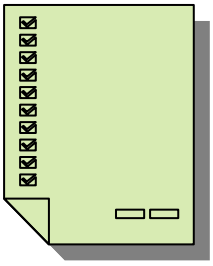
LABFORS 3 OPERATING MANUAL & USER GUIDE

INFORS EQUIPMENT
Read this FIRST!

SAFETY

COMPLIANCE



SPECIFICATIONS

MAINTENANCE & SERVICING

1. CE conformity declaration

INFORS equipments are CE-labelled from the factory and supplied with an EC declaration of conformity

INFORS equipment complies with the following Directives & Standards:

Directive Machinery 89/37EC
Directive Electromagnetic compatibility 89/336 EEC (IEC61326)
Directive Low Voltage 2006/95 EC EN61010

2. Quality Management Systems

All INFORS equipment is manufactured in accordance with INFORS' quality management system which is certified by BVQI in accordance with the requirements of ISO 9001.

3. Testing

All INFORS equipment undergoes electro-mechanical operational testing before it leaves the factory. The exact nature of the tests varies according to the equipment type.

All equipment is delivered with a signed test certificate. The tests described are conducted in accordance with the procedures set out in INFORS' quality management system and in accordance with international classification companies.

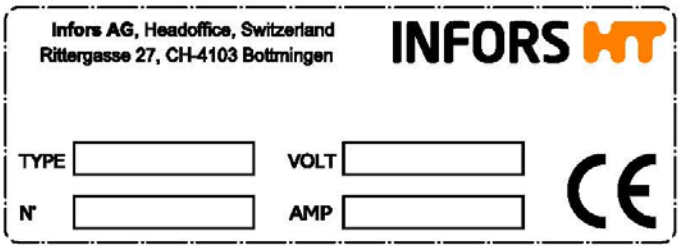
4. Applicability

This is specified in the specifications table included within this section of the document.

If any of these specific sections appears to be missing for your equipment please contact INFORS and this can be rectified.

Please be aware that if the equipment is acquired second-hand from an original user, it may have been modified, upgraded and enhanced such that some details of the configuration may differ to those described in this manual. We will provide any help and information necessary to bring the documentation up to date but individual options may not be the standard ones supplied by Infors. In this case, it is the responsibility of the previous owner to supply any additional manuals, configuration information and safety-related items. **INFORS disclaims responsibility for all equipment that is not in original condition i.e. modified by the user without prior agreement from INFORS.**

5. Identification plate



The identification plate is located on the housing of every piece of equipment.

The identification plate must <u>never</u> be removed from the equipment. If the name plate is removed, it is not possible to identify the equipment, and it will not be possible for warnings contained in this manual to relate to the specific applications for which the equipment is used.

The serial number of the equipment is also displayed on the vessel identification plate for stainless steel pressure vessels.

5.1 Vessel identification plate

6. Safety Points

6.1 General


NEVER open open or remove covers (internal or external) with the power switched on.


No operations beyond those expressly stated in this guide are authorised by INFORS as being suitable for the equipment.


All work on the equipment – including adjustments, repairs, pipe couplings, etc. – must be undertaken by professionally qualified staff.


When repair and maintenance work has been completed, any safety equipment provided must be refitted in its original state before the equipment is started.


The equipment must be installed in accordance with the instructions contained in this user manual.


The equipment's weight is over the permitted allowance of kilos/pounds that people may lift, so it must be lifted mechanically. –see specifications sheet.


Users are responsible for ensuring that the equipment is used in accordance with safety procedures applicable to their work and is free of any biological or chemical contamination if an examination by INFORS staff is requested.
INFORS will not be held responsible for any equipment which has been improperly used, maintained, modified or repaired; nor for any consequential losses arising.


All the housing covers of the basic unit and operating panel are, as they may cover critical areas, only to be removed by personnel explicitly authorised by INFORS to do so.
If in doubt about any aspect of the use of this equipment or its suitability for an application, please contact INFORS.


Please ensure a Risk Assessment is carried out according to your safety regulations before using the equipment.


Live steam under pressure can cause severe burns and physical damage due to release of pressure suddenly.

6.2 Safety points relating to installation & use


Electrical connections should only be installed and fitted by a qualified electrician to current electrical safety regulations.


Installation of all services lines should be made only with pressure resistant tubing retained with suitable tubing clamps.


Authorization for use of an oxygen supply and its operation in accordance with your own safety guidelines are the responsibility of the customer'


If previous operation was not subject to either an unexpected loss of power or closed down by turning off individual parameters but switched off using the main ON/OFF switch, then restarting the unit is analogous to a return of power after a failure and the last set points will be used for control. This means that the stirrer can operate and possibly that the acid and base pumps may deliver reagent. Therefore, a fermentation must always be terminated by explicitly turning off individual parameters.


Never use the main ON/OFF switch to end operation!


Never remove a top-mounted motor or work on the drive shaft of bottom drive units with the mains switch set to on.


Always use gloves when removing a top-mounted motor after a period of use, as it is likely to be HOT.


Always remove the tubing from the peristaltic pumps when cleaning the base unit or vessel in case acid or base may be accidentally released. Warning: do not autoclave reagent lines filled with corrective agent in case the tubing bursts. Use water instead. As part of the cleaning regime, the corrective reagent tubing MUST be rinsed with water. Storage and autoclaving of tubing containing acid or base represents a hazard to the user and can damage the tubing.


Take care regarding overpressure following autoclaving. Tubing can burst or come off inlet pipes. Ensure a pressure equalisation line is present for each reagent bottle, capped with a small filter.


Take care to avoid „stick" injuries when handling the piercing needles for the inoculation and reagent inlet lines plus fitting the antifoam electrode.


When inoculating or adding any peripheral by piercing a membrane, ensure the flame does not come in contact with hands, electrical wiring etc in the vicinity of the vessel top plate.


Always work to GMP and observe other appropriate standards


Observe all safety issues relating to hazardous chemicals, biological material and equipment under pressure, especially points regarding skin and eye contact.


The equipment is only to be operated by suitably qualified and trained personnel, both in terms of equipment use & microbiological expertise.


In normal use, operators should ware appropriate safety clothing, gloves, safety goggles and a face mask as appropriate to the degree of microbiological risk.


The nature of the microbiological and chemical risks associated with the use of individual units cannot be assessed by the manufacturer and its specification is the responsibility of the user.


The environmental hazards associated with the use of individual units cannot be assessed by the manufacturer and its specification is the responsibility of the user.

6.3 Safety points related to testing the equipment

 Should the previous fermentation not have been stopped using the Intervention/Stop menu but by turning off at the main power switch- analogous to a power failure- the set points in force at the moment of termination will be used on re-start. This means that the stirrer can run.

 The main power switch should not be used as a functional ON/OFF.

 Never put your hand into an operating unit – risk of injury due to high rotational forces.

 No reagent lines to be left connected to the pumps - if the pump operates or the contents spill out there is risk of damage from acid or base.

 Handle glass vessels, reagent bottles and other glass components with care to minimise the risk of breakage or other damage resulting is sharp edges

 Do not apply excessive pressure when handling any glass components in case of breakage and consequent sharp edges.

 Ensure hair, loose clothing etc cannot come into contact with any rotating parts.

7. Emissions and Warning indications

 Any loud and/or unusual noise from any part of the equipment should be taken as a sign of a problem and the equipment closed down and inspected immediately.

 Any smoke or smell of burning should be taken as a sign of a problem and the equipment closed down and inspected immediately.

A whistling noise could indicate a dry mechanical seal. Stop the stirrer and lubricate the seal immediately. Exchange if necessary.

In normal operation, some additional noise and heat may be generated, the extent depending on the phase of operation (see performance data)

Service & Maintenance

- Only fully qualified and authorised persons may repair the equipment
- Cleaning and routine maintenance information in provided in the main manual.

Before you begin…

Copyright

Modification

Due to the constant development and improvement of our products, the bioreactor system supplied to you may differ from the one described in this manual. INFORS explicitly retains the right to make such deviations and modifications.

Safety aspects

A number of points relating to the safe operation of this machine and its proper use/maintenance are contained in this manual. It is vital you read all relevant sections before bringing the bioreactor into use and comply with the instructions given during its operational lifetime. No operations beyond those expressly stated in this guide are authorised by Infors as being suitable for the equipment.

Infors will not be held responsible for any equipment which has been improperly used, maintained, modified or repaired; nor for any consequential losses arising.

Additionally, users are responsible for ensuring that the equipment is used in accordance with safety procedures applicable to their work and that the bioreactor is free of any biological or chemical contamination if an examination by Infors staff is requested.

All the housing covers of the base unit and control panel are, as they may cover critical areas, only to be removed by personnel who have been explicitly authorised by INFORS to do so.

Individual safety warnings are given throughout this manual where appropriate and clearly labelled with the following symbol ➲.These points cover both potential risks to the operator and the machine by mishandling or incorrect use.

➲ ***Please ensure a Risk Assessment is carried out according to your safety regulations before using the equipment.***

If in doubt on any aspect of the use of this equipment or its suitability for an application, please contact your local subsidiary or distributor.

Service and technical information can be obtained by contacting INFORS AG. See our web site www.Infors-ht.com for latest contact details.

Please note the serial number of your unit(s) plus the approximate date of purchase for use when making enquiries.

8. CONTACT DETAILS

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Contact details of our local dealers world wide can be found on our web site.

HOW THIS MANUAL IS ARRANGED:

- **The first section deals with installation requirements:**

  - Conformity & Safety points
  - Contact details
  - Connections to services
  - Location
  - Chemical resistances
  - Contents of Service Kit
  - Pre-checks

- **The next section tells you how to bring the LABFORS 3 into operation in a step-by-step manner, including**

  - Preparation
  - Sterilization
  - Operation
  - Closedown & cleaning.

- **The third section covers a detailed description of the X-DDC instrumentation:**

  - Offline Menus & Options
  - Online Menus & Options
  - Parameter Options
  - Description of control loops

- **The Appendices include (as appropriate to your system):**

  - Connections to software IRIS
  - Cold water quality
  - Gas mix options
  - Dimension drawing standard
  - Specifications table
  - Utility requirements
  - Pin configuration

  Possibly:

  - Cell culture features
  - Use of Analogue pump
  - Multi-bioreactor use
  - Labfors Lux information

Additional training notes, experimental protocols & practical training courses are available for users new to fermentation - please contact us.

TABLE OF CONTENTS

9. Installation

9.1 Delivery Information

Transport to final location
In view of the weight of the complete bioreactor and packing materials it should never be carried by one person alone. For moving the packed system over long distances, a low trolley or palette raiser is recommended. If the bioreactor is to be transported by a fork lift truck, **it is vital it is secured in such a way that there is no chance of it falling off or otherwise being damaged!**

Packing materials
The bioreactor and auxiliary equipment is shipped in a wooden crate. All packing materials are environmentally friendly and can be recycled.

Unpacking and Checking
Please make sure that no part of the bioreactor sustains damage during unpacking. Use the delivery documentation to ensure that all parts are present. If there is a discrepancy between the list and the contents and/or there are any signs of transport damage, please contact your local Infors representative **at once**.
If this advice is not followed promptly, any resulting costs will be the responsibility of the purchaser i.e. you!

9.2 Location

The bioreactor is best mounted on a laboratory bench of suitable working height. For the size of space required, see dimension drawings in Appendix) Access to air, water and power services should be nearby along with a suitable drain / sink.
A free space of 50cm should be left all around the bioreactor to make servicing both easier and quicker.
The unit should be sited away from potential sources of electrical noise

9.3 Services

The final connection of the bioreactor to the services will be made by INFORS, if installation is requested by the customer. This is aided greatly when all services outlets including the necessary pressure reduction system and appropriate filters (where necessary) are already installed by the customer.

9.4 Connections

9.4.1 Gas Supplies

For cell culture systems or bacterial systems with oxygen supplementation, the appropriate gas supplies must be available at the time of installation and commissioning.
➲The gas supply **must** be pressure regulated (max 2.0 bar) and have a suitable rotameter if constant gas flow into the bioreactor is to be maintained. Unless specified with the bioreactor, supply of these components is by the customer.

➲Authorization for use of an oxygen supply and its operation in accordance with your own safety guidelines are the responsibility of the customer.

Barbed pipes for push-fit connection to services are provided in a separate bag. Push these into the corresponding sockets in the base unit. Flexible tubing for these pipes is provided as part of the Service Kit.

9.4.2 Water Inlet

Cold water supplied at a pressure of >= 1,5 bar enters from the left side of the base unit of the bioreactor.
Push-on connector pipe, 10mm, secured with a jubilee clip.
➲ Only cooling water with no more than a slight hardness can be used exclusively. The use of drinking water supply for cooling purposes is possible, as long as it is “very soft” or “soft”. **Never use demineralised or distelled water!** Contact your INFORS Service for questions concerning water quality and calcification.

9.4.3 Water Drain

The drain outlet is on the top of the bioreactor jacket - if applicable. It requires a clear fall to the drain/sink with no back-pressure. For single-walled vessels with a heater pad, a "cold Finger" may be fitted.
Push fit onto glass the overflow connection arms (12mm) or the drain arm of cold finger, as appropriate.

9.4.4 Air gas Inlet

The air inlet can be found on the right rear side of the bioreactor. Use only clean, dry oil and dust free air with a pressure of >= 0.7 bar.
Push-on Connector pipe (6mm). Secure with a jubilee clip.
➲Never connect the gas supply in any way which bypasses the pressure reduction valves! Secure gas tubes with hose clamps.

9.4.5 Exit Air

The exit air leaves via the disposable filter attached to the exit gas cooler (if fitted).
➲ If oxygen supplementation is used, take care to pipe the exit air away safely to avoid explosion risk

9.4.6 Electrical Power

230V/ 50Hz,10A single-phase supply for standard LABFORS (or 115V option).
The power supply should be clean and constant - if not, it is recommended you fit suitable filters and or a UPS to the mains power supply.

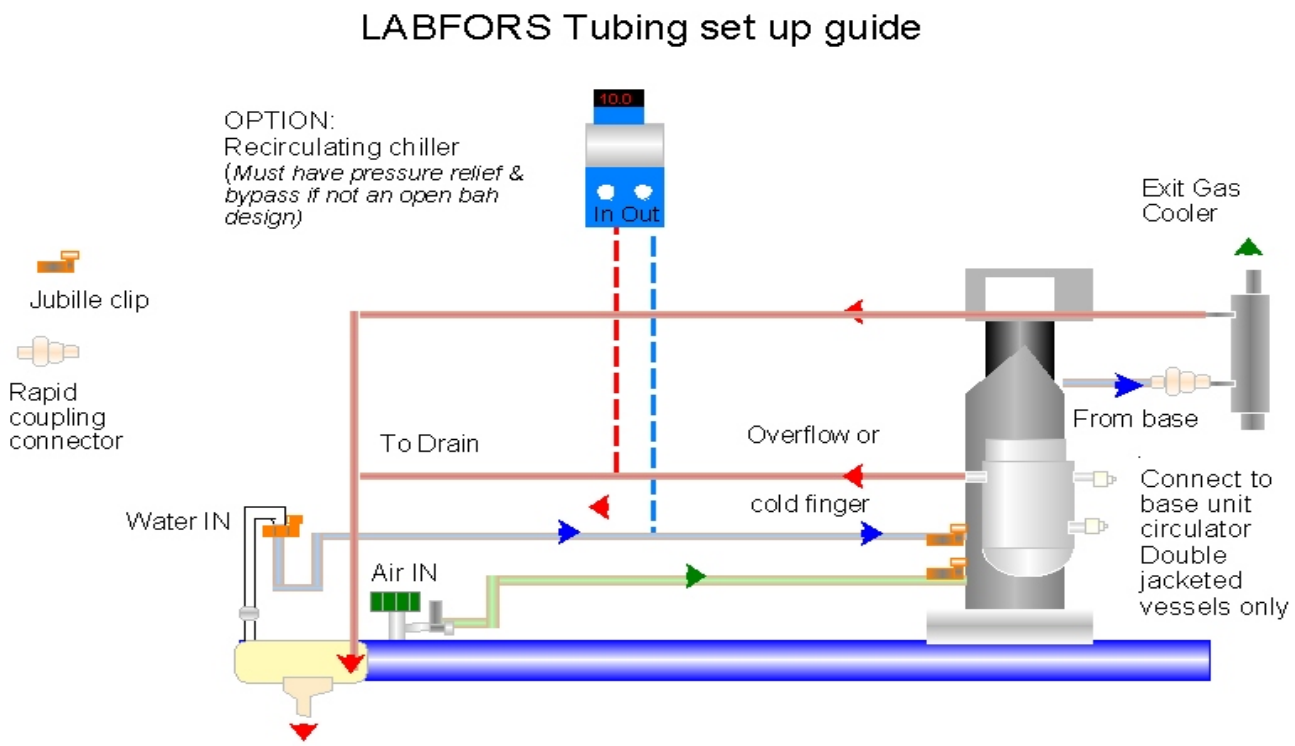
➲Electrical connection by a plug and socket, should be installed and fitted by a qualified electrician to current electrical safety regulations.

➲Installation of water and air lines should be made only with pressure resistant tubing retained with tubing clamps (jubilee clips).

You may need:

A Thermocirculator for supply of chilled water at eg. approximately 10^{o}C
NB Must have a pressure relief and bypass system fitted.

A diaphragm-type air pump for oil-free supply of air eg 0-15L/min


LABFORS Tubing set up guide
OPTION:
Recirculating chiller
(Must have pressure relief & bypass if not an open bah design)
10.0
In Out
Exit Gas Cooler
Jubille clip
Rapid coupling connector
From base
To Drain
Overflow or
cold finger
Water IN
Air IN
Connect to base unit circulator Double jacketed vessels only

9.5 Serial port connections

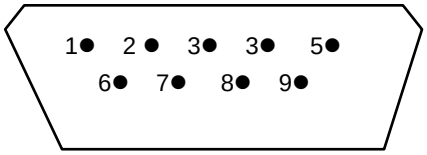
The X-DDC system can communicate directly over the serial connection or with a computer running the IRIS software

RS 232
On the underside of the instrument panel on the left side, a male, 9-pin connector is provided. Do **NOT** use the serial port connector in the base unit section - this is reserved.

Pin Configuration

Pin 2 receive data (RD)
Pin 3 send data (TD)
Pin 5 Signal ground

RS232 Connections



Description	PC Side 25 pin	PC Side 9 pins	Labfors 9 pins
Ground	7	5	5
Tx/Rx	3	2	3
Rx/Tx	2	3	2

Tx Rx

9.6 Memory-Card

The memory card is located in a slot under a cover at the left side of the X-DDC operating panel.

Its function is to store configuration data about the fermentation (eg. calibration values & PID settings etc). It does **NOT** store experimental data. Sufficient memory is available for the card to hold the configuration of multiple bioreactors, if the system is used in this way.

The facility is provided for the data on the card to be stored on a PC and uploaded when required (see Section Recipe).
The battery inside the card buffers the data and has to be exchanged regularly (durability up to 2 years). Otherwise data stored on the card will be lost whenever the bioreactor is switched off. Save your data (see section 5.1) before exchanging the battery.

➲IMPORTANT: After taking out the old battery, immediately replace it with the new one.

1. Remove the card from the slot in the X-DDC panel housing (noting the orientation of the card). A firm pull may be needed to release the card.
   ➲ Your bioreactor **MUST ALWAYS** be turned off before removing the memory card.
2. Put your fingernail or eg. a thin penknife blade into the small crack above the area containing the screw hole and pull backwards.
3. A battery holder is revealed with the battery sitting in position. Note the orientation of the battery and exchanged for a new one.
   ➲ Touch a new battery only sideways when exchanging.
4. Re-insert the memory card.
   Ensure the write protect tab on the back edge of the card is set towards the middle of the card in the "WRITE" position (not protected) before replacing it.
5. The card will be automatically initialised on starting the bioreactor. This will take a few seconds.

➲**NB. A memory card MUST be present for the bioreactor to operate. Never start the unit without a card in place.**


Push the button to release the memory card


Pull out the memory card

Memory card / battery holder / battery

9.7 Maintenance points

We recommend that all **O-Rings** and **Membranes** should be changed at least once a year.
Clearly, any deformed or damaged O-rings must be replaced before each run. Before fitting the O ring, wet the O-ring with alcohol. Pierced membranes must be replaced.

During operation, any spillages of acid or alkali should be cleaned up immediately.

At the end of the fermentation and emptying of the vessel and reagent tubes, the vessel should be sterilized and rinsed several times with water. 0,1 N NaOH is recommended for denaturizing proteins.

➲Observe all safety precautions regarding skin or eye contact and inhalation of such chemicals as defined by your local regulations regarding the treatment of hazardous chemicals.

9.8 Mechanical seal

The mechanical seal must be replaced at regular intervals.
The seal is destroyed by running dry. Therefore it is vital to ensure this component is always supplied with fluid (glycerine).
See instructions on the next page for topping up the seal.

9.8.1 Electrodes

The pH- and pO_2-Electrodes are described in the manufacturer's literature (in the box with the electrodes). There you will find information about cleaning and regeneration. The pO_2 electrode membrane cartridge **MUST** be filled on first use, then checked and refilled with electrolyte after every 2-3 sterilizations for normal operation.

