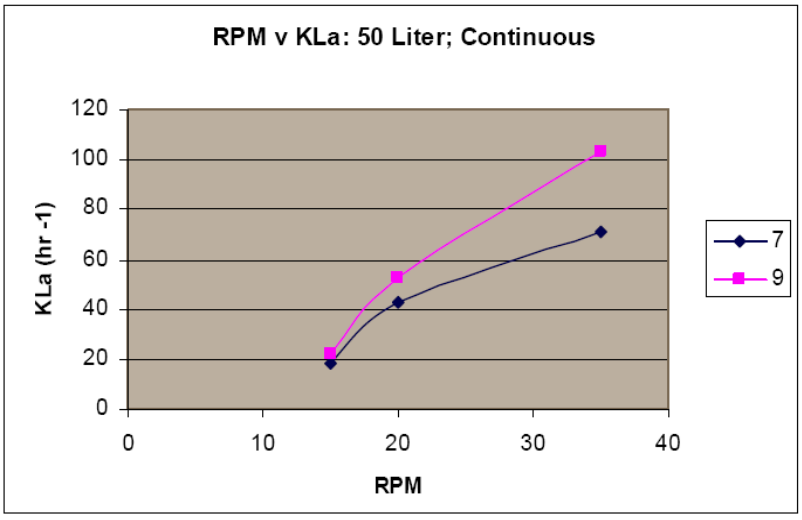


Figure A-8 shows the effect on oxygen transfer of the rocking rate at two different rock angles for a full volume (100 liters) Cellbag-200L. Figure A-9 shows the effect of angle at high set rocking rate of 30 rpm.

Fig A-8. 100 liter fill in Cellbag-200L.

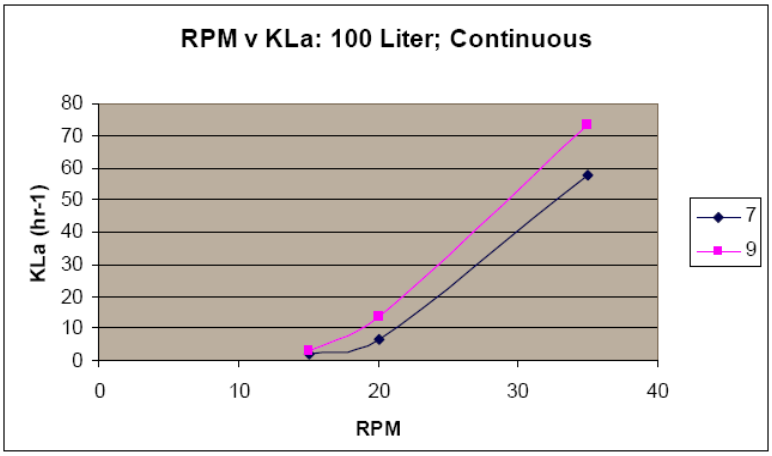
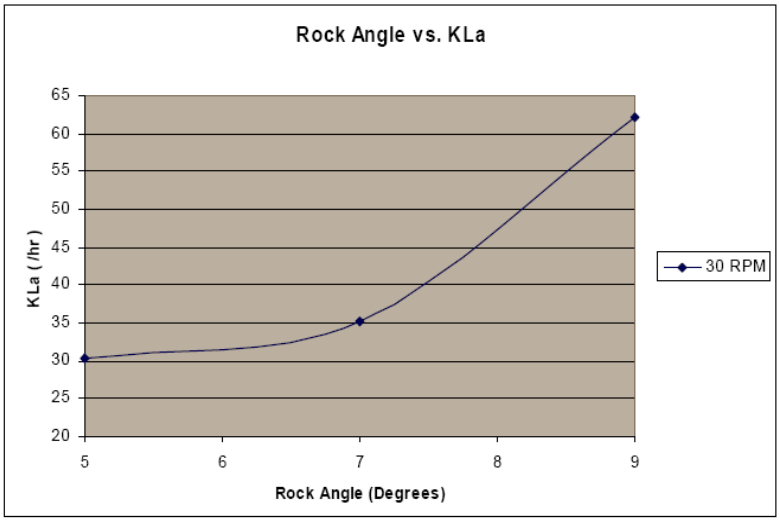


Fig A-9. 100 liter fill in Cellbag-200L.



Typical recommended operating conditions of 20 rpm and a rock angle of 7 degrees should result in a k_La around 25 hr^{-1}. Maximum performance of 60 hr^{-1} can be achieved at 30 rpm and 9 degrees. As observed with smaller bags, reducing the fill volume increases the oxygen transfer capacity.

Oxygen transfer data - 1000 liter Cellbag

Figure A-10 and Figure A-11 show data for Cellbag-1000L with a fill volume of 500 liters.

Fig A-10. RPM vs. K_La for 500 liter fill in Cellbag-1000L.