9.8.2 Chemical resistance

The housing was tested with various substances. In connection with the following substances, **no significant changes** were found:

-Water
-Inorganic acids, phosphoric, nitric, sulphuric, 10% solutions
-Organic acids: formic, vinegar and lactic, 10% solutions
-inorg. alkali: sodium hydroxide, 10% solution
-inorg. Chemicals in aqueous solution.
Hydrogen peroxide, 10%
Calcium chloride and concentrated bleach
-hydrocarbons. benzene, Benzene, motor oil & diesel

The housing should not be in contact with the following substances **for prolonged periods of time**

-Acids, alkalis and inorganic chemicals in higher concentrations than given in section
-Methanol, Ethanol
-Acetone
-Cyclohexanol
-Ethyl acetate
-Ethylene glycol acetate
-Methylene chloride
-Perchloro ethylene
-Carbon tetrachloride

9.8.3 Topping up the mechanical seal

1. The seal is lubricated with glycerine and must NEVER be allowed to run dry.
2. Some liquid in a loop of silicone tubing at the base of the drive hub on the top plate shows if any glycerine is still present .
3. To replenish the glycerine (no glycerine is visible anymore in tubing before autoclaving), pull off the tubing from the longer arm.
4. Connect a syringe filled with glycerine to the silicone tubing and pump glycerine in until it can be seen emerging from the open long pipe.
5. Remove the syringe and remake the loop.

1. Look for liquid in the tubing

2. Fill with glycerine if necessary

3. Replace tubing

9.9 General Points

Components not supplied directly by INFORS
All components sourced from outside the INFORS group of companies have been tested before incorporation into our products. As a general rule, INFORS only uses proven components. In exceptional cases, it is still possible that faults can occur in other manufacturer's components. In such cases, INFORS cannot accept any liability other than our normal warranty obligations. We ask our customers for understanding and do, of course, always strive for the highest possible quality.
This exclusion does not apply to safety matters.

9.9.1 Reselling

For reasons of operator reliability, legal considerations, smooth and continued supply of spare parts and the protection of INFORS' intellectual property rights, the customer is obliged to inform INFORS of any change of ownership or re-sale.
In return for this service by our customers, INFORS offers to support the new user and supply training for the new operator(s).
INFORS reserve the right to refuse to supply maintenance support or spare parts if this duty is not fulfilled.

9.9.2 Disposal

The LABFORS bioreactor is mainly constructed from metal with some glass, plastics and electronic components. Disposal by normal methods is possible along with the motor, heaters and other internal components.

Please ensure that all necessary steps have been taken to ensure microbiological safety before considering disposal.

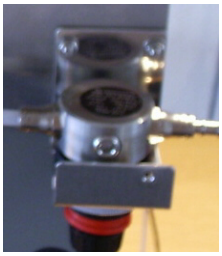
9.9.3 Identification plate and serial number

The ID plate and serial number can be found on the right side of the base unit

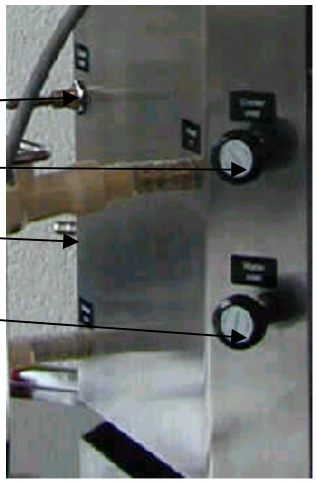
10. A Test Run

Before preparing the system for first use, a functional check on the services and basic operation of the stirring, temperature and gas supply systems can easily be carried out. Full details of the individual procedures can be found in the later sections.

1. Ensure that the mains switch on the base unit is OFF and the manual water inlet valves at the right bottom of the base unit (Green knobs) are turned off- fully clockwise Likewise, the rotameter needle valve should be fully closed (black knob below the glass rotameter scale).
2. Check all connections to services lines are tight and that air and water are turned on at the supply. **CHECK FOR LEAKS**.
3. Fill the vessel to the working volume with water and then connect to the base unit



Air Inlet at right rear



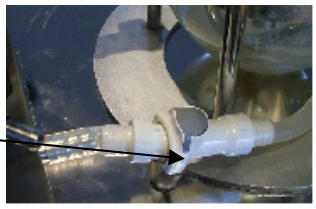
Exit Gas Cooler Water Inlet

Exit Gas Cooler Valve

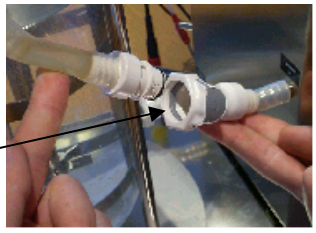
Water Supply IN

Water inlet valve to Jacket

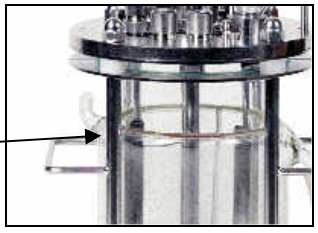
Double jacket:



Water outlet from double jacket to base unit / rapid coupling connector

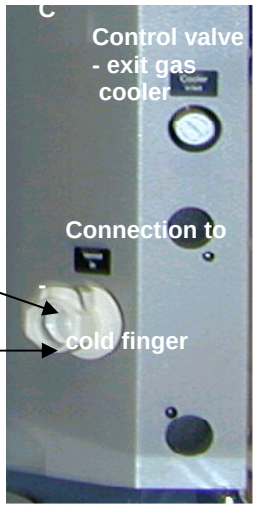


Water inlet to double jacket / rapid coupling connector



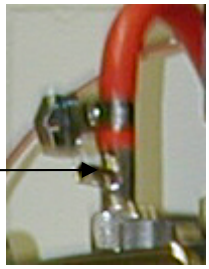
Place a pipe from the overflow to the drain
(using the silicone tube, 10mm inner diameter)

Single walled vessels should arrive fitted with a heater pad. Connect the optional cold finger in the top plate with the rapid coupling connector with the base unit.

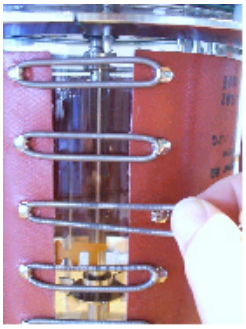


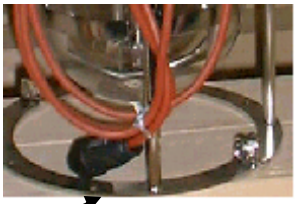
Connect the rapid coupling connector to the water inlet on the base unit (lower right side)

Connect the outlet of the exit gas cooler to a flexible pipe and run this to the drain/sink (or return to a thermocirculator, if used.

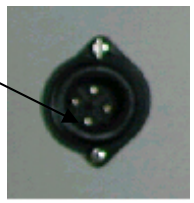


If not already fitted, locate and fit the heater pad around the vessel.



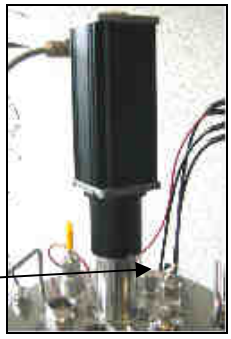


Connect the heater pad plug to the socket on the lower left



4. Connect the motor to the base unit using the steel DIN-type socket at the middle right of the base unit (front one).

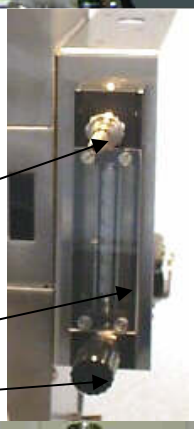
5. Mount the motor on top of the vessel, turning until it locates on the pin in the hub.



Locating pin on drive

6. Plug in the mains cable and switch on the electrical supply.

7. Connect the rotameter outlet (pipe above glass scale) to the sparger (tube with holes in the vessel) with flexible tubing and turn the rotameter needle valve knob anti clockwise until gas can be seen bubbling though the liquid. Filters are not needed at this stage but the inlet filter may already be fitted.



Outlet pipe to sparger
Glass tube with scale L/min (bottom of ball)
Needle valve-adjust
Sparger inlet

➲When using a Mass Flow Control Valve for gas flow (**option**):
If the Mass Flow Control Valve is activated without first turning on the gas supply, a huge control error will build up. This influences any adjustments necessary by the valve to reach the pre-selected set point for flow rate. When the gas supply is later turned on, this error causes the set point to be exceeded by a large margin, which is clearly undesirable.

→Therefore, always turn the gas supply on before setting "Output Active" to ON (in X-DDC), or otherwise activating the Flow parameter.

8. Open the Water Inlet Valve (BOTTOM green knob) so water can flow.

9. Insert Pt-100 temperature sensor into the pocket in the top plate until metal to metal contact can be heard and/or felt (orange cable with metal sensor end permanently attached).

10. The Operating panel will have completed its self test and you will see the **FERM** screen.
11. Select bioreactor 1…4, if applicable

12. Use the rotary knob, to go to the **START** tab, click to select, then turn the knob to move the cursor to **"Temperature"** and click the knob.

13. Type in a set point of 5 (^{o}C) and press 2 x the **ENTER** key.

14. Repeat this process for **"Stirrer"** and give a setpoint of eg. 200(rpm).

For temperature, ensure Measurement and Control Active fields are all switched ONFor antifoam, Regulation ON/OFF will be seen. If ON then it works with the Foam probe, if OFF then id can accept manual/software dose times based on a percentage time of 0-100% active.

15. Click knob or press **ENTER** key to automatically start the fermentation and the **Values** screen shows actual values

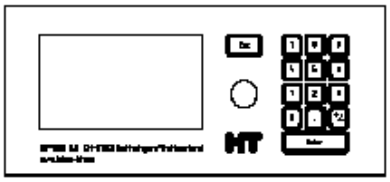
16. You should hear/see water rushing into the cold finger or vessel glass. When full, water will overflow and out to drain.

17. The stirrer should now be active.

18. Select **Temperature** using the cursor, then Setpoint. Enter a new value of eg 30(^{o}C). Carefully feel the jacket for signs of heating.

19. End the "fermentation" using the **Stop** tab and pressing "**1**", follow the instructions on screen.

A Preview of the Operating Panel.



Rotate to move position of cursor/ To navigate in menu. Highlight tabs

Click (press) to go from "Navigation" to main screen/option

Enter numerical value

Switch a function ON or OFF or AUTO

Enter Confirm change and leave menu option

Esc Allows return to the tabbed area of the screen or aborts current action

Select bioreactor

```
F1:STOP F2:STOP
 Info Select Config Passwrd Receipe

        Ferm1   Ferm2   Ferm3   Ferm4
Temp     24.0    26.0    28.0    29.0
Stirrer 180     180     180     180
pH        7.4     7.3     7.1     7.2
pO2      70.0    70.0    70.0    70.0
AFoam    0.00    0.00    0.00    0.00
Flow     0.00    0.00    0.00    0.00
Feed      Off     Off     Off     Off
Gasmix    Off     Off     Off     Off

Turn the rotary L-R: Scroll/Continue
```

Choose parameter

```
F1:STOP F2:STOP
 <<Back Start Param Pump Diagnose >>

  Selected fermenter:1

Temp     24.0        Stirrer  100.
■pH       7.0        pO2      100.
AFoam    0.00        Flow     0.00
Feed      Off        Gasmix    Off
FreeA    0.00        FreeA    0.00
FreeC    0.00        FreeD    0.00
FreeE    0.00        FreeF    0.00
FreeG    0.00        FreeH    0.00

Click: Menu entry
```

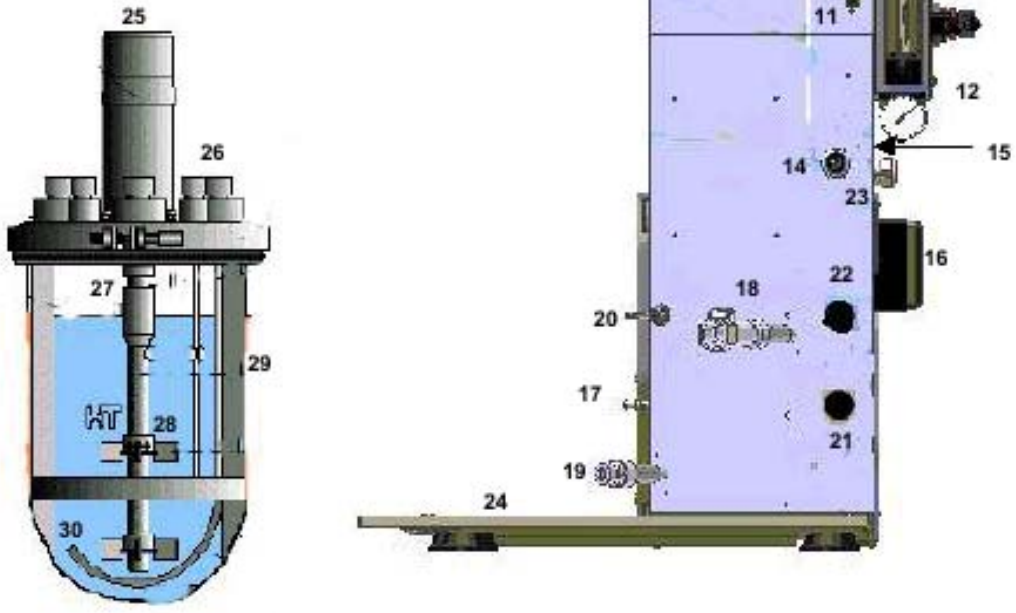
Type in setpoint

```
F1:STOP F2:STOP
 <<Back Setp Calib PID PConf Name SCasc.

  Selected fermenter:1
 Parameter:pH        Channel:2

Setpoint    :7
Alarm Lim.+-:5

Temp compensation (OFF:20°C) :On

Outp active :Off

Turn the rotary L-R: Scroll/Continue
```

Value screen

```
F1:RUNNING F2:STOP
 <<Back Select Values Pump Stop Diagnose

  Selected fermenter:1
 Name      Value    Setpoint      Output %
-------------------------------------------
 Temp    ¦  24.0 ¦  24.0        ¦Off
 Stirrer ¦   0   ¦   Off        ¦Off
 pH      ¦  7.00 ¦   Off        ¦Off
 pO2     ¦  0.0  ¦   Off        ¦Off
 AFoam   ¦  0.0  ¦   Off        ¦Off
 Flow    ¦   0   ¦   Off        ¦Off
 Feed    ¦  0.0  ¦   Off        ¦Off
 Gasmix  ¦  21.0 ¦   Off        ¦Off

Click: Menu entry
```

11. Preparation, Sterilization, Setup and Use



MAIN COMPONENTS

1. LCD Display
2. Keypad
3. RS232 port
4. Rotary Knob
5. Connection to Base Unit RJ45
6. Memory Card slot
7. Digital Peristaltic pumps
8. Manual Button for pump motor
9. Cables - Pt-100, pH,pO_2, Foam
10. Operational Amplifier Module
11. Status lights incl. Mains power ON
12. Air Inlet, & Rotameter assembly
13. OPTIONAL mass flow valve etc.
14. Motor connection socket
15. Analog/comms connections (rear)
16. OPTIONAL analogue pump
17. Water Inlet
18. Water inlet for jacket or cold finger
19. Water return for jacketed vessels
20. Water inlet-Exit Gas Cooler
21. Water inlet - manual control vavle
22. Exit gas cooler - manual valve
23. Main power socket & ON/OFF
24. Stainless steel drip tray on base
25. Drive Motor on top plate
26. Port fittings
27. Mechanical seal on drive shaft
28. Impellors
29. Baffles
30. Sparger

11.1 Checking Services Connections

For **OPERATION**, it is assumed that a correct installation and operational tests have been carried out and also that there are no mechanical alterations to the unit

Please make sure that you always inform us if the bioreactor is to be used for unusual applications eg higher temperatures, pressure control etc.

➲**Take care that:**
The supply of air & water are guaranteed and they are of sufficient quality.
Check the pressure regulation is correct.

11.2 Safety Points

➲If the current fermentation is not shut down from the start/stop menu i.e. by simply using the main ON/OFF switch, switching ON again will be analogous to a power failure and the operating conditions which existed before the interruption eg. setpoint values, will be used immediately on re-start.
That implies that the stirrer and reagent pumps (eg.acid & alkali) will also run!
Therefore:
➲ Never use the main ON/OFF switch to end operation!

When setting up or dismantling after a fermentation:
➲

- Never use the mains switch as an operational ON/OFF
- Never handle the motor system with mains power ON.
- Only pick the motor up by the handle or by using gloves - after prolonged use the casing will be HOT.
- A heater pad, if used, may get hot to the touch if elevated temperatures are use - take care when removing.
- No reagent lines to be left connected to the pumps - it may be that there is still some acid or alkali residue in the line
- Take care to avoid injury due to "needle sticks" when handling the inoculation, sampling and reagent delivery systems.
- Always work to GMP and/or other appropriate safety standards.
- Only allow suitably trained operatives to use the bioreactor

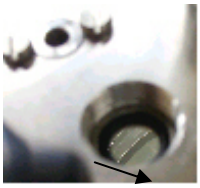
Please ensure a risk assessment is carried out if necessary.

11.3 The vessel

The vessels for Labfors3 bioreactor are normally delivered complete with internal fittings already in place (drive shaft, baffles, impellor, sample pipe and port closures. Other items for fitting to the top plate will be packed separately (eg. exit gas cooler, pH, pO2, antifoam electrodes and inoculation needle).

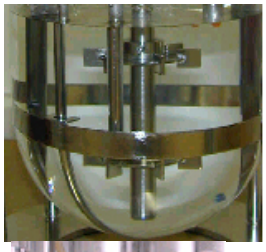
- Vessels come in several sizes, covering the range of 2-13L total volume. All are held in a metal support frame, to which the reagent bottle holder can be fitted.
- The body of the vessel is borosilicate glass and the top plate plus fittings are constructed from 316L stainless steel. All vessels have a round bottom.
- They can be equipped for bacterial or cell culture applications.
- The number and size of ports in the top plate varies with the size and type of vessel.
- Vessels can be jacketed to allow water circulation for temperature control or single-walled for use with a heater pad and cold finger. Connection to the water supply is via rapid coupling connectors (if these are stiff on first use, a little silicone grease applied to the "male" end will help).


Top plate


13.5Pg O ring & port


19mm port fitting & O ring


Vessel seal



Full details of vessel sizes, proposed port allocations and lengths of appropriate electrodes can be found in appendix.

Assuming the objective is to begin a new fermentation, a basic preliminary is the cleaning of the vessel and its peripherals.
Check the fit and connections of individual components before and after cleaning to prevent damage.

The vessel top plate is removed by undoing the four nuts around the circumference of the vessel and carefully lifting the top plate up far enough to clear the glass section.

The sealing of the vessel openings is achieved using **O rings**.
These are usually located in the parts to be installed such as the port fittings, electrodes etc. Only with 13.5Pg ports you will find them in the recess of the plate.

Please ensure that before installation, these O rings are undamaged. To make fitting and release easier, wet them with alcohol.

The following parts can be removed before cleaning,

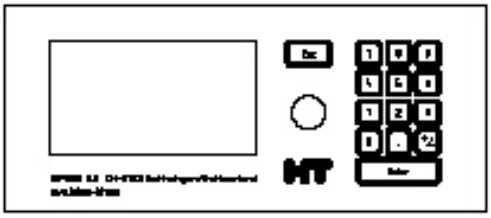
- Baffles, compress top slightly and pull out of vessel glass.
- Sparger, connected to the top plate
- Stirrer shaft, connected to top plate with impellors
- Port fittings, temperature sensor, also sample valve device can easily be disconnected from the top plate

NB. *The drive shaft can be removed by taking the top plate from the vessel and resting it on the bench with the drive shaft uppermost. Use a spanner to turn the flattened section of the drive shaft whilst keeping the bottom section still.*
The mechanical seal is exchanged by releasing the spring locking ring using a small hexagon key and taking the various seal elements way. When replacing, it is critical that you DO NOT touch the seal faces (or clean them with acetone to remove any grease from fingerprints etc).
If you are able to make this exchange yourself, we can provide information which provides step-by-step instructions on request. Alternatively, this exchange can be made as part of a preventive maintenance contract.

11.4 Switching ON at the base unit and Display

➲Before turning on, please note all safety instructions first!
The main power switch on the front right side of the instrument housing - press down to switch **ON.** A green light immediately shows in the small window above the switch on the base unit if power is present. I f not, check the socket and fuses.

The unit automatically performs a "self-test" diagnostic on start-up. If everything is satisfactory, "PASS" will appear next to the tested components such as 'CPU' , ' RAM' etc. If one or more components show 'FAIL', switch off at the mains switch, wait a few seconds and then switch on again to re-start. An information screen, **Ferm** shows.



The screen backlighting automatically switches off approx. 30 minutes after the last key press. The display is then dark. The backlight activates again as soon as any key is pressed. If desired, the backlight can be switched on permanently using Menu Options.

Rotate to move position of cursor/ highlight tabs

Press to go from "Navigation" to main screen/option

Enter numerical value

Switch the function ON or OFF or AUTO

Confirm change and leave menu option

Enter Leaves menu option without making any changes. Allows return to the tabbed area of the screen*

Esc *** some exceptions eg. Diagnostics**

Please note the following basic guidelines:

1. Numeric values, eg. 'Setpoint' pH '7.2' must be confirmed by '**Enter**' in Calibration mode. With a second 'Enter' the change is accepted and you leave the menu option. For other numeric fields, moving the cursor along is sufficient.
2. Switching On and OFF, eg. 'Output active ' - ON/OFF-does not require confirmation by '**Enter**'. Pressing the '**Enter**'- key confirms your input and immediately leaves the menu option.
3. Use the '**ESC'** to leave the menu option and any changes, including those values confirmed previously are not accepted.
4. Help on„How to proceed", can be found on the bottom edge of the display.
5. Tabs on the top line of the display give main options. >> = forward or << back
5. The main part of the screen is accessed by clicking the rotary knob, which can then be used to move up and down options.
6. Some screens allow direct entry of a numbered option from the tab.
8. Click the knob again to go back to the tabs or press the **Esc** key.

The operating panel is removable from the support frame at the back of the base unit. Undo the single bolt at the back of the panel to release . It can now be placed on the bench

The operating panel is connected to the base unit via a cable with twin RJ45 connectors running from the back of the unit to a socket at the rear of the base unit. Ensure this cable is securely plugged in before starting the unit.

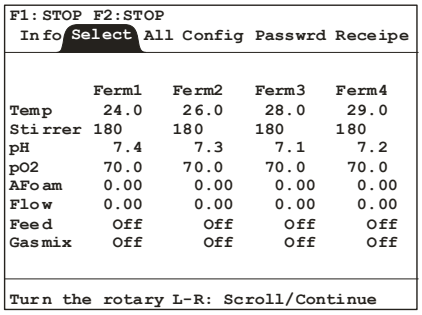
Diagnostic LED display

Acid pump On
Base pump On
Feed pump On
Foam pump On
Heater On
Cooling On
Water pump On
Coms status OK
Water present
Unused
Mains Power ON

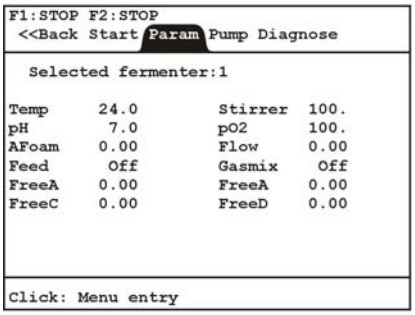
If communications have been correctly established a diagnostic red light will flash rapidly above the green mains light to indicate this fact. If the LED flashes slowly, the communication between the operating panel and the base unit is not properly established.

11.4.1 Starting a fermentation

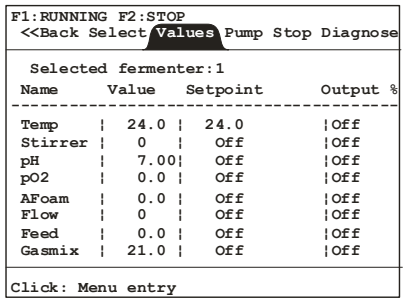
To start the bioreactor, select an individual bioreactor (bioreactor) (1..4) if necessary then move the rotary switch clockwise and the next tab to the right**, Start**, is highlighted.



Turn the rotary knob to the **Param** (parameter) field. Click to enter the menu. Choose each parameter by turning the rotary knob. Enter the Set points by using the numeric keys. Use the **+/-** key to turn on their output (**ON**). **Esc**, **Enter** or click to leave the menu.



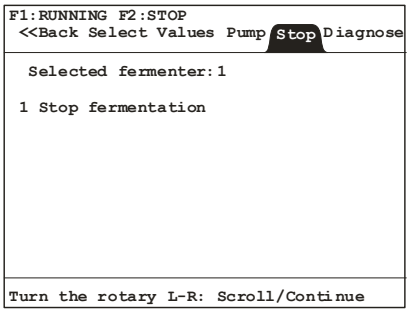
After starting the bioreactor by pressing **Enter**, the active screen is now displayed showing information about each parameters actual value, setpoint and controller output (a number from either 0-100 or -100 to +100) relating to how hard the controller must work to bring the actual value to the set point value.



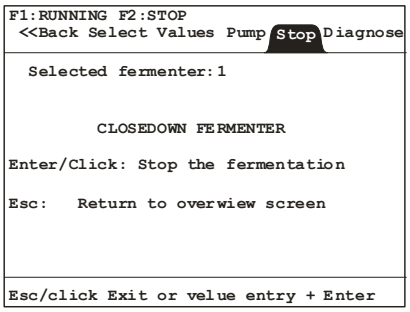
Stopping fermentation / Closedown bioreactor ("bioreactor").

➲ **Emergency** - use the main ON/OFF switch, being aware of any hazards making this difficult.
Alternatively, use the switch at mains power socket.

Normal closedown
Go to the **Stop** tab and press **1**. All parameter functions stop.



Press **Enter** to confirm when dialogue screen appears, bioreactor closes down and offline menu options are shown.

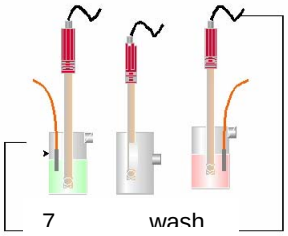


11.5 Before Filling

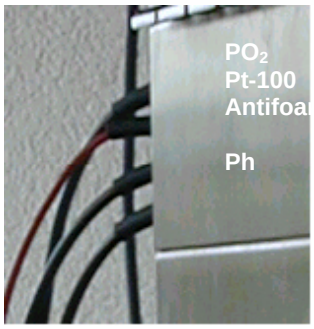
11.5.1 Preparation and calibration of pH electrode

The pH electrode must be calibrated before insertion into the vessel, before the vessel is filled at the latest.

If the pH electrode is to be used for the first time, the silicone seal over the diaphragm is removed using the small craft knife included with the electrode (shaped like a key). This can be scraped off rather vigorously to reveal the small white "dot" of the diaphragm beneath.



The silicone rubber sheath is removed from the bottom of the electrode and the red cap at the top replaced by the red connector attached to the cable on the base unit.
The electrode must be calibrated before sterilization, preferably using a two-point (High/Low) regime.

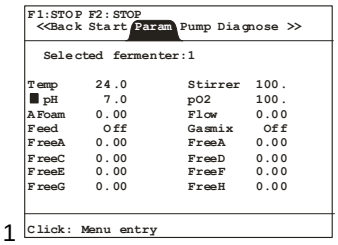


The electrode is connected to the cable at the left of the base unit which has the red co-axial connector (see diagram opposite for location of the right cable).

.
Hold the cable on its black collar.
Push carefully the connector onto the electrode.
Turn the red connector (2 – 3 turns) until the cable is connected firmly with the electrode.
Then start with the calibration.

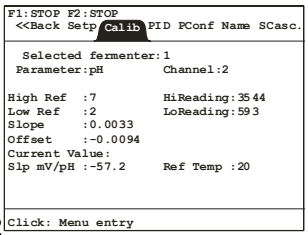
Mounting the pH Electrode in the Vessel
Fit the calibrated electrode directly into one of the 13.5mm Pg ports, or use an adaptor if necessary, for longer electrodes
➲**NOTE: Hand-tighten ONLY, do NOT use tools!**

The instrumentation is switched on and, following initialisation, the **Param.** tab is selected [1] followed by the pH parameter. Click to enter the main part of the screen then use the rotary knob to navigate to **pH** and click.



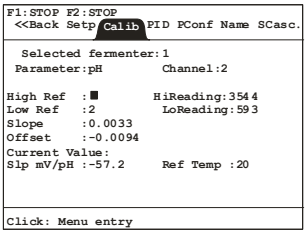
1

Move to the **Calib** tab using the rotary knob and click to enter the main screen [2].



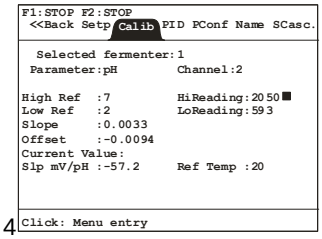
2

The cursor will automatically move to the **High Ref** field. Use the numeric keys to enter the **value** of the high reference buffer eg. pH 7 [3]. The electrode should now be placed in a beaker containing this buffer solution. Unless the Pt-100 temperature sensor is also placed in the buffer AND pH temperature compensation is switched ON, the buffer temperature is assumed to be 20°C



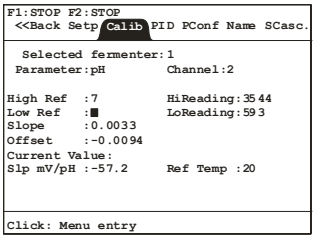
3

Press **Enter** and the cursor moves automatically to the **High Reading** field. A number is shown which is in the range of 0-4095 and represents the digitised signal from the pH electrode. When this reading is steady, press **Enter** to accept the value [4].



4

Move the cursor to the **Low Ref**. Field [5]. Rinse the electrode using a wash bottle with distilled water and place in the lower value buffer solution eg. 4.



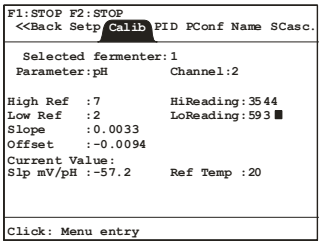
5

Repeat the steps as for the High value buffer and after the final **Enter** to accept the **Low Reading** value [6], move the cursor past the slope and offset fields (rotary switch two positions to the right) and click to accept the calibration [7].

The **Esc** key aborts the process and leaves the original values in place.

A one-point calibration only uses the **High Ref** and **High Reading** values. Move the cursor past the **Low Ref and Low Reading** fields without altering them and click to accept.

→NOTE: Using the ALL tab will help to facilitate work and save time calibrating sensors. This special feature of XDDC is described in section "ALL tab".

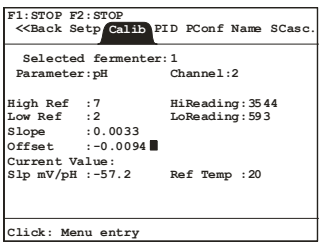


6

Rinse the electrode again and keep it protected and in electrolyte until ready to place it in the vessel.

➲At no times subject the electrode tip to damage while calibrating or rinsing.

NB: Slope is pH units per mV and so has a small value.



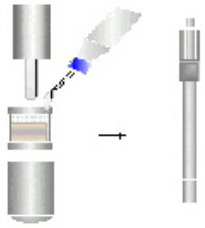
7

11.5.2 Dissolved Oxygen (pO2) Electrode preparation

- Before first use and after every longer unused period, the base of the electrode must be unscrewed to refill the electrolyte into cartridge pressed onto inner glass section (depending on type of electrode).
- Remove cartridge by turning the bottom section over and GENTLY pressing metal mesh in base.
- Fill silicone cartridge with electrolyte provided (*remove cover from under bottle cap first)* and make sure no air bubbles remain.

➲ Electrolyte is vitriolic!

- Press onto glass section gently (some liquid will come out) and reassemble
- For storage >4 weeks, empty the electrolyte and store dry.



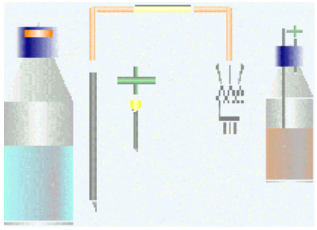
11.6 Reagent bottle preparation

The sterilization of supply vessels takes place in an autoclave.
As a rule, borosilicate glass flasks are used. The top plate of the flask is always fitted with a dip tube and some form of pressure relief system. Either a metal top plate or a membrane may be in place.

Choice of reagents and their strength varies according to which microbes are being cultured. Refer to literature

➲ Do not use hydrochloric acid and ammonia must not be autoclaved (explosion risk!)

Fill the 250ml reagent bottles approximately 2/3 full with either acid, base or antifoam as appropriate.



Fit the bottle cap, using a silicone membrane to seal the open top.

Prior to sterilization, fit a pressure relief outlet using the disposable needle and small disposable filter from the Service Set.

The pump tubing - sized to be long enough to reach from the final location of the container via the pump head and to the vessel inlet on the top plate. Use a Marprene insert in the area compressed by the peristaltic pump rollers for maximum tubing life. An example line is prepared in included in the Service Set.

Connects the tubing to the dip tube (long needle) in the reagent bottle.

The outlet from the flask dip tube is closed off during autoclaving due to pressure differences between the inside and outside of the flask. With glass flasks, the pump tubing must be clamped off with a suitable clamp eg. a gate clip.

The reagent bottles are now set up already connected to the inlets of the 2-way or single needle fittings and simply clamped off for autoclaving. The bottles and fitting are placed in the holder which is hooked onto the pins of the vessel support frame and the whole arrangement sterilized together with the vessel in an autoclave.

Operation, use and cleaning of corrective reagent tubing and pumps.

➲ WARNING: do not autoclave the reagent bottle lines filled with corrective agent, as this could damage the dip tube. Use water instead.

➲ Take care regarding overpressure! Tubing can burst or come off the inlet pipes.

➲ As part of the cleaning regime, the corrective reagent tubing **MUST** be rinsed with water! Storage and autoclaving of tubing filled with acid or base can result in damage to the tubing and also represents a safety hazard to the user!

11.7 Sample Device

This is prepared in the same way as the reagent bottles except a short pipe with a needle end replaces the dip tube.

A number of spare, capped empty bottles are prepared and autoclaved ready for use when sampling. How many bottles you need depends on the number of samples you wish to take but always prepare double what is required to allow for discarding pre-samples of culture.

The metal holder is screwed in place on the top plate support and a silicone tube connects the inlet to the sample bottle and the dip tube already located in the vessel. The line is clamped off for autoclaving and remains closed until a sample is to be taken.

The use of the sampling system is covered later in this manual.

11.8 Preparations for Autoclaving

The vessel is cleaned and assembled.
Medium is added to the required working volume (possibly with a 10% over dilution to allow for losses during autoclaving)
The top plate fittings are added to the assembled vessel. Assemble all sensors etc. needed before autoclaving to reduce risk of contamination.

11.8.1 Exit gas Cooler

This can be found packaged separately from the vessel. It prevents excessive loss of liquid from the culture through evaporation. The inner section has a series of cooling late to aid condensation of water which is returned to the vessel.

1. Depending on the vessel size and type, this can be fitted directly in either a 12mm or 19mm port.
2. Insert the base of the cooler into the base and push into place. Screw into the port fitting.
3. Add a short length of reinforced silicone tubing to the topmost pipe and fit the exit gas filter (green tape towards the pipe). Secure with cable ties at both ends.
4. ➲It is **VITAL** the exit gas line is left open and **NOT CLAMPED** shut as this allows pressure equalisation following autoclaving. ➔ **explosion risk!**

1

11.8.2 Inoculation Port

2

This will be packaged separately, along with a bag of membranes of a suitable size.

1. Remove port closure and insert a membrane of the correct size (12 or 19mm).
2. Screw collar into place to hold membrane securely
3. Re-fit port closure into collar for autoclaving.
4. Prepare the inoculation needle for sterilization, ensuring the needle section is covered with a sheath or foil or bag. It should be connected to the bottle/vessel to be used for transferring the inoculum.

3

11.8.3 Antifoam Electrode

This is packaged separately and should arrive assembled ready to fit onto the top plate. It detects foam on the surface of the culture using conductivity and this can trigger a pump to deliver a dose of liquid antifoam reagent.

1. Fit electrode into 19mm or 12mm (small vessel, magnetic drive) port complete.
2. Adjust height by loosening top part of clamp and pulling electrode gently upwards. Take care not to damage the transparent sheathing around the electrode (clamp still too tight).
3. It is better to be too low and pull up as the converse is not possible due to the contamination risk.
4. After autoclaving, fit the RED banana connector to the hole in the top of the electrode and the black one into the small hole in the top plate made to accept the connector.
5. The electrode is hollow so a silicone tubing line for antifoam can be fitted (not acid or base as these conduct)

11.8.4 pH.

4. Place electrode into 13.5Pg port fitting and screw into place
5. If a longer electrode is used, add a clamping adaptor and adjust the height to suit the vessel size before screwing adaptor just tight enough to hold the electrode securely. **Do NOT use tools**.
6. Cap electrode with red cap for autoclaving
7. Connect the cable by pulling back on red locking ring on the cable and pushing connector down onto the electrode. Screw into place.

11.8.5 pO_2.

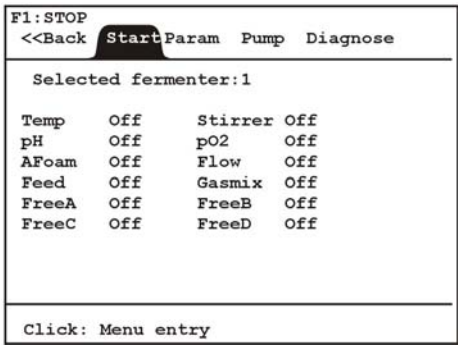
1. Place electrode into 13.5Pg port fitting and crew into place
2. If a longer electrode is used, add a clamping adaptor and adjust the height to suit the vessel size before screwing adaptor just tight enough to hold the electrode securely
3. **Cap electrode with aluminium foil for autoclaving.**
4. Connect cable by aligning orange line in connector cap with orange line on top of electrode connection.
5. Twist locking ring on cable down and clockwise to connect to electrode. Removal is the reverse.

11.8.6 Air Inlet

1. Fit a 0.22 micron filter (red line on filter) to the top of the sparger pipe using reinforced tubing secured at both ends with cable ties. Note the side of the filter marked INLET should be on the top, facing away from the vessel.
2. The air input filter may only be connected loose-fitting with the tube that is connected to the rotameter. ➲Danger of overpressure!
3. For autoclaving, this inlet line **MUST** be clamped shut with eg. a gate clip.

12. Switching Parameter on and off

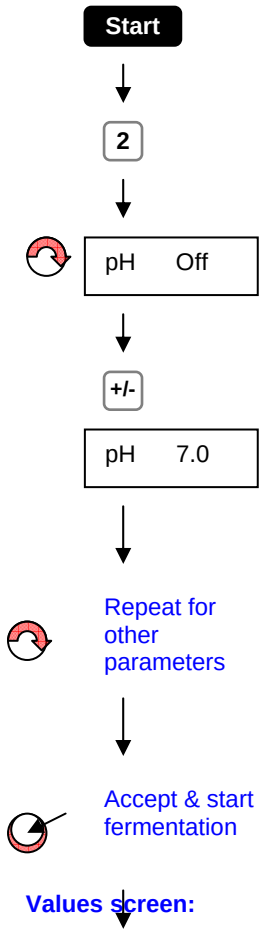
Move to the **Start** tab when the bioreactor is inactive. The screen now shows a list of parameters with Off after the name.



Switching parameters on & starting the fermentation

Use the rotary knob to move to those parameters you wish to switch ON and use the **+/-** key to toggle the setpoint On/Off for that parameter. When all desired parameters are switched on, click the rotary knob to accept and **start the fermentation**.

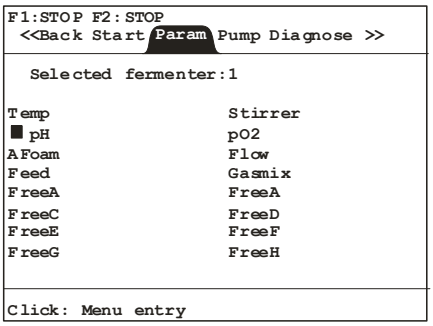
The setpoints and status for the parameters is set in the **Param**. screen (see below).



Values screen:

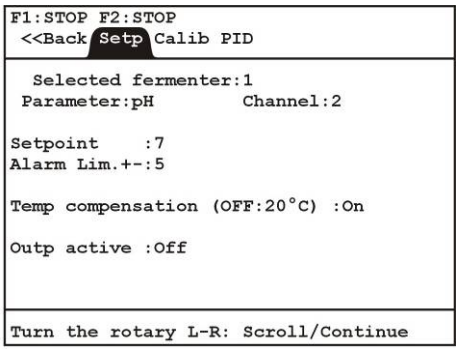
- **Shows parameter name**
- **current actual value**
- **current set point**
- **Controller output as a %**
  +100 to -100% typically

12.1 Entering or changing set point & alarm limits



Move to the **Param** tab when the bioreactor is inactive and click to enter the main part of the screen, which shows a list of parameters. Not all of these may be available for use on individual systems, according to specification.

Move the cursor using the rotary know to select the desired parameter eg **pH** and click to enter the parameter setup display with **Setpoint** as the first option. **Different parameters have different options esp. pH, PO_2 and antifoam eg. see opposite…**



Click to enter the screen and the cursor will move to the first field for alteration, which is the setpoint value

The value can now be changed using the numeric keys and then **Enter** is used to confirm the new value. Use the rotary switch to move the cursor to other fields such as alarm limits. A symmetrical alarm limit can be set here in the units of the parameter eg. for pH with a setpoint of 7, an alarm limit of 1 would be+/- 1 ph unit. I.e. between pH 6-8 and there would be no alarm.