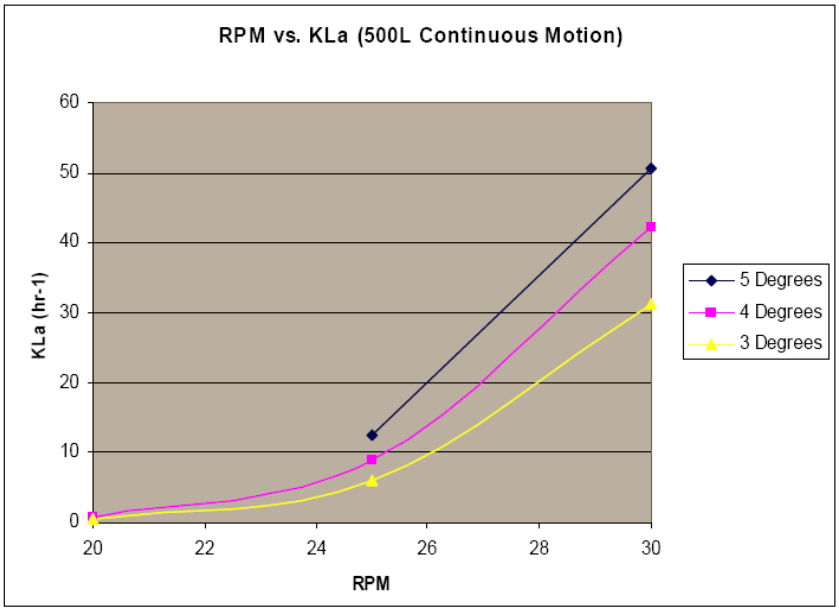
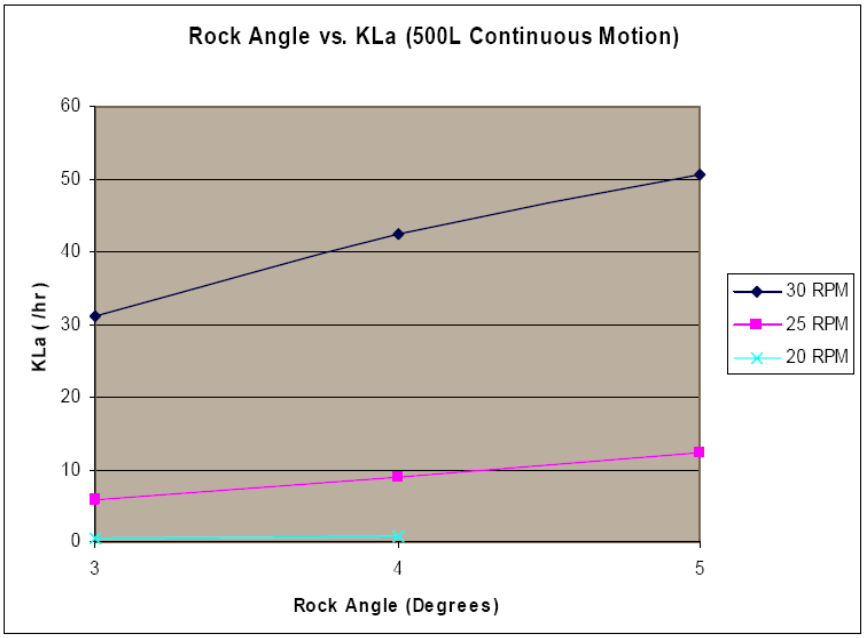


Fig A-11. Rock angle vs. K_La for 500 liter fill in Cellbag-1000L.



At typical operating conditions of 25 rpm and 4 degree angle, a k_La of 10 hr^{-1} is expected. For higher performance, the rock rate can be increased to 30 rpm and the angle to 5 degrees. This will result is increase in k_La to 50 hr^{-1}.

Overall guidelines

With an oxygen transfer capacity (k_La) of 30 to 40 hr^{-1}, and assuming a typical oxygen demand of 0.1 mM $O_2/10^6$cell-hr it is possible to grow up to 7×10^7 cells/mL while maintaining dissolved oxygen concentrations above 10% saturation.

With this knowledge of oxygen transfer it is possible to guarantee against oxygen depletion at any given cell density by selecting the appropriate rocking/aeration rate. Unlike stirred tank bioreactors, there is no bubble damage or danger of "over aeration." CO_2 stripping can be controlled by adjusting the CO_2 concentration of the inlet air. This effectively eliminates the need for a dissolved oxygen probe/control system. Samples may be taken and assayed periodically on an off-line blood gas analyzer to verify the DO and pCO_2 levels.

These data are with air (20.8% O_2) in the headspace. Enriching the headspace with oxygen changes the saturation value C* and will increase the oxygen transfer in proportional with the oxygen concentration in the headspace.

A.1.1 Mixing in WAVE Bioreactor system

Mixing times in the bioreactors at various rocking rates were determined by injecting a fluorescent tracer dye and videotaping the dispersion of the dye. UV light was used to enhance the contrast. Time-tagged images were captured from the videotape and the mixing time was determined visually from photographs. Mixing time was defined as the time required after injection to achieve complete homogeneity.

These experiments showed that the wave-induced motion was very effective in mixing the liquid in the bag. Mixing time was typically 5 to 10 seconds at rocking rates above 15 rpm. Rock rates below 6 rpm resulted in poor mixing. Liquid volume in the bioreactor was also critical. When the liquid volume exceeded 50% of the total volume of the bioreactor, mixing efficiency was substantially reduced. This is the reason why the maximum operating volume in the Cellbag-2L is 1 liter and Cellbag-10L is 5 liters (=50% of total volume).