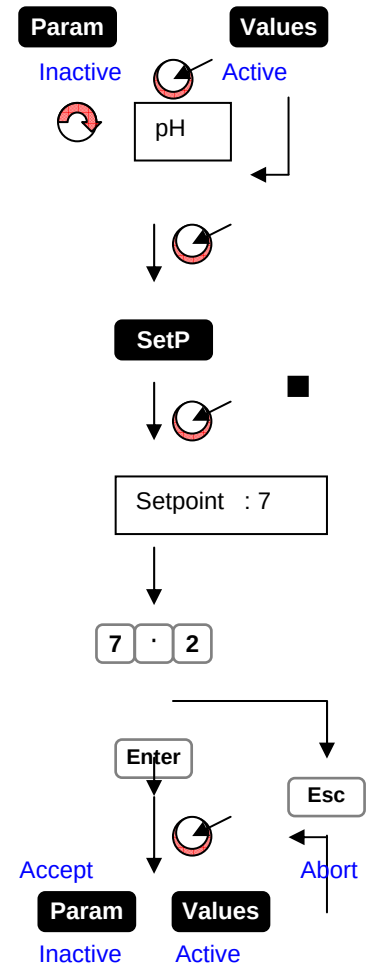
For control as well as measurement of any parameter ensure that **Output Active** is also switched to ON. (Exception: "**regulation**" for stirrer speed, must be switched off (**OFF**).
Click to accept and the use the **Back** tab to return to the main offline menu tabs.

The Programming of additional parameters is analogous the procedure described for pH above.

A point to consider concerns the stirrer speed and gas flow set point values used when working with media which are prone to form foam. Set the values appropriately i.e. not too high. Determine the highest rate necessary following sterilization by increments whilst using antifoam reagent.

NB. When the bioreactor is running, the parameter can be selected from the **Values** display by clicking to enter the main part of the screen, moving the cursor to the required parameter and clicking. This leads to the parameter setup sub-menu tabs

With all setpoints entered ready for operation, the next task is to sterilize the vessel and peripheral items by autoclaving.



13. Sterilization - Autoclaving the vessel etc.

The Standard Labfors3 bioreactor is sterilized in an autoclave at 121°C for a minimum of 30 minutes and typically for one hour. For water circulation units, the vessel **jacket must be filled** for heat transfer and efficient sterilization.

During this process, approximately 10% of the liquid volume will be lost due to vaporization. This must be allowed for either by over-diluting or adding extra liquid during inoculation.

Reagent bottles, the sampling device and any other peripheral items are sterilized in the same way, generally along with the vessel. Tubing should NOT be filled with liquid.

Any heat-labile components are normally filter sterilized and added afterwards by injection or with the inoculum. If the whole medium is heat labile, several strategies are possible:

- Sterilize the vessel with a small quantity of PBS -phosphate buffered saline (enough to cover the electrode tips) and remove it after autoclaving using a sample/ harvest pipe. Add medium at the correct strength & correct volume.
- If the autoclave is too small and the vessel must be tilted, it is possible to put a very small amount of liquid in the vessel and allow that to generate a moist environment to protect the electrode tips.

Either of these methods could be used for sterilization of a Cell Culture system.

13.1 Cooling down and replacing the vessel

➲ After autoclaving, the vessel **must** be allowed to cool to a comfortable handling temperature before replacing it on the base unit of the bioreactor.

It will be several hours before the electrolyte within the electrode cools despite what the overall vessel temperature may fall to.

This time can be used for polarizing the pO_2 electrode by connecting it to its cable (grey lead) and leaving the instrumentation switched on for at least 6 hours.

The **temperature control** for the vessel can be switched on AFTER the following procedures have been completed:

1. The vessel is located on the bioreactor base
2. The **heater pad** is wrapped around the body of the vessel and secured **OR**
3. The rapid coupling connectors on the **vessel jacket** are connected to the base unit pipes. Tubing is connected to the overflow pipe and run into a drain.
4. The pipes for the **exit gas cooler** and/or cold finger are connected to the water supply and drain
5. The main water tap (or circulator) is switched on
6. The **inlet valves** for water/exit gas cooler supply are turned ON and adjusted for flow rate.

The stirrer is placed on the vessel drive hub and turned until it locks into position.

Final Checks before Autoclaving:

VESSEL:

- *➲ Exit gas line has a 0.45u filter fitted and is OPEN*
- *Inlet gas line (sparger) has a 0.2u filter fitted*
- *All unused ports are fitted with a closure.*
- *Membranes are fitted to ports used for piercing with a needle eg. Inoculation*
- *Sample device and reagent bottles are connected to vessel if they will be autoclaved together.*
- *➲ Lines to sparger, sample pipe, and reagent inlets are all clamped SHUT*
- *pH and pO2 electrodes are capped or covered with foil*
- *Drive shaft top is covered with foil/cap*

SAMPLE DEVICE

- *Filter fitted onto needle for pressure equalisation*
- *Inlet needle connected to harvest pipe in vessel via silicone tubing which is clamped SHUT*
- *Spare bottles prepared*

REAGENT BOTTLES

- *Filter fitted onto needle for pressure equalisation*
- *Dip connected to inlet line in vessel via silicone tubing which **is** clamped SHUT*

Or

- *Line is prepared for aseptic coupling to the vessel following autoclaving and is clamped SHUT*
- *Bottles are filled with reagent at the correct strength and clearly labelled*

The same principles apply for medium feed and harvest bottles for continuous culture, although these are almost always sterilized separately.

13.2 Preparation of Corrective reagent lines

If the reagent bottles have been autoclaved with the vessel, they should be connected to the inlet pipes on the top plate.

The stages for setting up the pumps to deliver liquid reagents (acid, base and antifoam) are as follows:

1. Unwrap tubing from bottle and **unclamp**.
2. Pass tubing FROM bottle to the slot at the underside of the correct pump (labelled Acid Base AF and Feed) so the white connector stops it from pulling though.
3. Pull on white knob at bottom of pump head and then lift the black cover right upwards.
4. Pass the Marprene (yellowish) tubing around the pump rollers.
5. Replace black cover and re-clip
6. Pass the tubing through the slot in the top of the black cover so that the white connector again prevents the tubing from pulling right through.
7. Press the black knob (with power switched ON) and the pump motor starts manually. Keep pressing the button and push the black cover backwards so the pump rollers rotate. This primes the pump. When liquid from the pump falls into the vessel release the button and black cover. The pump is ready for use.

The pumps are labelled (acid, base etc.) Make sure the correct reagent bottle is attached to its appropriate pump.
Should any liquid (base, acid etc.) leak into the pump housing, remove the tubing instantly and clean the pump head. After removing the crosshead screw (rear right side of housing) you can extract the steel drip tray for cleaning.

➲**IMPORTANT:** Tubing used in the pump heads must have a wall thickness no greater than 0.8mm for correct operation.

13.3 Connection of electrode cables

Pt-100 temperature sensor

1. This is the only sensor on Labfors3 bioreactor which needs no connector.
2. It is added to the vessel after autoclaving by pushing the end of the electrode into the special 10mm pocket on the top plate.
3. Push until metal to metal contact can be heard and felt to ensure accurate readings.

pH sensor

4. Connect the cable by pulling back on red locking ring on the cable and pushing connector down onto the electrode.
5. Screw into place.

➲Buckling or twisting the cable while mounting will destroy its delicate sheathing and lead to faulty measurements.

pO_2

6. Connect cable by aligning orange line in connector cap with orange line on top of electrode connection.
7. Twist locking ring on cable down and clockwise to connect to electrode. Removal is the reverse.

➲Buckling or twisting the cable while mounting will destroy its delicate sheathing and lead to faulty measurements.

➲Important: do not touch the metal ends of the banana connectors with your bare hands without “grounding” yourself first by touching the equipment.

Antifoam

8. After autoclaving, fit the RED" banana" connector to the hole in the top of the electrode and the black one into the small hole in the top plate made to accept the connector.
9. The electrode is hollow so a silicone tubing line for antifoam can be fitted (not acid or base as these conduct)

➲Important: Always loosen firstly the clamping device before adjusting height of the sensor. ➔A damaged sensor insulation would cause faulty measurement and lead to permanent addition of antifoam medium.

13.4 Polarization, electrode calibration & pO2 control

Polarization
Before the dissolved oxygen (pO_2) electrode can be used it must be connected to the base unit and the electrical power turned on for a minimum of 6 hours. Without this polarization time, any attempt at calibration will be unsuccessful.
Calibrationa) Zero
Before fitting:

- **With special gel from the manufacturers** (METTLER/INGOLD). The electrode tip is pushed into the gel and the zero point set using the 'Low Reference' calibration. This calibration is guaranteed by the manufacturers for applications where the partial pressure of oxygen is >5%. Where the same electrode is always used, a single calibration is enough unless a problem occurs.
- **With nitrogen**, the electrode must be placed in a measuring cylinder and **N_2** introduced.

Or
b) After fitting

By gassing with Nitrogen in the growth medium. This is achieved after the vessel has been cooled to the operating temperature and the reagent lines are closed. Calibration of the 100% point follows on from this.

100% Calibration - see description opposite.

As a rough approximation, calibration of 100% in air prior to autoclaving is possible, especially for cell culture work.
The oxygen-partial pressure value - as opposed to the zero-point claibration - depends critically upon the values for

- Temperature (the higher the temperature the lower the O2 solubility)
- Stirrer speed
- Air flow rate
- Pressure (the higher the pressure, the bigger the O2 solubility

These parameters must be set to the correct operational values before the calibration (maximum values for this specific fermentation) to provide the 100% calibration point

Control

PO_2-control can be mediated by

- Stirrer speed * Standard
- Mass flow control valve altering air flow rate
- Supplementation with oxygen pulsed from a valve.
- Gas mix with CO_2, O_2 , N_2 and air (Cells version)
- Control of feed rate ***special setup required***

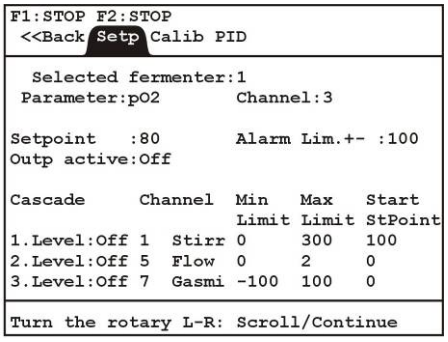
A Cascade function exists as a special section in the pO_2 Parameter Setpoint menu

This can be up to three-stages, providing **sequential** cascade, with the ability to define which parameters is to be used at each stage (by its channel code). Each parameter used can have a starting set point defined within selectable minimum and maximum limits. Individual steps can be turned on or off in sequence depending on need.
The order of listing of the parameters determines the sequence of their usage. Switching the first parameter in the cascade off deactivates all the other stages. No parameter can appear in the list twice, EVEN if it is switched off.

Calibration of the pO_2- Electrode 100%

First, you must:

1. Locate the drive motor on the top-plate
2. Set the stirrer speed (RPM) set point value to the maximum to be used during the fermentation.
3. Switch on air supply pump (if necessary)
4. Set the air supply on the rotameter to the maximum value to be used eg. 1.5vvm
5. Set temperature and pressure if necessary
6. Wait 5-10 minutes for the medium to saturate with oxygen.
7. Go to the calibration display, press for a one-point calibration and set the value at 100



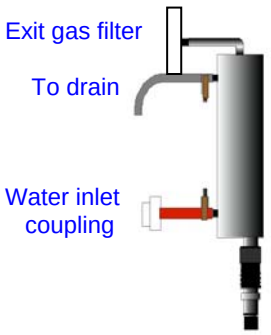
Cascade Setup (in pO_2 Setpoint Screen

Cascade:	Param. Name	Min Limit	Max Limit	Start St point
1. Level OFF	0 Stirrer	100	1000	500
2. Level OFF	5 Flow	2	10	5
3. Level OFF	7 GasMix	-100	100	0

Specially for use with eg. E. coli bacteria:
The minimum value can be higher than maximum value for reducing the feed rate by pO2 control. E.g. Min.limit: 100, max. limit: 0

13.5 Setup of the Exit Gas Cooler after autoclaving

This is used to prevent excessive losses of liquid from the culture in the form of water vapour carried out in the exit gas stream.
The Infors thermodynamic cooler has a special internal structure which increases the efficiency of the re-condensation process.



The cooler is connected by braided flexible tubing from its lower inlet pipe to the specific water supply pipe in the base unit. A manual valve controls the flow of cooling water. When the outer wall of the condensor feels cold to the touch the flow rate is adequate.

A second pipe, of normal silicone tubing, links the outlet at the top of the condensor to a drain/sink. This must have an unobstructed fall to drain at a shallow angle.
➲ Only cooling water with no more than a slight hardness can be used exclusively. (see details in appendix).

13.6 Set up of the Digital Feed Pump

This is the forth pump - additional to the installation of the Acid, Alkali and Antifoam pumps, which operate automatically. They are all digitally controlled. This means that flow rates must be set through shot/delay cycles of pump operation.

For the Feed pump, the cycles can be set as 0-100% On time (in seconds) of the total cycle time, with intervals of 0.1 seconds.

Example:
The desired flow rate is eg. 0.2L/Hr (3.3ml/min). Therefore, the pump must run for approximately 37 % of the total time -
(Desired rate/Max. rate x 100) A set point of 37% leads to 22.2 seconds of dosing time in every 60 seconds

The pump can deliver up to 8.89ml/min, using the standard size of tubing provided with a Marprene mid-section of 2.79mmID (options available). The pump can be calibrated from the **Pumps** tab.

The **Dose** time as a percentage 0-100% is set in the set point screen of the **Feed** parameter. Ensure **Regulation** (if present) and **Output Active** are set to ON.

Example:
The actual value displayed for the fermentation is pH 6.5. The external measured value is 6.9.
Therefore, the difference is +0.4.
In the Field 'Offset' the displayed value relates to the actual pH value of 6.5. It must therefore be corrected by adding 0.4 to the offset to give a new value 6.9

If a constantly variable flow rate is required, it is better to work with a pump under analogue control. Either the optional analogue pump must be fitted, or an external analogue pump must be used with the analogue connector on the base unit.

13.7 Other Possibilities

There are other possible options, displays and settings not covered in this part of the guide. The information provided is sufficient for you to prepare, sterilize and then operate the bioreactor with the minimum of delay.

Particularly in the case of fermentations of long duration, it is usual to check the internal pH measurement for "drift" by comparison with an external measurement. This can be corrected for using the **'Offset Value'** in the **Calibration** screen.

In Use and Afterwards

13.8 Inoculation

Before inoculation, the correct operational status should be verified. Are the pH corrective bottles connected and is there enough reagent to last for the desired fermentation time?

The precise method of inoculation depends on the configuration of the system. At its simplest, it is connection of the inoculation needle to the top pate by piercing membrane and overpressuring the vessel containing the inoculum to transfer it into the vessel. The volume of inoculum is typically 5-10% of the working volume of the bioreactor.

What you should check before inoculation

- Correct Temperature?
- Correct Stirrer speed - cascaded to oxygen control?
- Correct pH?
- Correct pO_2 partial pressure?
- Have you checked the growth medium concentration is correct after autoclaving losses (not Cells version)?
- Are any heat labile supplements to be added eg. glucose solution?
- Are the pumps primed?

1. After autoclaving, when ready remove port closure
2. Add ca. 1ml of 70% ethanol to surface of membrane
3. Use a Bunsen flame near to the port to create a warm updraft OR set fire to the ethanol held in the collar.
4. Pierce membrane with needle and screw into place. Use gravity or overpressure to add inoculum.
5. Clamp off line to inoculum vessel.

Optionally, the inoculum can be concentrated into a small volume and added by syringe OR can be poured into the open port (no membrane) under a laminar flow cabinet.

1.

2

3

4

13.9 Aseptic Sampling

Sampling takes place at various times after inoculation. Usually, a sample is taken immediately after inoculation to determine the initial number of organisms in the culture.

To use the sampling device, a syringe and portable Bunsen burner are needed. Spare bottles which have been previously sterilized are also required.

The size of sample should be determined by your analytical needs but also taking account of the volume of culture and the duration of the fermentation

eg. 100ml samples every 4 hours from an experiment using a 1.2L working-volume vessel which is destined to last for 3 days is clearly not possible.

Typical sample volumes are in the area of 10-20ml for a typical bench-scale bioreactor.

If the bioreactor is used in continuous mode, it is possible to make a "sample bypass" in the harvest line and so allow the sample device port to be used for other purposes.

The sample device can also be used for inoculation if a longer dip pipe is inserted and the syringe used to "blow" the inoculum over.

1. Remove any aluminium foil covering the air filter and connect a syringe eg 25ml on the open end of the small filter.
2. Loosen the clamp on the line and draw back on the syringe until liquid flows into the bottle.
3. When 1/2-2/3 full, push the syringe plunger down to blow the contents of the sample line back into the vessel.
4. Re-clamp the line, remove the bottle and cap it aseptically.
5. Fit a new bottle to the cap (➲CARE! Sharp needles) and screw into place
6. For the next sample, draw a little culture into the bottle the DISCARD it. Use a new sterile bottle to actually take the sample.

Start

Unclamp

Attach

Pull

13.10 Ending a fermentation

Take care to follow this sequence when closing down for safety reasons.

- Ensure that services lines (eg. Gases, cooling water etc.) are not pressurised.

➲ Make sure that no medium can get into the gas supply system!

- Then initiate „Closedown“ at the bioreactor operating panel (see description down right) or via the software IRIS.
- With biologically dangerous organisms, the disposal or subsequent use of the organisms must comply with laboratory safety procedures.
- Empty the corrective reagent lines means direction through manually expelling the liquid from each pump in turn (acid, base, antifoam, feed)
- Turn off the device at the main switch.
- Removing the cultivated organisms in a living and uncontaminated condition for further experiments requires that you connect a „harvest pump“ to the sampling system outlet and pump the cultivation fluid into a sterile container over.
- Remove the tubing of the corrective reagent bottles without dismantling it from the pump head

➲ only using gloves and safety glasses!

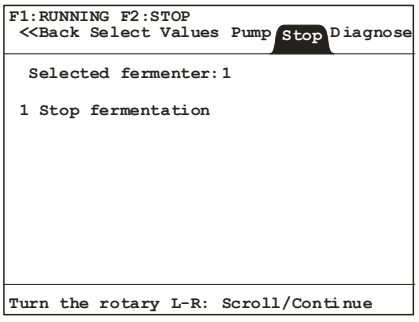
and rinse the tubing thoroughly with water. Before further dismantling the containers, they should be sterilized in an autoclave according to local biological security directions before cleaning.

Push

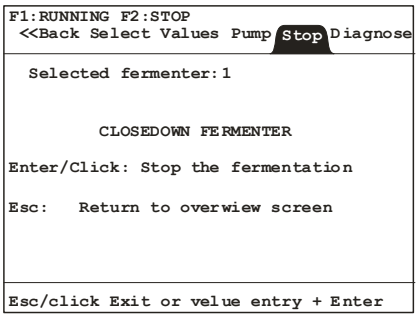
Clamp

Change bottle & replace

To stop an active bioreactor, go to the **Stop** tab.



Then press “**1**”, and confirm with **Enter** (instructions on the screen.)



13.11 Cleaning & Storage

After autoclaving the vessels the leftover liquid can be disposed down the drainage.

Remains of dried foam and proteins become denaturated and sticky. Fill up the vessel with 0,1 N NaOH and stir it for about 2 hours.

➲Attention: Strictly follow the laboratory safety procedures while working with NaOH

If foam has reached the gas cooler: Remove the gas cooler from the top plate and put it then for 4 hrs into 0,1 N NaOH. Rinse it afterwards thoroughly with water and put it then into a ultrasonic bath for about 2 to 5 minutes. Finally wash through with 70% Ethanol and rinse again with water.

The tubes must be completely empty – no corrective reagent should remain before cleaning and rinsing the tubes and pump heads. Basically, all screw-in components of the top plate should be dismantled and cleaned.

➲Do not forget to loosen the clamp nozzles before you remove the sensors. Any use of force can destroy the sensors – especially the insulation sheath of the antifoam sensor, which has to be handled very carefully.

After removing the retaining nuts, take the top plate off the vessel at right angles and put it on a clean underlay. Inserted parts of the stirring system must not come off.

.

➲Attention: Any deformation of the stirrer shaft can damage the mechanical seal and will harm the bearings in the hub.

Loosen both grub screws on the stirrers with the provide Allen wrench (do not unscrew!) to eliminate biological organisms in the space between stirrer and stirrer shaft in order to clean the shaft and stirrer blades separately

Check all O-rings and replace them if necessary.

➲Do not clean the top plate in a washing machine, as water must not get into the bearings. (For severe problems, the drive hub should be sealed against liquid ingress to allow automated washing).

➲Disconnect all electrical connections with the console before wiping or cleaning the surface of it. Reconnect them only after the console is completely dry again. The console must not be doused with water.