Studies with micro carrier cultivation showed good off-bottom suspension with some particle gradients in the liquid. However, no significant settling of microcarriers or cells was observed.

A.2 WAVE Bioreactor system - Cell culture reports

WAVE Bioreactor system has found applications in human T cell expansion, insect cell culture, virus production, growing pathogens or other high containment systems, inoculum scale-up, gene therapy, protein expression, primary cell line expansion, monoclonal antibodies and cell therapy.

Cellbag bioreactor has been optimized to be low cost and easy to use. There are many applications in industry, academia, hospitals, and biologics manufacturing.

Many types of cells have been produced in a WAVE Bioreactor system. Some reports are presented here. Contact us or check our website for culture protocols and the latest data.

A.2.1 Monoclonal antibody production

An important application for cell culture is the production of monoclonal antibodies in vitro. Spinner or shake flasks can be used for 1 to 3 liters or culture, however oxygen transfer limitations in these systems preclude further scale-up. For larger volume it is necessary to use complex bioreactors based on stirred tanks, hollow fiber, or immobilized technology. The applicability of WAVE Bioreactor system for lab and pilot scale (10 to 580 liter) production of monoclonal antibodies has been evaluated.

Operating parameters

WAVE Bioreactor system was placed inside a temperature controlled incubator room maintained at 37°C. Headspace gas was CO_2 mixed with air. Initial gassing was with 10% CO_2 in air using minimal aeration rates (0.001 vvm). Once cell growth was above 1×10^6 cells/mL, the gassing rate was increased to 0.01 vvm and the CO_2 composition was changed to 5% CO_2 in air to better control culture pH. Initial rocking rate was 10 rpm. Rocking rate was increased during the cultivation to maintain dissolved oxygen levels above 70% saturation. The maximum rocking rate necessary was 20 rpm. There was no need to supplement the headspace air with oxygen.

A similar cultivation was also performed in a bench top WAVE Bioreactor system using the HEATER20 temperature control system. CO_2 conditioned air was pumped into Cellbag.

Cell line and culture conditions

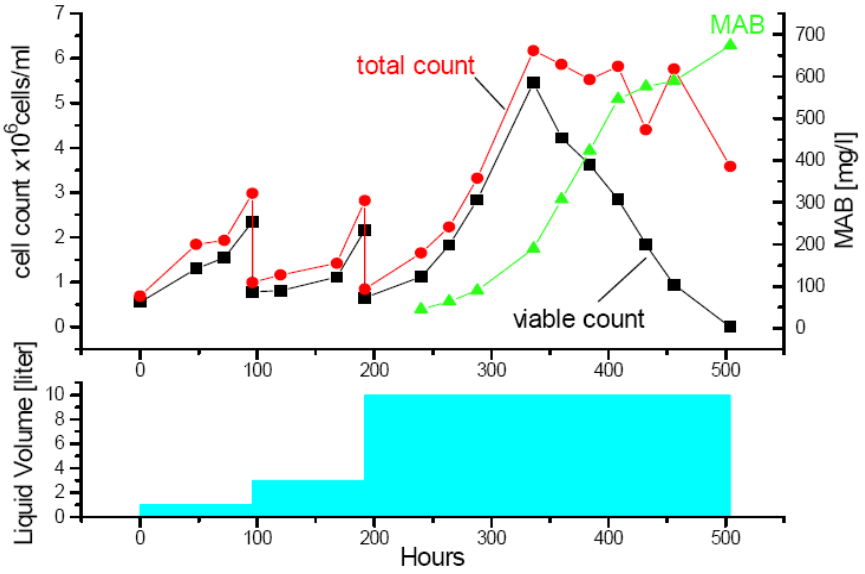
The cells used were a NS0 cell line expressing a humanized monoclonal antibody. Culture was performed in serum-free media commonly used for this cell line. Media contained bicarbonate buffer for pH control using CO_2 in the headspace. Cultivation was started at a 1 liter culture volume in a 20L Cellbag with 250 mL of inoculum from a 500 mL spinner flask + 750 mL media. After 96 hours the cell density was 2.3×10^6 viable cells/mL. Two liters of fresh media were added to the bioreactor and the cultivation continued. After an additional 96 hours the cell density again reached 2.1×10^6 cells/mL and 7 liters of fresh media were added to the bioreactor to bring to the final culture volume of 10 liters. Cultivation was continued until zero viability (300 hours). Figure A-12 shows the cell counts and monoclonal antibody production.

The stepwise dilution technique used demonstrates how the large turndown ratio (maximal volume/minimum volume) of the bag enables large culture volumes without the need for transfers as would be the case using spinner flasks. In this example inoculum scale-up from 1 to 10 liters was done inside the bioreactor itself.

Performance

Culture profile was very consistent with smaller scale spinners. Cell densities exceeded 5 × 10^6 cells/mL. Dissolved oxygen levels remained above 50% saturation. Antibody expression was normal at over 600 mg/l. The system is clearly well suited for suspension cell cultivation and monoclonal production.

Fig A-12. Antibody Production in WAVE Bioreactor system, Cellbag-20L.



100 liter scale operation

The same cell line was evaluated at pilot scale in 100 liters of culture in Cellbag-200L. Inoculum was made in a Cellbag-20L. Ten liters of this cell culture was added to the 200 liter disposable Cellbag bioreactor along with 20 liters of fresh media. Additional fresh media was added whenever the cell count rose above 2 × 10^6 cells/mL to a final volume of 100 liters. Aeration and rocker speed control were done in a similar manner to that used for the 20 liter system. Cell growth and antibody production were very similar to the 10 liter scale. The maximal cell density achieved was 5 × 10^6 cells/mL. Dissolved oxygen levels remained above 50% saturation indicating adequate oxygen transfer.

Benchtop operation

Identical performance in terms of cell growth and productivity was obtained in the heated bench top unit.

A.2.2 Virus production

The potential of WAVE Bioreactor system for virus production was tested using a recombinant adenovirus system.

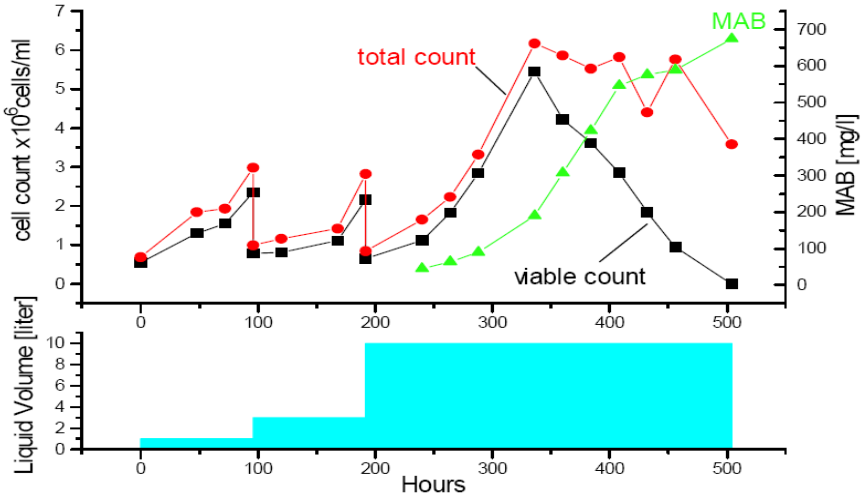
Operating parameters

WAVE Bioreactor 2/10 with Cellbag-2L was used in this evaluation. The system was placed in a conventional cell culture incubator maintained at 37°C in a 5% CO_2 environment.

Cell line and culture conditions

A human embryonic kidney cell line (293) grown in suspension was used as the host. Initial seeding density in a 2L Cellbag containing 1000 mL of serum-free media was 0.3 × 10^6 viable cells/mL. The culture was grown for 5 days at a rocking rate of 10 rpm and 0.1 vvm aeration. At that stage, the viable cell count was 2.7 x 10^6 cells/mL. 500 mL of the culture was then removed and replaced with 500 mL of fresh media. After an additional 24 hours the culture was infected at an MOI of 30 virus particles/cell. The culture was harvested three days post-infection at which point the viable count was zero (Figure A-13).

Fig A-13. Adenovirus Production in a WAVE Bioreactor system.



Performance

The final virus titer was 10,000 particles/cell, which was very similar to the titer obtained in conventional cultivation systems. From Figure A-13, it is apparent that the system is quite capable of maintaining adequate dissolved oxygen levels at these cell densities. The pH remains constant due to the excellent gas exchange as evidenced by the constant pCO_2 until late in the infection when the pH drops rapidly beyond the capability of the buffer system as lactate builds up to high concentrations.

Special considerations for virus production

All additions and sampling were done in the incubator. No biosafety hood was required. The standard 0.2 micron exhaust filter was supplemented by a Pall DFA cartridge filter attached after the vent pressure control valve. The integrity of the exhaust filter system was tested by passing the exhaust gases to an uninfected 293 culture. Absence of any infection in the second culture confirmed the effectiveness of the exhaust filtration in containing adenovirus.

A.2.3 Insect Cell/Baculovirus Cultivation

The sF9/Baculovirus system is an excellent method for the rapid expression of large amounts of recombinant proteins. However, the oxygen requirements of sF9 cells are higher than mammalian cells and this restricts the volume of spinner or flask culture to around 1 liter. Typically, larger volumes are required to produce sufficient protein for isolation. This requires the use of more complex bioreactors. Experiments were performed to demonstrate that the simple WAVE Bioreactor system could be used to scale-up sF9 cells, and produce baculovirus in 10 liter culture volume.