Specifically check the insulation of the antifoam tube and replace it if necessary.
➲Be particularly carefully when sliding the tube into the clamp nozzle.

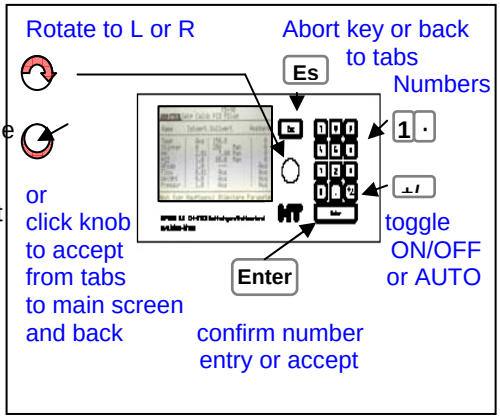
If the bioreactor is not used for a long period (1 month or more) then store the individual parts separately.

Strictly follow all manufacturer's instructions concerning operation, cleaning and storage of the sensors.

NOTES:

14. XDDC Instrumentation

14.1 Main Menu Offline Options



The **X- DDC** panel is mounted above the main instrument cabinet. It can be removed completely. An RS485 connector links the panel to the base unit via a cable with a pair of RJ45 connectors. An RS232 port is available under the bottom left of the panel for programming and data transfer. A slot on the left side houses a memory card which is used to store all the setup and calibration data etc. The system must NEVER be used without a memory card in place. Switching on the mains power at the base unit also activates the rotary DDC panel. The bioreactor should be inactive at this point. A POST (Power On Self Test) screen appears briefly and all the fields should show **PASS** next to them. If not, please switch Off/On again after checking a memory card is fitted.

Screen Display of Rotary XDDC (active)

- The top line shows the tabs which lead to screens with more options
- The main section of the display is accessed by clicking the rotary knob inwards. Other than the Values screen which shows live data of all the process parameters, there are options to be set using the numeric keys or the **+/- key** to toggle options such as **On/Off or Auto**(matic).
- Return to the tabbed area is by pressing the **Escape** key or clicking the rotary knob, depending on the screen options.
- Navigation up and down in the main section of the screen is by moving the rotary knob left (up) or right(down). As the screen scrolls, a cursor block indicates the current position.
- Clicking using the rotary knob will select the process value or option next to the cursor if a further screen can be reached.

```
F1:RUNNING
 <<Back Select Values Pump Stop Diagnose

  Selected fermenter:1
Name      Value    Setpoint       Output %
-------------------------------------------
Temp     |  24.0 |  24.0         |Off
Stirrer  |    0  |   Off         |Off
pH       |   7.00|   Off         |Off
pO2      | 100.0 |   Off         |Off
AFoam    |   0.0 |   Off         |Off
Flow     |    0  |   Off         |Off
Feed     |   Off |   Off         |Off
Gasmix   |   Off |   Off         |Off

Click: Menu entry
```

The bottom 2 lines of the display list options of what to do next and how to access the choices

14.1.1 Initial Choices from the X-DDC Menus (offline)

Info: Information about the X-DDC version of the firmware in your bioreactor is also provided here.

```
F1:STOP F2:STOP
 Info Select All Config Passwrd Receipe

INFORS AG        Copyright(c) Aug 07 2007

            INFORS XDDC SYSTEM
                LABFORS 3

                Rotary switch
                VERSION 1.51

Labfors 3 Controller Copyright Infors AG
      Version 1.40 Nov  09 2006

Turn the rotary L-R: Scroll/Continue
```

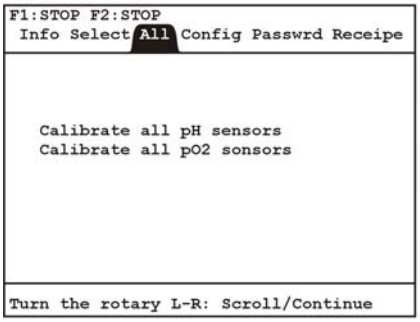
Select: This is the first screen where options are available to select an individual bioreactor (multi-bioreactor system).A number of "offline" options are listed as a series of tabs running from left to right across the screen. Moving the rotary know to the right will highlight the name of each in turn and clicking the knob downwards (where appropriate) will allow the main part of that screen to be accessed.
Active bioreactors show the current actual values and inactive units display a line of dashes.
Arrows **>>** indicate that more options are available
<< Back indicates that the original options can be reached again by turning the rotary switch left.

```
F1:STOP
 Info Select All Config Passwrd Receipe

         Ferm1    Ferm2    Ferm3    Ferm4
Temp      24.0       |        |        |
Stirrer   0.00       |        |        |
pH         7.0       |        |        |
pO2       100.       |        |        |
AFoam     0.00       |        |        |
Flow      0.00       |        |        |
Feed       Off       |        |        |
Gasmix     Off       |        |        |

Turn the rotary L-R: Scroll/Continue
```

Other tabs available are **All**, **Config. Password & Recipe,**

14.1.2 ALL tab

Two options are provided in the main screen of the ALL tab. They are accessed as usual by clicking, navigating to the desired option and clicking again.



Location of the ALL tab

Calibrate all pH sensors
Calibrate all pO2 sensors

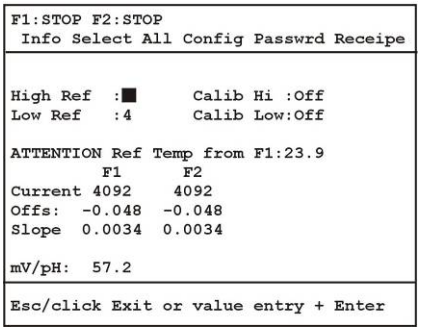
This is an extension of the normal calibration screen (which is still available for use with individual vessels, as normal).

The first difference is that as well as entering the reference values, each stage of the calibration (high/low) has to be explicitly switched on and will automatically revert to off after leaving this screen.

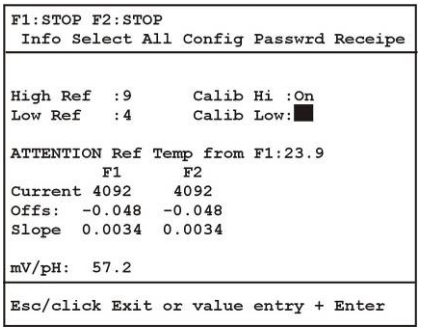
It can also be seen that the temperature compensation is now provided from just the first vessel's Pt-100 temperature sensor (which can be placed in the beaker of buffer). This is not user-adjustable although individual calibration still works in the conventional way.

The display includes information about the status of the probes (mv/pH for pH and nA reading for pO2.)

When the reading has stabilized, individual units must have the calibration confirmed by pressing the corresponding bioreactor number.
Enter each bioreactor number (normally in the order 1 to 4) when the reading is stable.



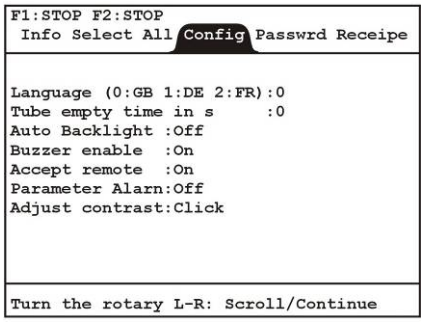
Set Reference



Switch ON calibration mode

14.1.3 Configuration

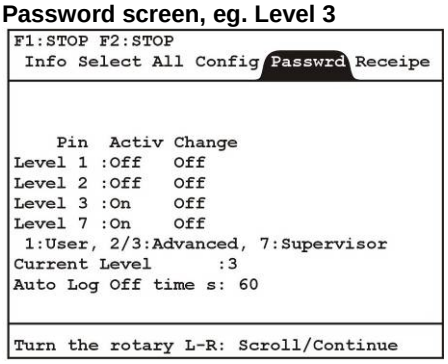
The **Configuration** menu is where a number of "housekeeping" utilities are found.



1) ***Language*** *settings: 0 = English, 1 = German, 2 = French*
2) *The* ***Auto backlight*** *is switched on, - approx. 20 minutes after the last key is pressed it switches off until a key is once pressed.*
3) ***Buzzer enable*** *is the acoustic alarm, depending on the alarm settings of the equipment, independent on the software (IRIS).*
   by selecting'OFF', the acoustic alarm is deactivated.
4) ***Accept Remote:*** *When connected to a computer, either data only is sent - OFF -, or control commands can also be accepted -ON-*
5) ***Parameter Alarm:*** *if set to ON, any parameter which goes into an alarm state will trigger the external alarm relay built into the 25 pin D socket of the base unit. If OFF, only "generic" alarms, such as a power failure, will be output externally. Also an alarm in software IRIS will be shown via this output.*
6) ***Adjust contrast****: Click to enter the screen. This option can be set visually using the UP/DOWN keys to increase and decrease the contract.*

Click to access the main screen. Use the rotary knob to move the cursor block to the desired parameter. Use the number keys for data entry or the ***+/-*** key to toggle between On and Off.

Password screen, eg. Level 3



14.1.4 Password (Pin)

The password (Pin code) allows to specify different authorisations for different users. Critical parts of the control system can be protected from unauthorised access.
There are 5 different password levels (see manufacturer's pin setting) Level 1, 2, 3 and 7 have each an individual and modifiable pin code (password) which is possible to activate. New pins can only be activated and defined in the offline mode (no active process/**OFF**).
It is possible to change a level during an active process. Simply choose an option that needs a more advanced level.
Once entered, a pin code is only valid until the "**Auto Log Out Time**" has expired (this is manually adjustable). Afterwards the system falls back automatically to level 0/**Log OFF** and all functions are locked again. As soon there is a function that needs a higher level, a new log in is necessary. It is also possible to login to any level after "**Auto Log OFF**" during an active process. Entering a false pin code leads to an automatic Log Out.
A pin code consists of a maximum of 9 digits. The numbers are shown as ******** on the display. Below the first level whose pin is activated, no pin is needed. The pin code of the next higher and activated level must be entered when higher levels are deactivated (**OFF**) and the lower ones are protected. This setting is not recommended because many functions would ask for the "Administrator" pin (7). A high level – pin combination leads to free access to all functions that are equal to or below the chosen level.

Certain aspects of configurataion can only be made when in Service mode, only accessible on Administrator Level 7. Please note:
➲**The number of people with supervisor access (Administrator) has to be limited. Anyone with this level of access must not make changes unless they are certain of the outcome. If there is any doubt, they MUST first consult an INFORS representative or the INFORS Service department.**
➔**Non compliance with this point could render your warranty void.**

The following information is shown on the display when no sequence is active (manufacturer's setting):

	Pin Active	Change Pin
Level 1	On/Off	On/Off
Level 2	On/Off	On/Off
Level 3	On/Off	On/Off
Level 7	On	On/Off

Auto Log Off time(s) 900

(Current level 0 = no functions unlocked, current level is editable)

Explanatory note to level 7:
It is not possible to deactivate the pincode for level 7 !
A fixed INFORS in-house pin code exists additional to the "Administrator" pin code. Alteration to level 7 is always possible as soon as a password (pin code) is asked for on the display

Setting **Change pin** to **ON**, the pin definition display shows the following information:
New Pin
Pin change of level
Current Pin code *****
New pin code ****
Repeat Pin code ****

Only level 7 allows a Pin code change (Change Pin). Users with lower levels can neither change this nor anyone else's Pin code.

Basically, every function that needs a pin code will automatically and visibly ask for it on the display, This is not dependent on active or inactive sequences.

Pin protected domain
Level 1/2/3/7
Pin ****

The lowest needed level is automatically shown after "Level" on the display.

General view access authorisation

Standard : user level 1
- Start
- Set points
- Calibration (only pH and pO2)

Advanced : user level 2
- Diagnose

Advanced : user level 3
- Recipes
- PID settings
- Configuration

A detailled scheme can be provided on request.

14.1.5 Recipes (advanced level 3)

The Recipe tab allows access to the various communications and configuration storage options available within X-DDC.
Simply press the number of the required option to go directly to that screen.

The entire information relating to a fermentation process can be stored on the Memory-Card. You can store the data from the card onto an external computer via RS232, either for security or when you wish to run another sort of fermentation with-out an additional card for exchange.

1) Store a X-DDC configuration on a memory card. Up to 9 configurations can be stored on a single memory card 1-9, with 0 as the default (which cannot be used). All setpoints, calibration data, PID settings etc are stored.
2) Retrieve the configuration from a memory card to a X- DDC system. Be sure to safe current values before reloading an existing configuration back into use. After using this option, switch the bioreactor Off and On again to ensure the settings are re-initialised.
3) Send the contents of the memory Card to a computer
4) Read the contents of an archived Memory-Card from a computer back into the card in the bioreactor.
5) Comms Statistic and Settings lets you set up communications protocol for a two-way transfer of data between rotary DDC and a PC running IRIS software (or a one-way transfer to a printer) and view the status of this connection.
6) Controller card options. For each Labfors3 base unit, this should display the card number and “Working” if the bioreactor is switched on.

Option 1 allows a configuration to be stored directly onto a memory card. Up to 9 complete configurations can be stored for retrieval as needed eg. one profile for bacteria, one for yeast, one for fungi etc.

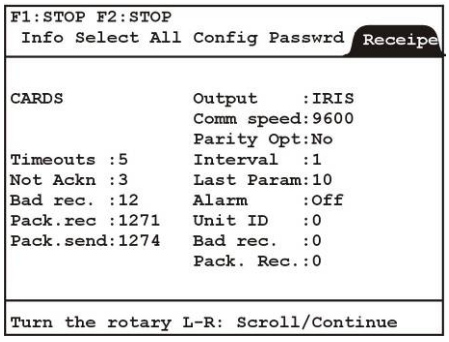
Option 2 allows retrieval of a setting from a particular location on the memory card.

Options 3 requires a file name to be given and a simple terminal program to be available on the PC (not Hyperlink).
A pattern on the display indicates data transfer

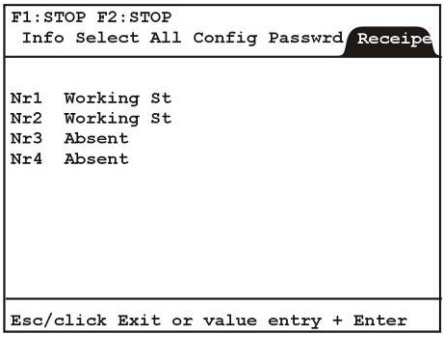
Option 4 allows a file name to be selected for upload to the memory card. A pattern on the display indicates data transfer

➲Options to send data to, and receive data from, recipe files stored on a PC may only be used when a computer is connected and **NO bioreactors are active**. Otherwise, errors in operation can occur due to a system „crash“. Data cannot be read into a card when the bioreactor is active.

Option 5
Explanations:



1) The number of **timeouts** (within a fixed time interval) when a request for communication has not been answered.
2) **Not Ackn**. Is the number of time the recipient has not understood the request for communication.
3) **Bad rec**. is the number of time a communication packet has not been fully understood (an indication of a bad contact or cabling fault).
4) **Pack Rec**. is the total number of communication packets successfully received.
5) **Pack Sent** is the total number of communications packets successfully sent.
6) With **Output** it is possible to switch between data going IRIS software or to a serial printer.
7) **Comm Speed** shows the serial baud rate and should remain set at 9600 (this does not influence performance).
8) **Parity Opt** shows the parity option for serial communication and is set to NO as standard.
9**)** **Interval** gives the time between data strings being sent to the printer in minutes (Min. 1 Minute).Does not work with IRIS.
10) **Last Param** determines the number of parameters sent to the printer. Does not work with IRIS.
11) With **Alarm**, you can decide if alarm messages will be sent to the printer. Does not work with IRIS.
12) **Unit ID** - here you can assign a unique ID number to the bioreactor. When more than one bioreactor is connected to the PC by a common serial port, all bioreactors must have a different Unit ID (beginning with 0 and matching the bioreactor list within IRIS
13) **Bad rec**. (I**RIS**) is the number of time a communication packet has not been fully understood (an indication of a bad contact or cabling fault).
14)**Pack Rec**.(**IRIS**) is the total number of communication packets successfully received.

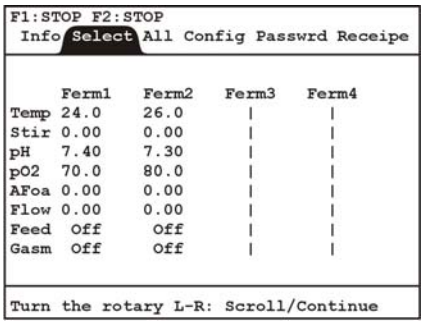


Option 6
Display screen to show how many bioreactor base units are present and their current status i.e. working if switched on and functioning normally.

.

14.2 Selected Bioreactor ("fermenter")

For a single bioreactor, click to enter the main screen. For multiple bioreactors, first select the unit number required.
When an individual bioreactor is selected, additional options become available for offline set up, namely



Switching parameters on & starting the fermentation

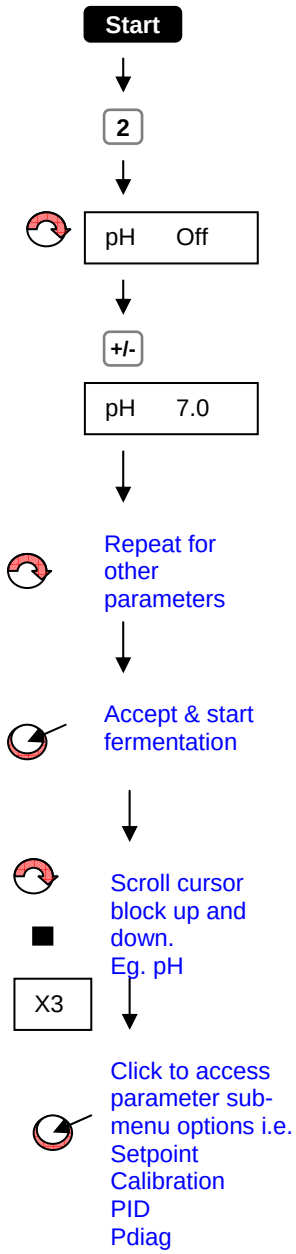
14.2.1 Start

For multi-bioreactor operation, up to 4 Labfors3 bioreactors can be controlled from a single X-DDC operation panel. Consequently, the number of the bioreactor to be started first has to be selected. The **Start** screen can then be accessed:

Click the rotary knob to move to the main part of the display. Turning the knob will now let you move up/down in each column of parameter names, with the order being temp, stirrer, pH, pO2… etc.

Use the **+/-** key to toggle between Off and the current setpoint for each parameter (equivalent to switching the parameter On). Entry of set points is done using the **Param**. tab, prior to starting the bioreactor (or directly by entering numbers into the OFF field for a parameter).

Previously entered set points from a completed fermentation will be stored and appear at this point. If your bioreactor has been under remote control, it will be the **last** external set- point given which will have been stored.



When complete for all parameters, pressing ENTER or clicking the rotary knob will automatically start the fermentation and the tab options change. The bioreactor details for online operation will be shown on the Values screen.

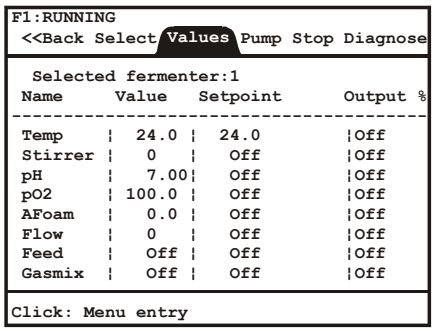
A number of shortcuts are available:

When a parameter has been selected using the rotary knob, setpoints can be entered directly using the numeric keys.

The +/- key can be used to turn individual parameters On or Off

Click on a parameter for the full range of options.

14.3 Values Screen - Online Operation

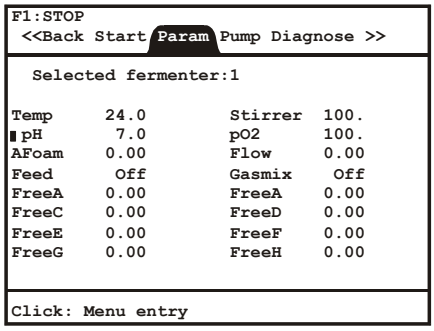


Clicking to enter this screen will cause a block cursor to appear to the left of the fist parameter. Clicking again will take you directly to the parameter submenu for that parameter. Pressing **Esc** will take you back to the tabs.

Parameters not shown on the initial display can be reached by simply turning the rotary knob to the right until the screen scrolls upwards to reveal the remaining 8 parameters in turn.
Current set points are shown here along with the output from the controller. If control is not active, "Off" will be shown and the set point will be replaced with a series of dashes - - -.

14.3.1 Closedown

from here requires moving to the **Stop**. tab and pressing **1** followed by **ENTER** to confirm closedown.