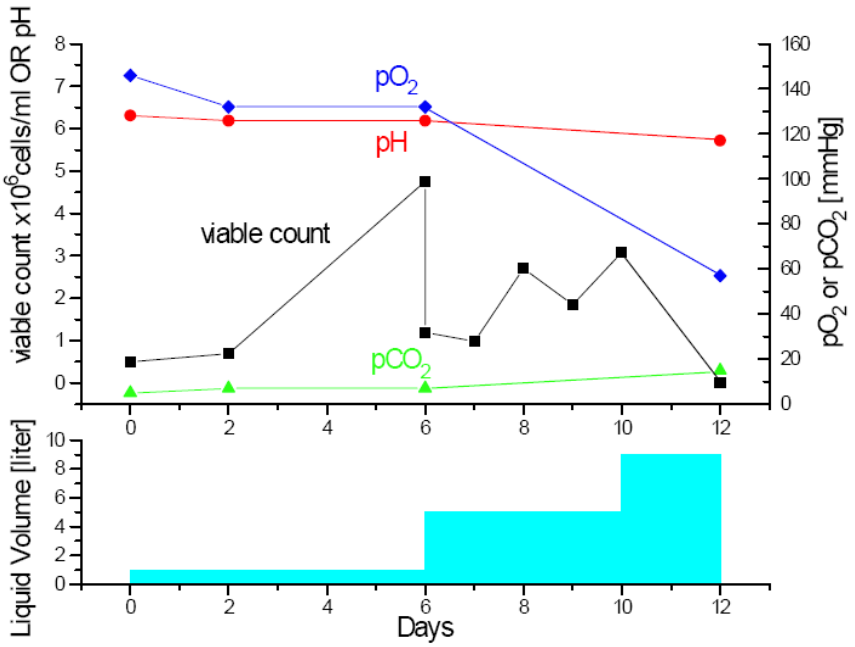
Operating parameters

WAVE Bioreactor 2/10 with Cellbag-10L was used in this evaluation. The system was placed in an incubator controlled at 27°C. Aeration rate was 0.01 vvm. Rocking rate was varied between 15 and 25 rpm depending on dissolved oxygen levels. An alternate system was also operated on the bench top using the optional heater/ temperature controller.

Cell line and culture conditions

The gene sequence for a recombinant protein was cloned into the pAcHLT-B baculovirus expression vector, transfected into sf9 cells, and used to generate a high titer virus stock.

Fig A-14. Insect Cell/Baculovirus Production.



One liter of Bio-Whittaker X-Press Insect Cell Media was added to a 20 liter WAVE Bioreactor. This was inoculated with 0.5 × 10^6 sf9 cells/mL. Rocking rate was 20 rpm in a humidified incubator at 27°C. After 6 days of cultivation the cell count reached 4.75 × 10^6 cells/mL. At this point 4 liters of fresh media were added to the bag. Within an additional 4 days, the cell count reached 3.0 × 10^6 cells/mL in this increased volume. Then another 4 liters of fresh media were added along with the virus at a MOI of 0.5 bringing the system to a culture volume of close to 10 liters. Harvest was done 2 days post infection.

Performance

This 10 liter culture in a WAVE Bioreactor system produced a titer comparable to 100 mL shake flasks. It demonstrated the ability of WAVE Bioreactor system to increase cell mass by simply adding fresh media to the system. No conventional series of flasks or splits were required, reducing labor and potential for contamination. All additions and transfers into the bag were done in the incubator. Figure A-14 shows that oxygen transfer and CO_2 desorption were not limiting.

Benchtop operation

Since typical insect cell culture media does not need CO_2 overlay for pH control a CO_2 controlled incubator is not needed. With the optional heater/controller the incubator is also not needed to maintain temperature. This option provides bench

top convenience with no temperature gradients or compromise in cell performance.

A.2.4 Anchorage dependent cells

The experiments reported so far were all performed with cells in suspension. In order to demonstrate that WAVE Bioreactor can also be used for anchorage-dependent cells, an experiment was performed with cells growing on microcarriers.

Operating parameters

WAVE Bioreactor 2/10 with Cellbag-2L was used in this evaluation. The system was placed in a conventional cell culture incubator maintained at 37°C with 5% CO_2. Attachment was done at very low rocking rates (< 6 rocks/minute) operated intermittently every few minutes for 10 minutes. Then the rock rate was set to 6 to 8 rocks per minute. Rock rate was raised to a maximum of 10 rpm based on DO levels.

Cell line and culture conditions

Human 293 cells were cultivated on Cytodex™ 3 microcarriers. Growth media was DMEM with 10% bovine calf serum. Inoculum was collected by trypsinizing micro carrier cultures grown in spinner flasks. Sixty mL of inoculum were added to 3 g Cytodex 3 in 900 mL of media and then transferred to WAVE Bioreactor system with Cellbag-2L. 10 mL of the inoculum were added to a 250 mL Bellco spinner flask with 0.45 g Cytodex 3 and 140 mL media that was run in parallel at 40 rpm. A conventional stirred-tank bioreactor containing 3 g/L microcarriers was also run with the same inoculum.

Performance

Growth in WAVE Bioreactor 2/10 was very similar to the spinner run in parallel. Cells grew to confluency (>50 cells/bead) in both systems. WAVE Bioreactor did however show some clumping of micro carrier beads and also some free clumps. Comparison with microcarriers grown in conventional stirred showed a much larger number of microcarriers with attached cells and a lower number of dead cells in WAVE Bioreactor.

Cell counts, glucose consumption and lactate formation were equivalent with the exception of slightly higher cell counts in WAVE Bioreactor supernatant.

Sampling considerations

One of the difficulties experienced in WAVE Bioreactor was obtaining a representative sample. The syringe sampling system is quite capable of aspirating

microcarriers however the contents of WAVE Bioreactor are not homogeneous at the low rocking rates needed for good cell growth.

The recommended procedure for sampling is to raise the rocking speed to 15 rpm 5 to 10 minutes prior to sampling. This ensures that the microcarriers are well-mixed. The rpm can be lowered after sampling to reduce cell damage.