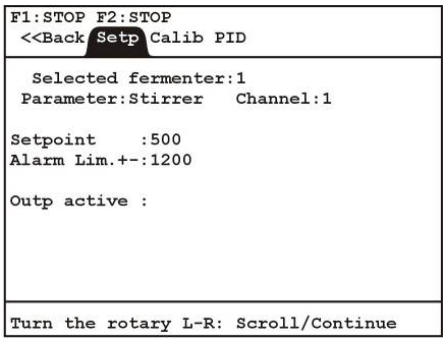
14.3.2 Parameter

The Parameter tab is really a gateway to the series of screens which provide the opportunity to alter a large number of options relating to one particular parameter eg. temperature or pH.

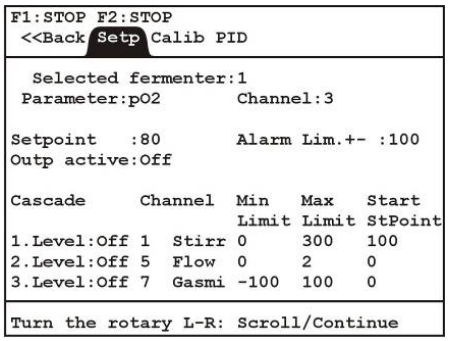
When the fermentation is active, this screen is not necessary as access to individual parameters can be gained via **the Values** screen. Therefore, the option is not displayed when the bioreactor is in "Running" mode.

The main part of the screen is accessed by clicking the rotary knob. A cursor block now appears by the first parameter on the list and rotating the knob to the right will take the cursor block to each parameter in turn.

Select the chosen parameter by clicking when the cursor is next to it. Pressing **Esc** will take you back to the tabs. Selecting **<<**Back will return you to either the main menu options (bioreactor inactive) or the Values screen, if active.



A new set of tabs now appears with the screen for the leftmost tab (**Setpoint**) displayed. Other options are **Calib(rate), PID and Pdiag**



The options available in the Parameters submenu are described in detail in a separate section.

Which Parameters are available

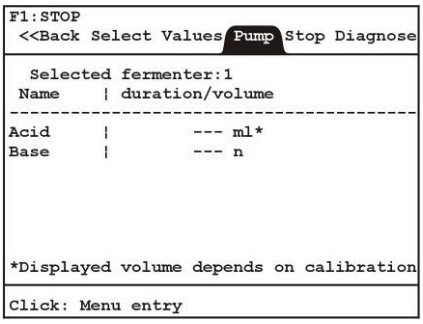
Pre-allocated
- Stirrer Speed
- Temperature
- PH
- pO2
- Antifoam
- Feed
- Flow *option
- Gas Mix

4 Free for eg:
- weight
- Redox
- Analogue Output for Pumps
- Biomass/Optical density

PO2 is Special:
A cascade option at the bottom of the Setpoint menu allows a range of parameters to be used sequentially for control of dissolved oxygen at a given setpoint.
The numbers before parameter name refer to the channel code of the parameter to be used for this control at a particular level. This must be entered manually.

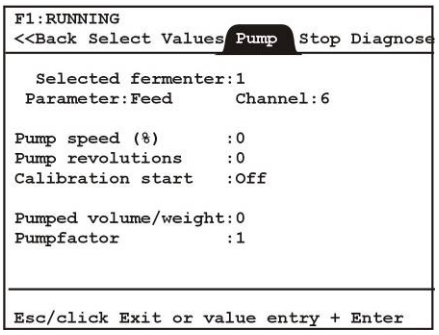
14.3.3 Pump.

This screen shows the total on times (in seconds) for active pumps. Click to enter the main part of the screen. A cursor block will appear by the first parameter option. Acid and Base pumps are displayed separately.



The running time for the pump(s) associated with that parameter will be displayed. Only the Feed pump can be set manually to deliver manually, using a does and delay cycle set in the **Feed** parameter.

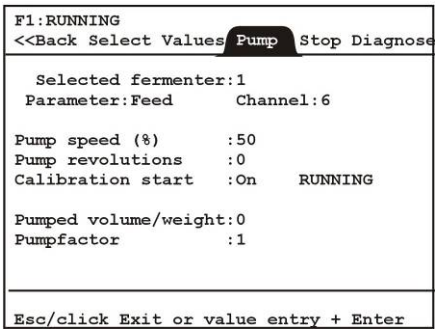
Explanations:
1) Pump 1 (+) = Acid pump, Pump 2 (-) =Alkali pump
2) Antifoam pump. This is under the control of the antifoam probe. It is turned on in the Pdiag. display for the AF parameter.
3) Medium dosing pump. The flow rate is set via the Set-point]-Menu Feed', or from an external signal. It must be explicitly turned on in the Pdiag display.



Calibration
Any pump can be calibrated to display weight in grams or millilitres delivered by first selecting the pump then pressing the [.] decimal key.

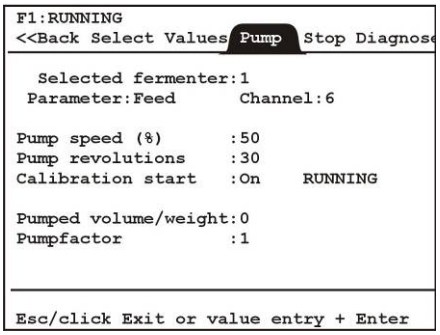
A new screen can then be seen.

Enter a pump speed in percent (0-100%) eg 50
Enter a pump rotation eg 500 for the pump eg. a value of 500 will allow delivery of approx. 2ml using standard tubing sizes (based on Labfors3 pumps)



When Calibration is turned from OFF to ON using the +/- key, the message "RUNNING" appears in the display.
After the calibration run time has expired, end, enter the weight dispensed or ml delivered and the pump factor is automatically worked out to allow display in the desired units.

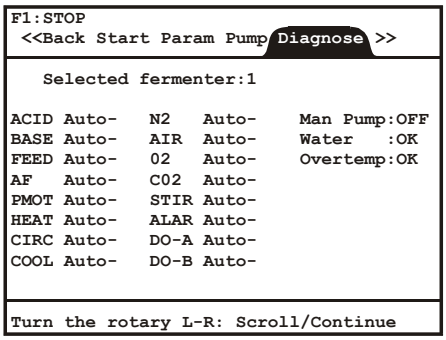
The example opposite causes the calibration to last for a total of 1 minute (as 30 seconds is 50% On)



If a pump has been successfully calibrated, the value displayed is shown with "ml" after the numbers. This indicates a pump factor which is not equal to one.

If a pump is uncalibrated, "s" for seconds will appear after the name to indicate this (i.e. pump factor =1)

14.3.4 Diagnose (Advanced Level 2 or 3)



This menu indicates the status of pumps, valves relays and other inputs/outputs. It is also possible to “force" the operation of valves. Please take care if using this menu as unauthorised interference can make the bioreactor un-usable.

Type of stirrer is now also displayed here **Bsti** bacterial or **CSti** for cell culture.

The additional inputs and outputs allowed by the system are also configured from this screen (see Appendix).

To test an actuator function eg. Acid pump

- Click rotary switch to enter main part of the screen
- When the cursor is against Acid the +/- key can be used to toggle the status from OFF - ON - AUTO
- When Off the should not operate (even if the pH controller requires acid)
- When set to ON the pump should run continuously
- When set to auto, the pump will only work when the pH controller requires acid.

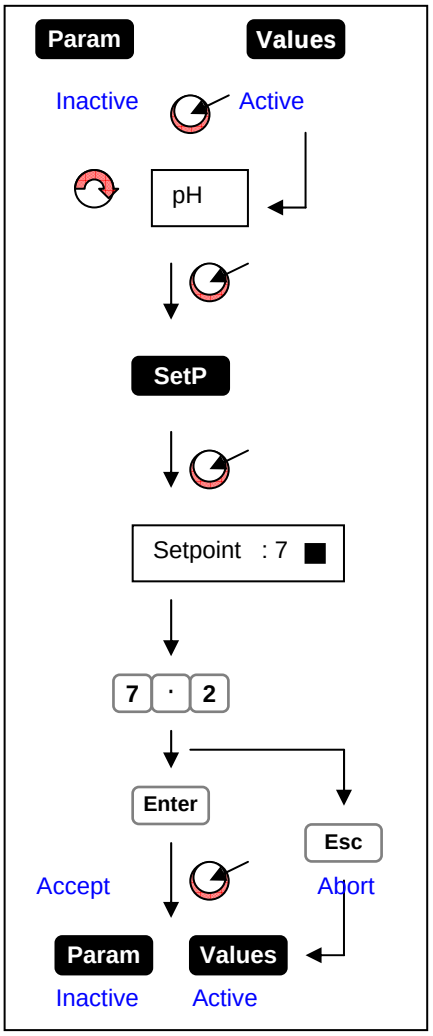
If a pump is active, The motor is shown as ON automatically.

Additional Indicators:

- **Man Pump** = The push button on top of the pumps is shown as active on the display when pressed
- **Water** = OFF Water cannot be detected by the sensor in the thermo-circulator and heating is therefore turned off (running dry or distilled water has been used).
- **Overtemp** = OFF the 80^{o}C over-temperature cut out has been activated (resets itself on cooling).

NOTE: An additional mechanical over-temperature device is fitted (se to 105^{o}C) and this may have to be re-set manually. Check by opening the bottom panel of the base unit and pressing the red button on the thermo-circulator.

14.4 Parameter Submenu



14.4.1 Setpoint

There are two ways to access this series of tabs:

1) When the bioreactor is inactive, via the Param. tab and selecting a parameter name from the list. Click to enter the main part of the screen. Move the cursor block to the required parameter and click.
2) From the Values tab, when the bioreactor is running. Click to enter the main part of the screen. Move the cursor block to the required parameter and click.

Use the << Back tab to return to the main offline menu or Values tab.

The Setpoint tab is automatically highlighted. The format is similar for most screens but can vary in the details according to the functions needed for individual parameters.

Explanations:
1) **Setpoint** value in the parameter units
2) **Alarm limits** in whole parameter units. This means eg. '2' will cause an alarm for a pH < 5.0 and > 9.0
3) **Regulation** On shows the parameter is controlled by a calculation from the processor within the X-DDC system.
4) **Output Active**: ON shows that control of the actuator (pumps or valves) is active.

** Stirrer speed and Flow MUST have regulation switched OFF to function correctly as they send set points to controllers specific to those parameters.*

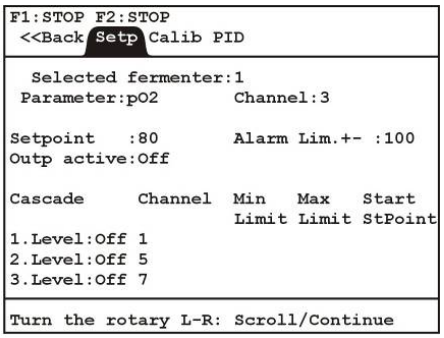
Everything below Output Active is a special option for pO2.
With the parameter pH, Temperature Compensation is included. This MUST be ON for pH to work properly during a fermentation.

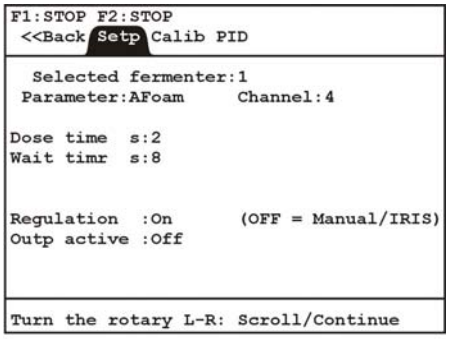
With the parameter [AF] :
1) Instead of 'Setpoint': '**Dose Time'** = Pump run time
2) Instead of alarm Lim: '**Wait Time'** = Pause between dose
3) An addition **Regulation** option ON/OFF is provided. If OFF, it provides for IRIS control or manual, with 0-100%, this value being the percentage of dose time during a 9 second cycle eg 50% = 4.5 seconds dose, 4.5 secs delay.

With the parameter [Feed]
1) A value between 0-100% ON time is given.

With the parameter [Gasmix]
This controls either oxygen supplementation for bacterial systems or the switching of Air/Oxygen/Nitrogen for the 3 gas mix system used for the Cell culture systems. The set point is this case is the percentage oxygen in the gasmix:
Set to -100% only Nitrogen would flow
Set to 0% only Air would flow
Set to 100% only oxygen would flow
Normally, this is set automatically by the dissolved oxygen control loop.

Remote control IRIS
If setpoints are sent remotely from the IRIS software, the external values will overwrite anything entered at the control panel of the bioreactor. Please bear this in mind when attempting to alter set points





14.4.2 Calibration pH Value

Explanations:

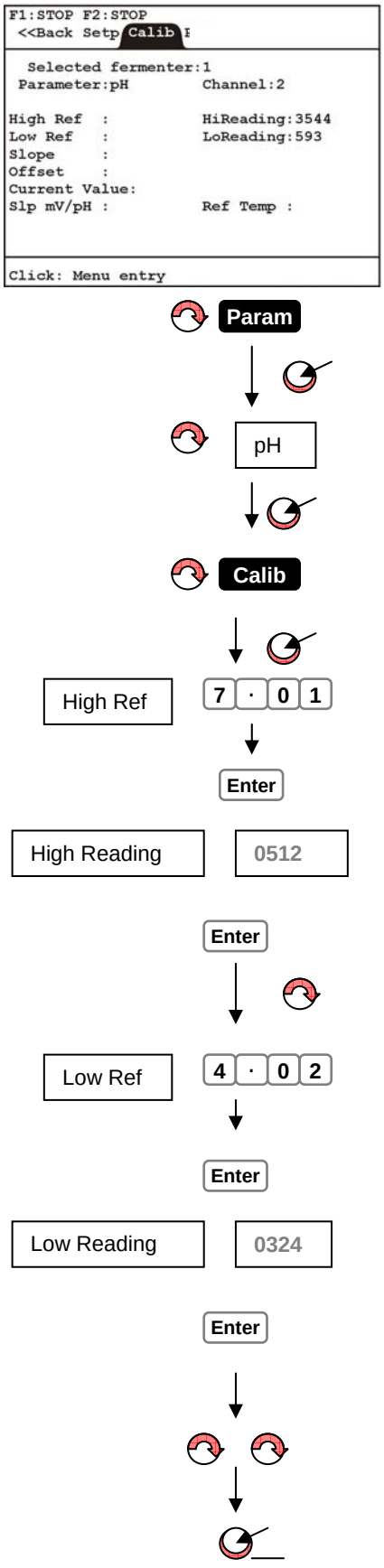
1) **High Ref:** Calibration buffer 1,e.g.. 7.0
2) **Low Ref:** Calibration buffer 2, e.g. 4.1
3) **Slope** (as pH units/mV, hence the small value)
4) **Offset** or Zero point. Using this value, a drifting pH electrode can be "rehabilitated".
5) **Current Value:** This shows the current pH-value as a digital number converted by the transmitter (0 -1024, mid-point pH7 ~512).
6) **Slp** Slope of the electrode in millivolts per pH unit.
7) **Channel:** This is the channel No. in the controller card assigned to 'pH'. This cannot be altered from this menu.
8) **Hi Reading:** The pH-value shown as a digital reading
9) **Lo Reading**: Ditto.
10) **Ref Temp**: Temperature of the buffer.

A one-point reading only uses the High Ref values. This can simply be whatever the current reading is (although accuracy across the range may then be compromised).
Move the cursor over the Low Ref and Low reading without changing the values.

The calibration for pH and dissolved oxygen 100% should be done for every fermentation. Temperature readings from a Pt-100 are much less likely to need recalibration.

Temperature compensation for pH can be switched On or Off. If Off, a buffer temperature of 20^{0}C is assumed. If On, the Pt-100 temperature sensor should be removed from its pocket in the vessel and placed in the buffer with the pH electrode.

Parameters such a Feed, with no sensor to provide feedback, clearly need no calibration.



14.4.3 PID

(additional document available for PID explanation)
Advanced Level 2/3

Explanations:

1) **Max Value:** display parameter max. measured value
2) **Min Value:** display parameter min. measured value.
3) **Output Max**: Set the maximum controller output with the value given here as a % (usually 100% or full range)
4) **Output Min:** Set the minimum controller output with the value given here as a % (usually zero)
5) **P term**: Proportional term (P): The greater the difference between the actual value and the set point, the greater the controller output. The given value is a multiplier in the differential equation. In most applications, a suitable P-control value is critical.
6) **Off State.** The setting here provides a default for when this parameter is switched OFF, or when the bioreactor is not in an operational condition..
7) **I Term:** Integral term (I): Sums the error over time. Should the setpoint not be regained using the P-term, the I term enables the output control to carry on working until the setpoint is reached. The I-term is only set to a small decimal value and small changes can have very large effects.
8) **Int. Limit** To ensure that the I-term cannot increase indefinitely, a limit can be set on the error to be summed. Normally leave set at 100% of full range of the parameter
9) **Output ramp.** A slow, stepwise activation of an actuator can be activated here (e.g. Stirrer speed or mass flow control valve action) .
10) **D Term**: Differential term (D): Accounts for the rate of change of the error over time and compensates for this to limit any overshoot. In most cases, it is not needed.
11) **Dead Band**. Inside the dead band range (Setpoint +/- given value) the controller is inactive.
12) **Step size**. This value is given as a % of the selected controller out-put. The control output will then be increased by this amount at regular intervals (the Value in 'Eval Time' in seconds).
13) **Neg. Factor**. If a controller has two actuators of differing strengths (e.g. strong acid, weak alkali), this value can compensate by adding a weighting factor or bias to the weaker controller output.
14) **Eval Time**. This value gives the time interval in seconds between re-calculation of the PID equation and, hence, changes in the controller output. 10 seconds is a good average value.

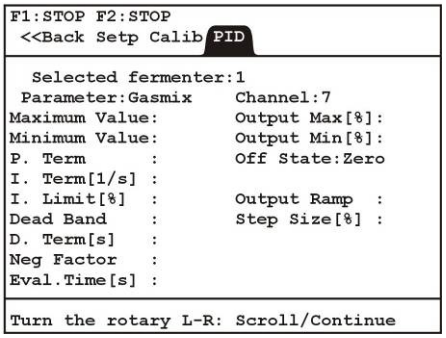
INFORS PID: P is actually the Gain (P=100%/gain), the bigger the value, the sharper the control. I=1/sec (hence a small number, as any increase/decrease has a large effect) & D is set in seconds (often zero).

➲You should only alter these settings if you are confident about using PID control, and the precise sequence and consequences of any changes can be evaluated.

The PID-control is governed by the following formula:

$$\text{Error}_n = (\text{Set} - \text{Act}) / (\text{Max.Value} - \text{Min. Value})$$

$$\text{Output}_n = \text{P.Term} \cdot \{ \text{Error}_n + \text{I.Term} \cdot \Sigma \; + \text{D.Term} \cdot (\text{Error}_n - \text{Error}_{(n-1)}) \}$$



Tuning a PID loop - a simple approach.

1. Make changes one step at a time. Changing the **proportional (P) term** will exert the most influence on the process variable. Increasing the P band too much will cause a gradual drifting of the actual value and too small a band will cause "hunting" e.g. for pH, a little acid is added followed by a little alkali, followed by acid...
2. The **integral band** should normally be a small value eg. and changed a little at a time.
3. The **D-term** is often set to zero to begin with and may not be needed. Alternatively, where large changes take place rapidly, a high value may be necessary.
4. An **Evaluation time** can be set which determines the time interval between recalculation of the PID terms.
5. The **Integral limit** can sometimes be increased if control is not good.
6. The **Negative factor** can introduce a bias eg. for pH a negative factor of 10 would imply a strong base was being used and 0.1 that a strong acid was being used.
7. A basic manual approach to tuning a PID loop when you don't have any previous settings as a guide relies on setting the P value so the proportional band is as wide as possible and the I and D terms are zero.
8. Gradually narrow the P band until the controller causes oscillation in the process value. Now adjust the P term back a little and repeat the process for the I term starting with a long time interval and gradually shortening it until oscillation again sets in. If required, the D term can be added.

14.4.4 Additional Parameter Setup

Feed Pumps

The Feed parameter can be used to set the operation of the digital pump option. The tubing used can be varied but MUST have a wall thickness of 0.8mm and a Shore hardness of 64°. Flow rates for the standard Marprene tubing are shown opposite with some information on other sizes.

If the optional analogue pump is used by allocating one of the free channels, this is what should be considered:

- Control range
  This defines the range of control signals to be used for the controller output eg. 0 and 100 corresponds with a control output of 0 -20mA

- Volume
  If the flow rate of the pump is known, this can be entered directly. This is normally in ml/min.

In the PID screen, these values should be used

Maximum Value	100
Minimum Value	0
P.Term.	1
D.Term[s]	0
I. Term[1/s]	0
I.Limit[%]	100
Dead Band	0
Neg. Factor	1
Eval. Time[s]	1

The desired setpoint value can now be entered, conforming to the range defined in PID.

Pumps can have a setpoint range of eg. 0-100% and the steps can be set in 0.1 increments. However, an analogue pump cannot turn less than 1 rpm (below this a fault condition will occur). For example, if the analogue pump can operate from 0-100 rpm, the minimum possible step will be 1).