Media exchange

Media may be exchanged easily when using microcarriers. Simply remove Cellbag cultivation chamber from the rocker and place it so that the microcarriers can settle in one corner. This typically takes 5 to 10 minutes. Then connect a pump to the tubing and draw off the supernatant. With 500 mL of cell culture it is possible to remove up to 350 mL of spent media in this fashion while only losing 5% of the cells. After removing the spent media, connect fresh media to Cellbag and refill.

WAVE Bioreactor has been used with many different microcarriers, such as Cultispher™, Hillex™, and Fibra-Cel™ disks.

It is been our experience that more vigorous rocking promotes better attachment and prevents micro carrier settling. Use at least 8 to 10 rpm for micro carrier culture. Each micro carrier type requires slightly different conditions.

A.2.5 T lymphocytes perfusion culture

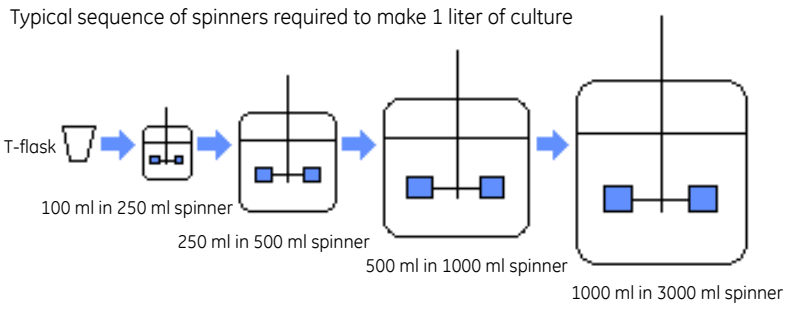
Perfusion culture of T lymphocytes in the WAVE Bioreactor™ System 2/10, Article no. 28-9650-52.

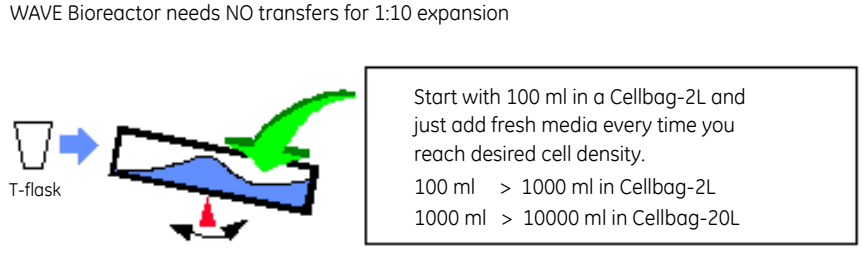
This application note presents a robust process for manufacturing 10^{10} to 10^{11} human T cells at cell densities of more than $1x\ 10^{7}$ cells per mL using the WAVE Bioreactor 2/10. The expanded T cells remain biologically functional and can be reactivated to produce high amounts of cytokines.

A.2.6 Other applications

WAVE Bioreactor system can be used for many other applications. Here are some examples:

1. Scaling up inoculum for conventional bioreactors

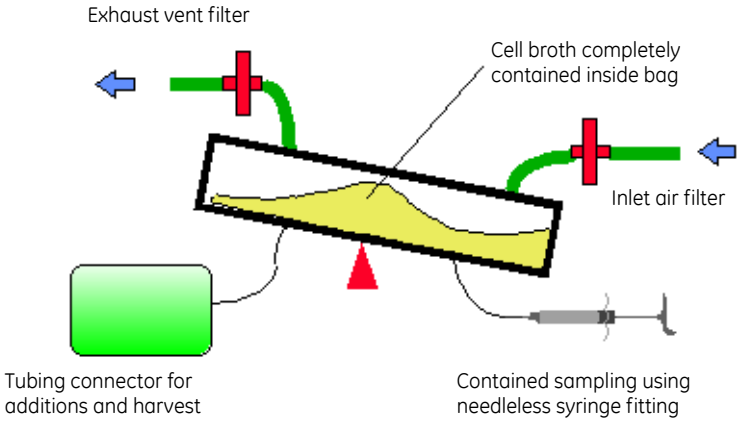




WAVE Bioreactor can save time and effort in preparing inoculum. It also reduces the risk of contamination.

2. High containment applications

WAVE Bioreactor operates as a completely closed system making it ideal for virus, vaccine, and other high containment applications.



WARNING! WAVE Bioreactor system may not be used under any circumstances for biological warfare applications. This is a violation of your purchase agreement.