The integral analogue AMSP 300 can operate from 0-100 rpm using Bioprene tubing of ID 3.2mm with a wall thickness of 1.6mm. This has a flow rate of 48ml/min at 60rpm (range approximately 1ml/min at 1rpm to 80ml/min at 100 rpm).

The same general procedue would apply to any signal having a 0-20mA input or output eg. input from a biomass probe, dissolved CO2 analyser, glucose probe, exit gas analyser etc.

NB: Parameter names can be changed by an Infors specialist, if desired.

Digital pump, Marprene tubing D2.97 x 4.39mm wall 0.8mm (part 22899)

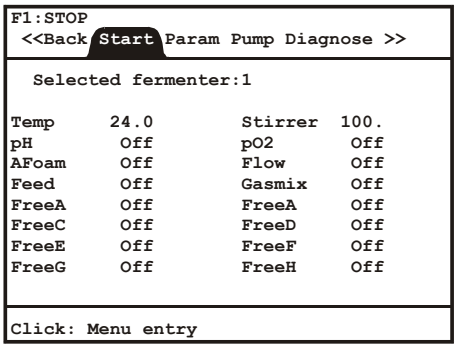
%-Output	ml / h	ml / min
5	22.000	0.367
10	48.600	0.810
20	102.800	1.713
30	156.400	2.607
40	210.000	3.500
50	266.600	4.443
60	323.200	5.387
70	374.200	6.237
80	425.200	7.087
90	479.500	7.992
100	533.800	8.897

Digital Pump –other tubing sizes (ID) 100% flow rate output

mm	Material	ml/ h	ml/ min
0.63	Silicone	32.4	0.54
1.14	Silicone	88	1.47
1.42	Silicone	154.6	2.58
1.52	Marprene	180.4	3.01
2.05	Marprene	322.8	5.38

14.5 Online Options

Select the required bioreactor



If an incorrect choice is made a message screen appears to tell you the selection is not valid.

14.5.1 Start

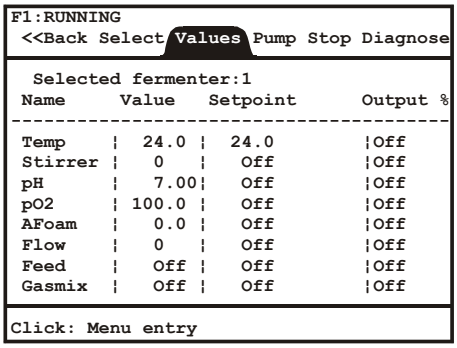
This is described in a former section. When online, it displays the current status of the parameters eg. setpoint or OFF.

14.5.2 Values

This is the default screen when a fermentation is active. The screen is described in the Offline Section

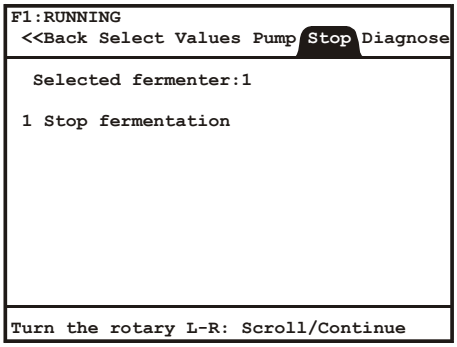
To quickly reach the Set point option, move the cursor to the desired parameter and press ENTER. For calibration, press the decimal [.] key.

To switch a parameter OFF, go to the Setpoint menu and turn regulation plus Output Active to OFF. This will then be reflected in the Parameters screen. The parameter is now only measured.



14.5.3 Pump

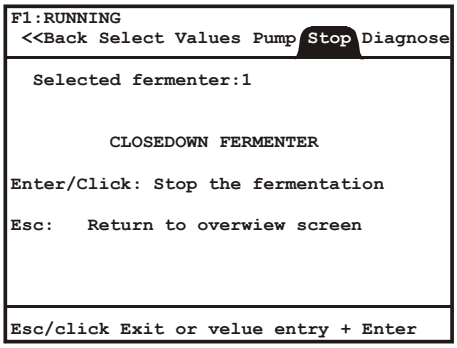
As offline.



14.5.4 Stop

SEE OPPOSITE

This allows the bioreactor to be turned off. Press 1 then ENTER to end a running fermentation



14.5.5 Diagnose

This is the same as Offline.

15. APPENDICES

15.1 Labfors3 Cells (Including Gas Mix)

The special version of Labfors3 for culture of mammalian and insect cells has a number of differences to the standard unit. Not all of these may be present for each installation:

- No baffles (for low shear)
- Magnetic coupling system (Infors axial system)
- Slow speed option (max. 20-300rpm)
- Spin filter (for cell-free removal of culture supernatant).
- Single, marine type impellor (several designs) for low shear and good mixing at slow speeds
- Sinter sparger or special system for smaller air bubbles
- Dissolved oxygen control by air flow control using a thermal mass flow control valve
- Special mixing options for dissolved oxygen and pH
:

  a) A two-gas supplementation system for oxygen and carbon dioxide
  b) A three gas mix unit for air, oxygen and nitrogen
  c) A three gas mix unit with an additional gas inlet for carbon dioxide

These options can be used with or without mass flow control.

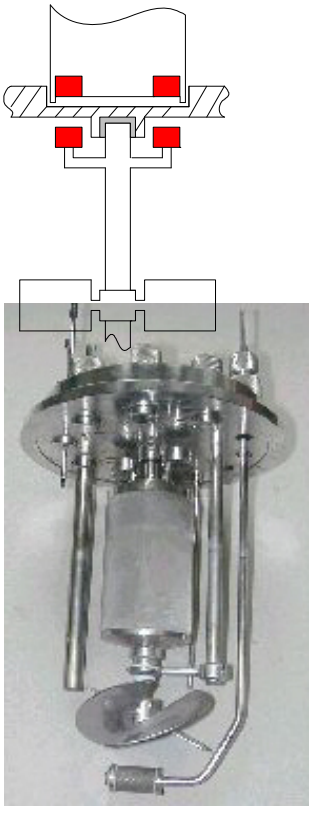
Some or all of these supplementary gases may be added to the head space through a separate inlet.

Most options are self-explanatory but the magnetic drive and spin filter need some additional explanation:

Magnetic Coupling

This kind of magnetic drive is based on the experience of the development of the multi-bioreactor, SIXFORS. Up to date technology provides a secure, strong coupling in a small space. The **INFORS axial magnetic drive** is crevice-free due to its open design and, therefore, can be cleaned in place.

Open axial magnetic drive



Spin Filter

A spin filter option is available for the removal of culture supernatant free of cells. This is important for applications such as the production of monoclonal antibodies where the cells remain in the culture vessel and the culture medium containing the antibody is removed.

The filter part is made of a mesh with a pore size too small for animal cells to pass through. It is mounted on the drive shaft using the small screw in the base collar at a height which ensures it dips into the culture. The rotation of the drive shaft creates a centrifugal force which keeps the cells from settling and growing on the mesh.

A separate harvest tube is mounted in the top plate so that the end is inside the cup created by the mesh filter. Culture supernatant collects in this cup free of cells and can be collected via the harvest tube. Fresh medium can be added via either the Labfors3' in-built feed pump or external pump

15.2 Setting up the Gas Mix Options

a) Control of dissolved oxygen by mass flow control

This is the simplest system for cell cultures and may be sufficient for cells with a low growth rate and undemanding oxygen requirements. Gas flow can be set to a constant value manually or changed according to the dissolved oxygen level.

In this latter case, setting a maximum on the rotameter as well as passing the gas through the mass flow controller will ensure that the system is not gassed at too high a flow rate.

Setting of the gas flow controller is covered in the next section.

b) Oxygen only or Two-gas supplementation:

Bottled oxygen and carbon dioxide gases are connected directly to the relevant valve with flexible pressure tubing (with suitable flow meters and pressure reducers connected to the gas bottles). An air pressure of 0.5-0.9 bar and a flow of eg. 0.1 vessel volumes per minute (VVM) ensures the addition of each gas does not cause problems when it blends with the main air flow from the rotameter (even if both valves work simultaneously).

Set the main air flow via the rotameter typically for 0.1 to 0.3 VVM maximum (and/or adjust the mass flow controller to this value used).

Once configured, control of the valves is automatic from the dissolved oxygen and pH controllers. For pH, the acid pump will operate at the same time as the gas valve. This allows maximum flexibility of use if a non-gassed pH control is needed for different tasks in the future.
➲ Inlet pressure should be reduced to 2.0 bar using a pressure reducting valve!
Be sure that you follow any standing instructions regarding the use of O2 and CO2 in your laboratory.

c) Three gas and three plus one gas mix.

Connections are made to the base unit with the supplied cables and the gas bottles connected for oxygen, nitrogen and carbon dioxide (if fitted). .

After configuration in the X-DDC instrumentation to cascade gasmix to the dissolved oxygen controller, the gas mix unit creates an artificial air mixture which is passed to the vessel at a constant flow rate via the mass flow controller. A manual setting can be given (eg. 20% oxygen) or the process is automatically controlled based on the set-point for dissolved oxygen.

The supply of carbon dioxide for pH control in place of acid is made separately to the main gas flow.
Carbon dioxide is added to the head space rather than entry via the sparger or special gassing system as standard.

Head space gassing with air/oxygen is also possible, as is the entry of CO_2 via the sparger. These and other possible options are on request.

15.2.1 Detailed Description of the Infors Gas Mix System.

The gas mix from Infors can be configured according to requirements. The standard configuration is suitable for all types of cell, especially mammalian.

An air/oxygen/nitrogen mix is added is delivered directly to the air inlet filter of the sinter sparger in the vessel via a thermal mass flow control valve mounted on the side of the box.

Carbon dioxide is delivered to a separate inlet pipe in the head space above the culture liquid as a pulsed flow according to the demands of the pH controller.

Alternative options and arrangements are available so please check the delivered configuration matches your requirements eg the mass flow valve has the right range.
Note: In the gassing system for O2 and CO2 needle valves have been integrated after the magnetic pulse valve. On delivery the valve is open. It can be adjusted manually by turning the screw clockwise to restrict the flow.

Setting Up

1. Connect the gases to the corresponding inlet pipes and secure FIRMLY with jubilee clips to ensure there are no leaks (expense and safety reasons). Make sure the metal pipes on the inlet have enough exposed metal to connect firmly when pushed to fit.

2. Connect the outlet pipes for the mixed gas and CO2 but do NOT connect to the vessel just yet.

3. Start the bioreactor with a test set up, using only pO2 control at first and set Flow to ON, providing a suitable set point eg. 0.5NL/min in the Pump/Valve menu so gas can be felt escaping from the outlet pipe.

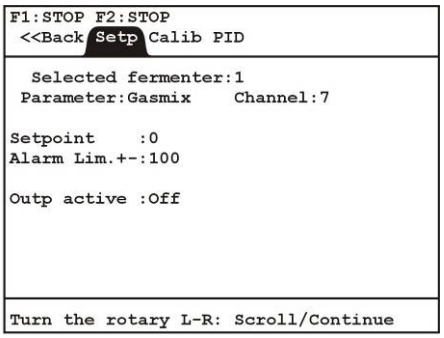
4. Test each gas in turn by adjusting to get Air, O2 and N2 to flow individually. When each gas flows, adjust the pressure roughly with the regulator on the gas cylinder or compressor. Each gas needs an input pressure regulated to 0.7-0.9 bar.
➲ Make sure inlet pressure does not exceed the max.of 2.0 bar!

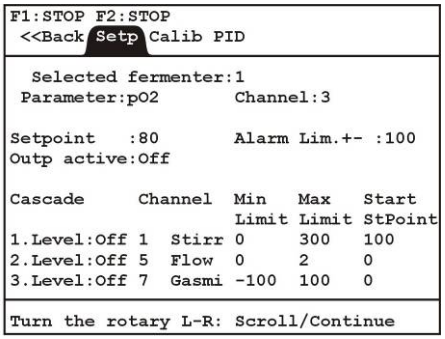
Gasmix Setpoint -100% = Nitrogen only flows
0% = Air only flows
+100% = Oxygen only flows

5. CO2 is set by turning pH control to ON and giving a low set point eg. 4. The gas should flow for up to 24 seconds in every minute and the acid pump will be seen to turn.

6. Attach the gas inlet pipe to the inlet air filter and the outlet for CO2 (if fitted) to the filter on the pipe/needle for head space gassing.

7. Set Gas mix as the first cascade level in the setpoint menu for pO2. Adjust flow rate to 0.1-0.3 vessel volumes per minute. Ensure the pO2 electrode has polarized properly (minimum 2 hours) before turning pO2 control to ON for the actual fermentation.





15.3 Connection to IRIS Software

The LABFORS system can communicate directly over the serial connection with a computer running IRIS software

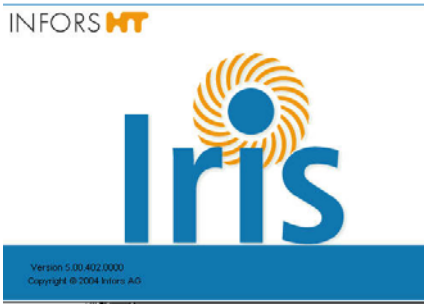
The following communications protocol is used:

Communication settings

BAUDE RATE	9600
DATA BITS	8
STOP BITS	1
PARITY BIT	NONE
FLOW CONTROL	NONE

1. Connect one end of the serial cable to the RS232 serial port at the bottom of the operating panel (left side). Screw in tightly
2. Connect the other end to the corresponding serial port of your PC (laptop or desktop). Screw in tightly
3. An earth lead may be provided on the cable. For desktop PC's. This can be connected to a casing screw. For laptop computers, the casing is plastic so this is not possible.

Install the software as for any Windows (98, NT3.5, 2000, Me & XP) PC - based program

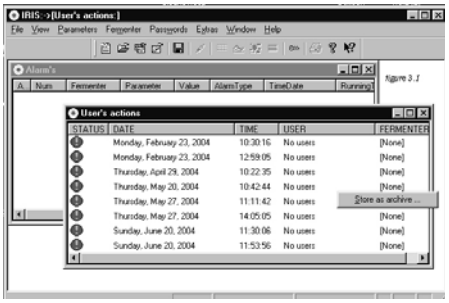


4. Insert the IRIS CD. If autorun is set for the PC, it should start automatically/
5. If not, locate the file Setup.exe on the CD and double click to run.
6. Follow the instructions given, choosing standard installation and accepting the defaults for the simplest installation.
7. Place a shortcut to the IRIS icon on the desktop for easy access.

The software installs in approximately 27MB of hard disk space but additional capacity must be available for the data files which will be created. It is not possible to predict exactly how much space is needed as different conditions of use will apply to different fermentations. As an example, data-logging 5 parameters for one hour at one minute intervals each (a relatively high log rate for most parameters) will create a file approximately 50K in size. A simple control sequence will create a file of only a kilobyte or so.

All IRIS data files have the structure FILENAME.iri and show the Infors IRIS icon. IRIS creates a new file for every fermentation. It is advisable to use a different directory than C-programmes for installation.

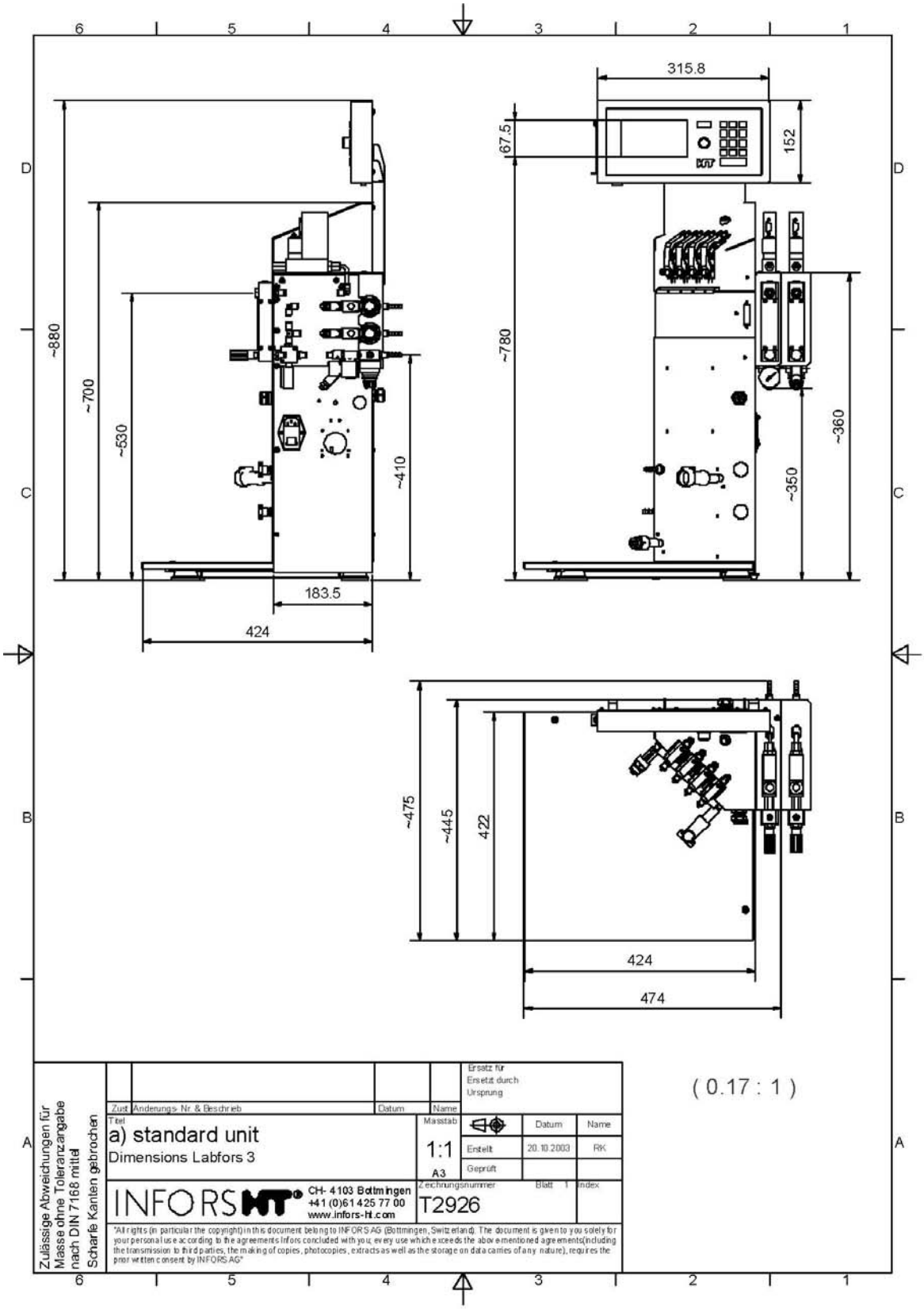
Double click on the IRIS icon from a desktop shortcut, the Programs section of the START menu or the Infors AG folder.
An opening screen will then load and IRIS will open with the default user logged in. A first, only the Alarms window and User Log windows open within the main IRIS window



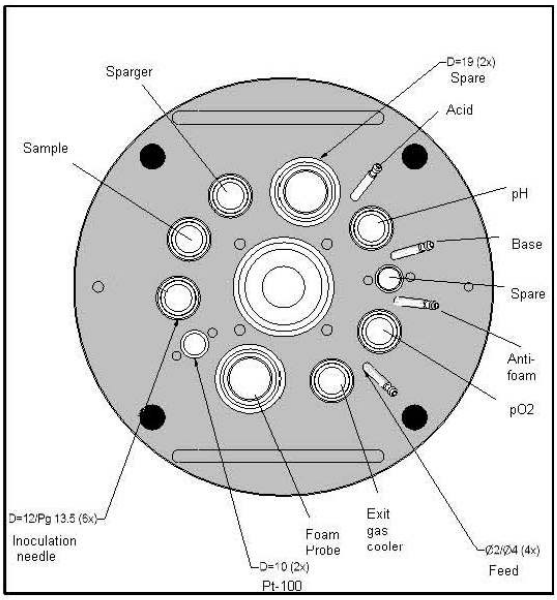
The main window comprises a menu bar with a toolbar beneath. The bottom part of the window is a status display with descriptive information about menu options and details of the active fermentation. The position and range of options provided on both the menu and toolbar areas is fully Windows compliant.

See the IRIS Operating Manual for further information.