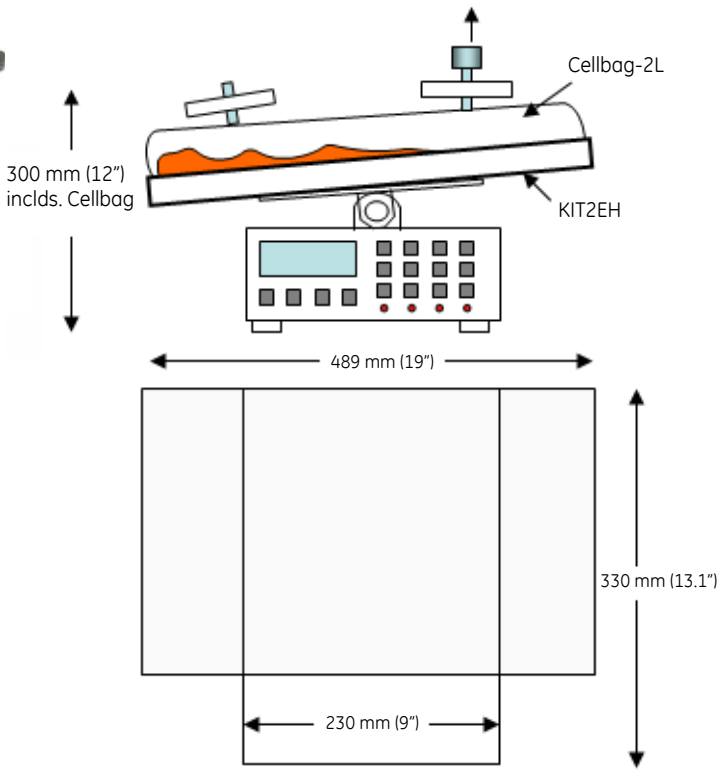
3. In-process pooling

In many situations it is necessary to pool cells prior to the next processing steps. For example, cells may be collected from several bioreactors and pooled prior to being used as inoculum for the next scale bioreactor. Cellbag offers an ideal way to keep these cell pools agitated and aerated during collection. The disposable nature of the system minimizes cleaning, sterilization, and validation.

Appendix B Specifications

B.1 WAVE Bioreactor 2/10

The electrically operated rocker unit is the base component of the WAVE Bioreactor 2/10 system. It is made of high quality stainless steel and aluminum. Disposable bioreactor chambers (Cellbags) are placed into a stainless steel/ fiberglass holder (KIT2EH) that fits on to the rocker base. The rocker unit is designed to provide optimal mass transfer and mixing. Unit can use Cellbag-2L or Cellbag-10L for up to 5 liters culture. For smaller culture volumes, Cellbag-500mL and Cellbag-1L are also available. Temperature control is provided for bench top operation or use in incubator.



Voltage:	110 to 120/220 to 240 VAC (For 110 to 120 VAC use 115V position) (For 220 to 240 VAC use 230V position)
Frequency:	50/60 Hz
Power:	145/210 VA
Maximum Current:	4 A
Fuse:	2 × T 4AL 250V
Performance:	Adjustable rock rate 3 to 40 rocks/min Adjustable angle from 2 to 9 degrees. Integral air pump with mass flow meter. RS-485 communications port LCD display and control interface. Temperature control with heater and sensor.
Dimensions:	230 mm × 330 mm × 160 mm (9 × 13.1 × 6 inch) With KIT2EH: 489 mm × 330m × 200 mm (19 × 13.1 × 8 inch)
Environmental:	Operating conditions 5°C to 30°C with lid, 15°C to 30°C without lid. <95%rh non-condensing Storage conditions -40°C to +80°C
Weight:	4.2 Kg (9 lb.)
Options:	Optional PERFCONT2E weight-based perfusion controller with integral feed/harvest

Appendix C Spare parts

WAVE Bioreactor rocker unit is designed for continuous reliable operation. Mean Time Between Failure (MTBF) is estimated at greater than 6000 hours. Please contact GE Healthcare Customer Service for spare parts.

Description	Part Number
Air line tubing with fittings	WV003465
Filter heater	28411639
Filter heater power supply	28411641
Fuse 250V, 4A slow blow, 5 × 20 mm	WV000772
Perfusion module cable DB9M-F 2.5ft	WV001154
RTD immersion-type temperature probe	WV000536
SRTD surface-type temperature probe	28411665
Stainless tray	28411542
Temperature probe cable (yellow 3-pin)	WV004036
EU Power cord	WV000449
UK/IRL Power cord	WV002924
US Power cord	WV002282

Appendix D Communications

D.1 Data acquisition

WAVE Bioreactor 2/10 has two ports for data acquisition. Both are located on the back panel. Either of the two RJ11 6-pin modular jacks can be used to daisy chain up to 247 instruments using RS485 multidrop communications for data acquisition via MODBUS. The GE Healthcare PCDAQ software provides a simple MODBUS data acquisition package and includes connecting cables for the RS485 communications port. All variables on the MODBUS register table can be accessed.

D.1.1 Communication protocol

The instrument modules communicate using RS-485 protocol. The communications parameters are:

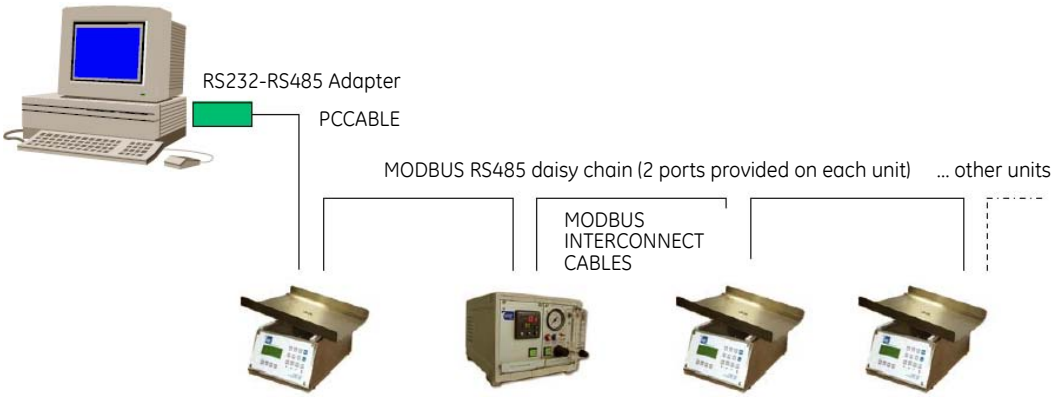
- 38400 baud
- No Parity
- 8 data bits
- 1 stop bit

The communication protocol used is standard MODBUS RTU.

D.1.2 Communication wiring

Multiple instrument modules and WAVE Bioreactors may be daisy-chained together to form a RS-485 network. Each module has two identical MODBUS connectors located in the back. Connect either MODBUS port on the first bioreactor on the network to the PC using the supplied PC cable. The RS-232 end has a gray converter that plugs into a PC COMM port. Plug the PC cable into the modular jack on the adaptor.

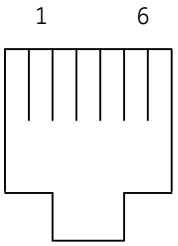
Connect the second module on the network using a MODBUS interconnect cable. Connect other WAVE Bioreactor instruments and instrument modules using the provided MODBUS interconnect cables. These are RJ11 modular straight cables that plug directly into RJ11 RS485 jacks located on the rear of the equipment.



The RS-485 to RS-232 adaptor uses power from the PC port. In some laptops there may not be sufficient power to operate the adaptor. Please contact GE Healthcare for information on alternative externally powered converters.

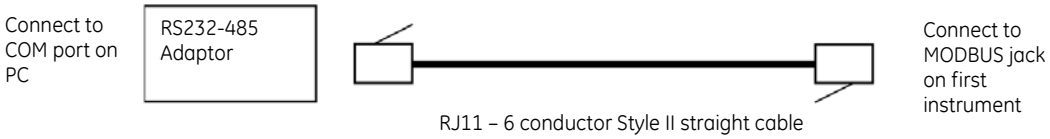
RJ11 RS-485 MODBUS JACK PINOUT

RJ11 - pin 2	DATA A(-)
RJ11 - pin 5	DATA B(+)
RJ11 - pin 4	SIGNAL GND

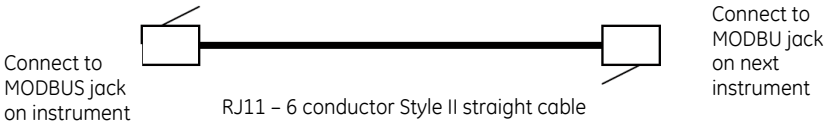


RJ11 jack pinout
viewed looking at jack

D.1.3 PC connection



D.1.4 MODBUS interconnection



D.1.5 WAVE Bioreactor 2/10 addressing

Each WAVE Bioreactor 2/10 is assigned a unique address.

The instrument's addresses start at 101. The first unit will be 101, the next 102 and so on. The highest address possible is 110. The factory default address is 101.

To change the address it is necessary to reprogram the PLC inside WAVE Bioreactor 2/10. This can be done from the ***SETUP*** Menu.

The GE Healthcare PCDAQ program provides preconfigured data acquisition for all WAVE instrumentation and bioreactors, including instruments 2/10, 20/50, 200 and 500/1000. This allows data acquisition to be set up on any Windows-compatible PC in minutes.

D.2 MODBUS registers

SIGNAL	DESCRIPTION	MODBUS ADDRESS
ROCKING SPEED PV	Current rocking speed	41400
AIRFLOW PV	Current airflow rate	41401
TEMPERATURE PV	Current temperature	41402
NET WEIGHT PV	Current net weight	41403
SPEED SP	Rocking speed setpoint	41411
ROCK ANGLE	Rock angle setpoint	41412
AIRFLOW SP	Airflow setpoint	41413
TEMPERATURE SP	Temperature setpoint	41414
PERFUSION ENABLE	Perfusion module enabled 1=enabled	41415
FEED SHOT ML	Specified feed shot volume	41416
FEED RATE ML/DAY	Specified feed rate	41417
WEIGHT SP	Weight setpoint	41418
FEED TIME	Calculated feed time	41419
FEEDON	Feed pump ON	40017.2
HARVON	Harvest pump ON	40017.3
ROCKON	Rocker ON	40018.1
AIRON	Airpump ON	40018.2
TEMPON	Temperature controller ON	40018.3
PERFON	Perfusion controller ON	40018.4
HIPRESALM	High bag pressure alarm	40068.1
HTROUTALM	Heater disconnected or over temperature alarm	40068.2
RTDFAILALM	Temperature probe failed	40068.3
SPEEDDEVALM	Speed deviation from setpoint	40068.4
AIRFLOWDEVALM	Airflow deviation from setpoint	40068.5

SIGNAL	DESCRIPTION	MODBUS ADDRESS
TEMPDEVALM	Temperature deviation from setpoint	40068.6
WTFAILALM	Weighing system failure	40068.7
FEEDALM	Feed failure	40068.8
HARVALM	Harvest failure	40068.9
LOW WEIGHTALM	Low weight alarm	40068.10
HIGH WEIGHTALM	High weight alarm	40068.11
MASTER ALARM	Master unit alarm	40069.1

For contact information for your local office, please visit
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