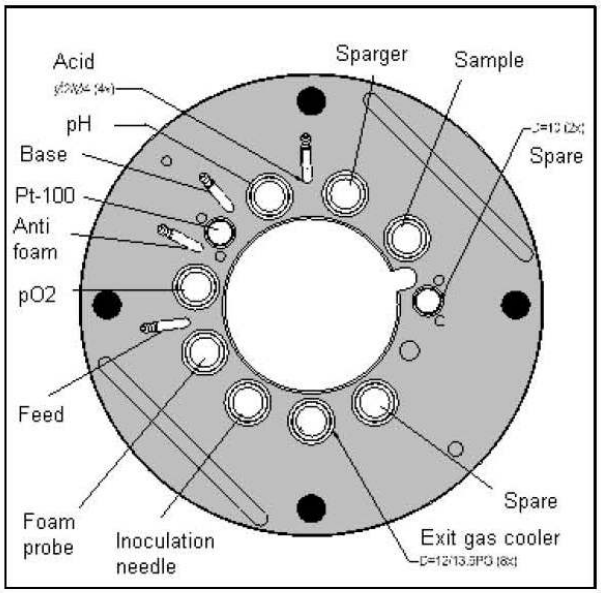
15.4 Dimensions standard unit



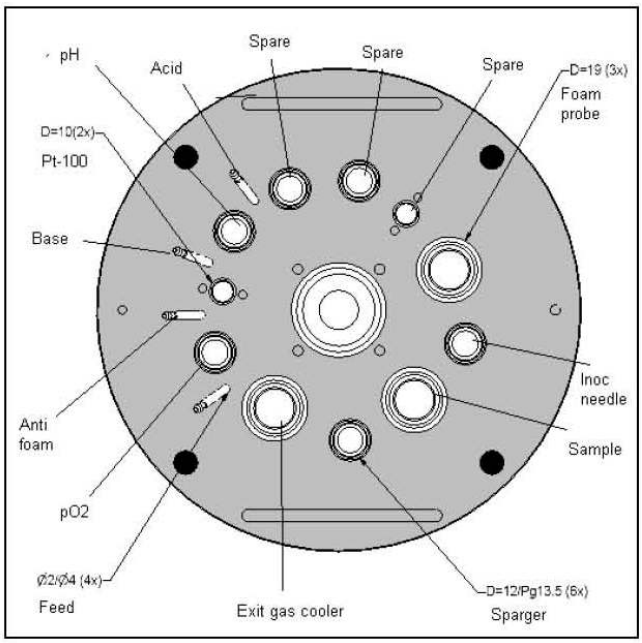
15.5 Dimensions Vessel Top plates



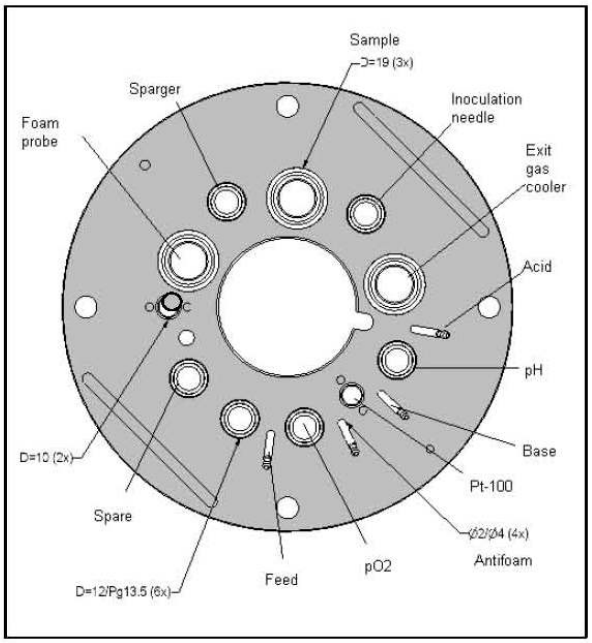
NW 115 NORMAL



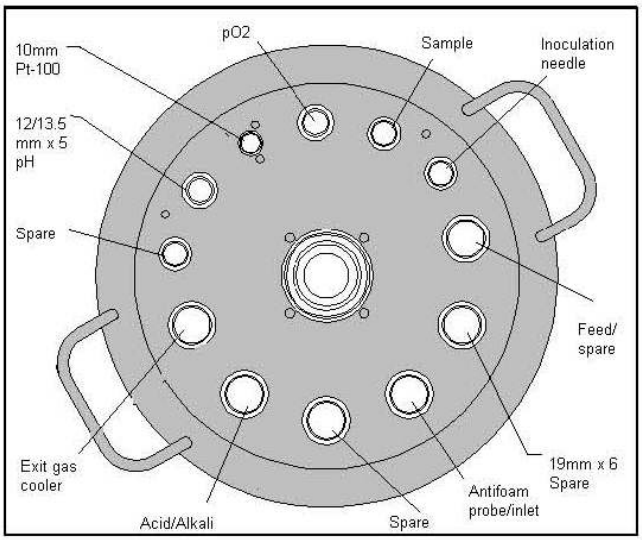
NW 115 MAGNETIC



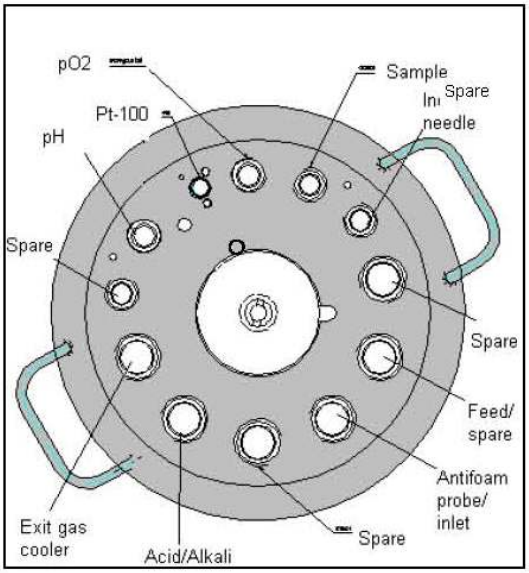
NW 150 NORMAL



NW 150 MAGNETIC



NW 200 NORMAL

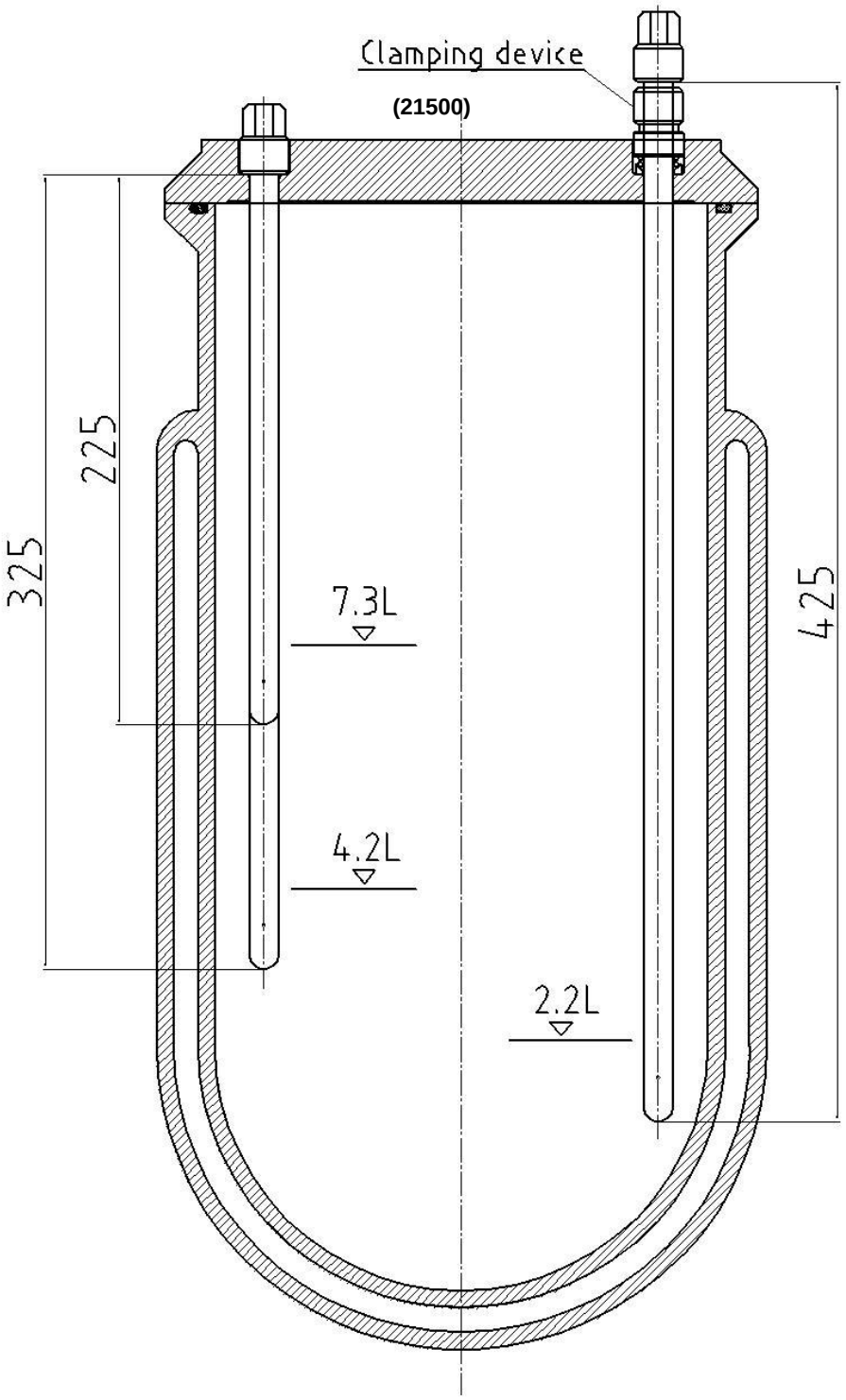


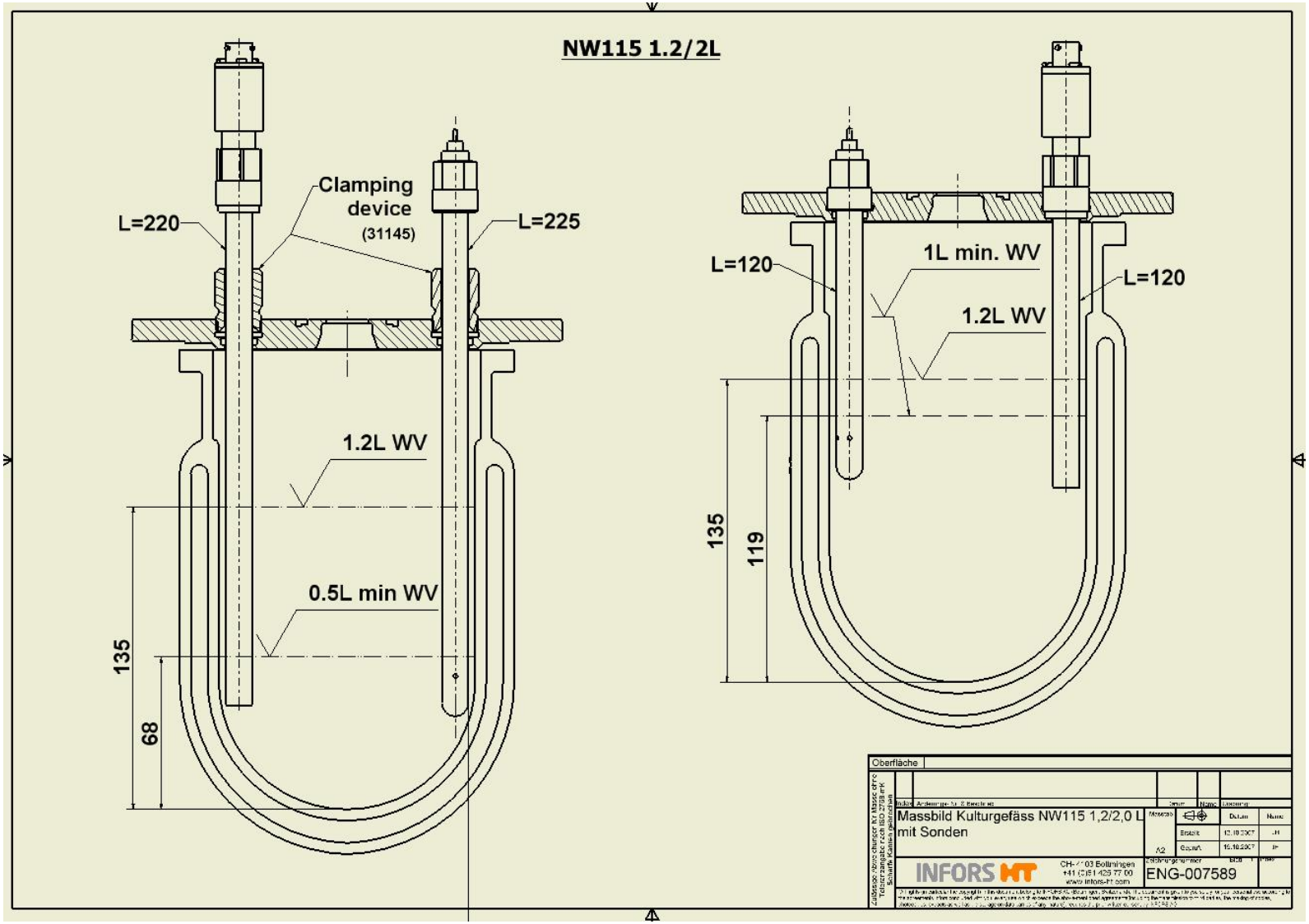
NW 200 MAGNETIC

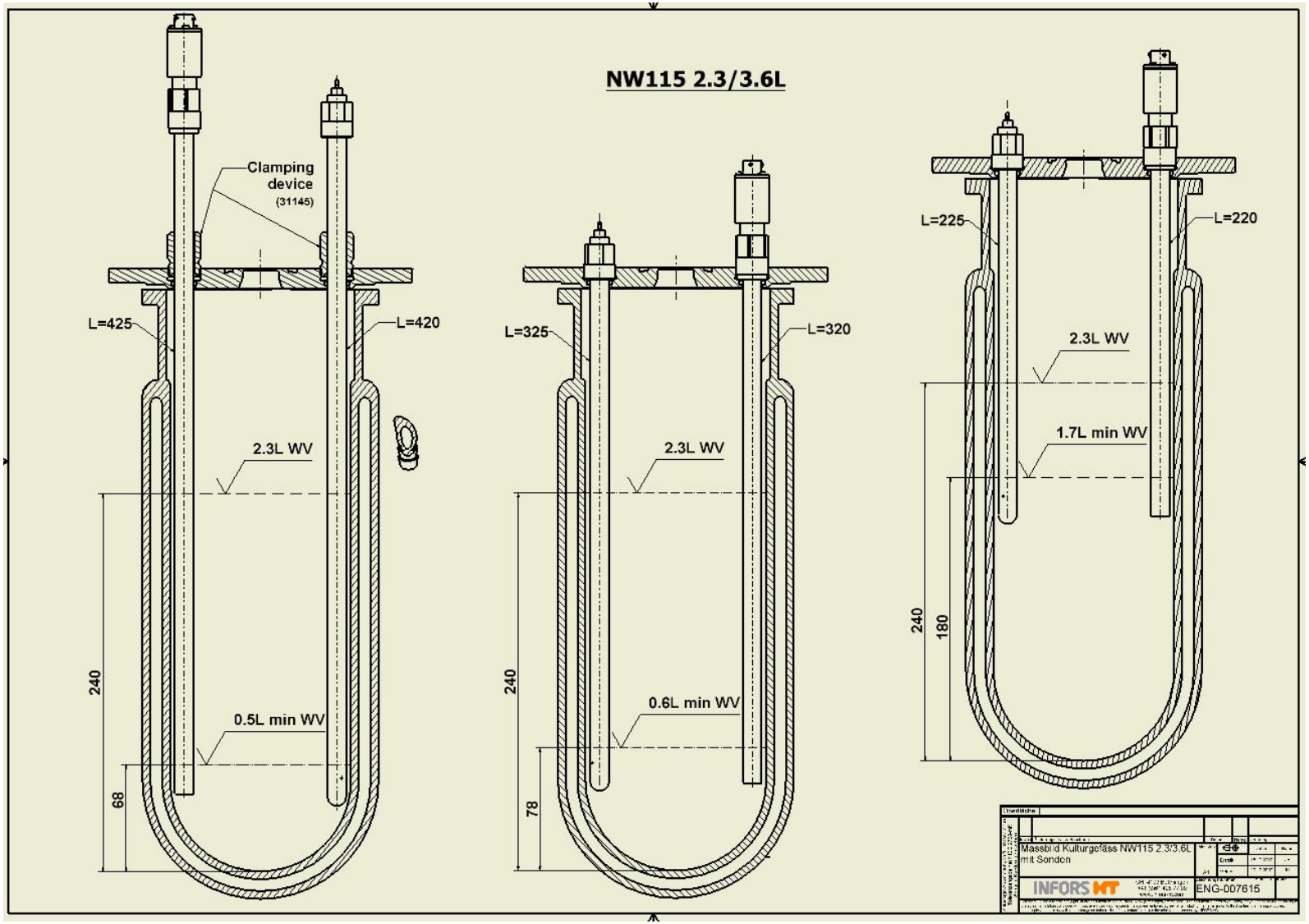
Nominal diameter	NW 115mm		NW 150mm		NW 200mm
Vessel / Tot. V	AMKL 2005/2 L	AMKL 206/3,6 L	AMKL 214/3,6 L	AMKL 216/7,5 L	AMKL 218/13 L
Impellers	2	2; Option 3	2	2; Option 3	2; Option 3
Ratio h/d for TV	1,8 for TV	3,2 for TV	1,6 for TV	3,1 for TV	2,1 for TV
Exhaust gas cooler	ASZZ 216 (D=12)	ASZZ 216 (D=12)	ASZZ 005 (D=19)	ASZZ 005 (D=19)	only ASZZ 005 (D=19)

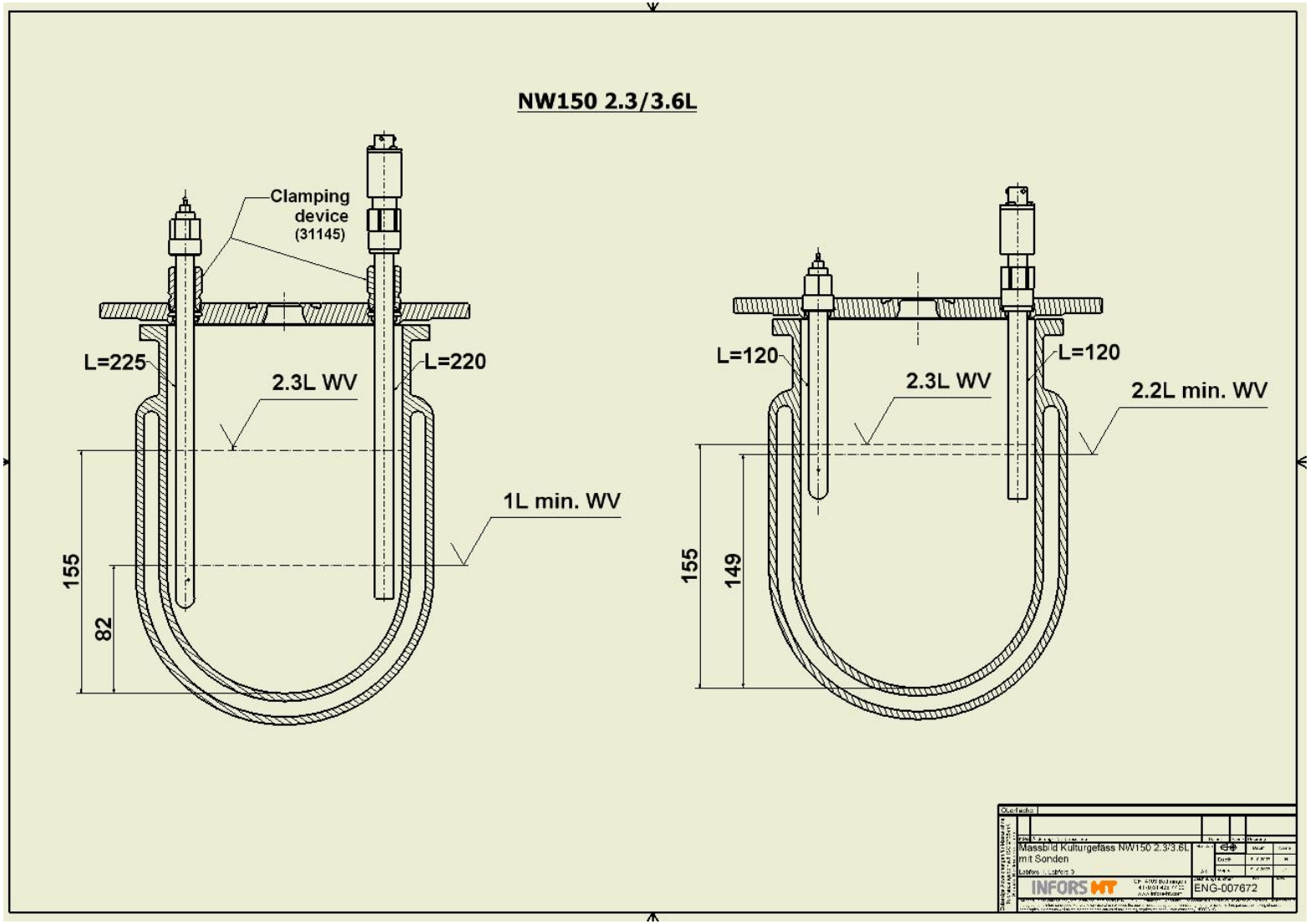
15.6 Labfors II and III Minimum Working volume with electrodes

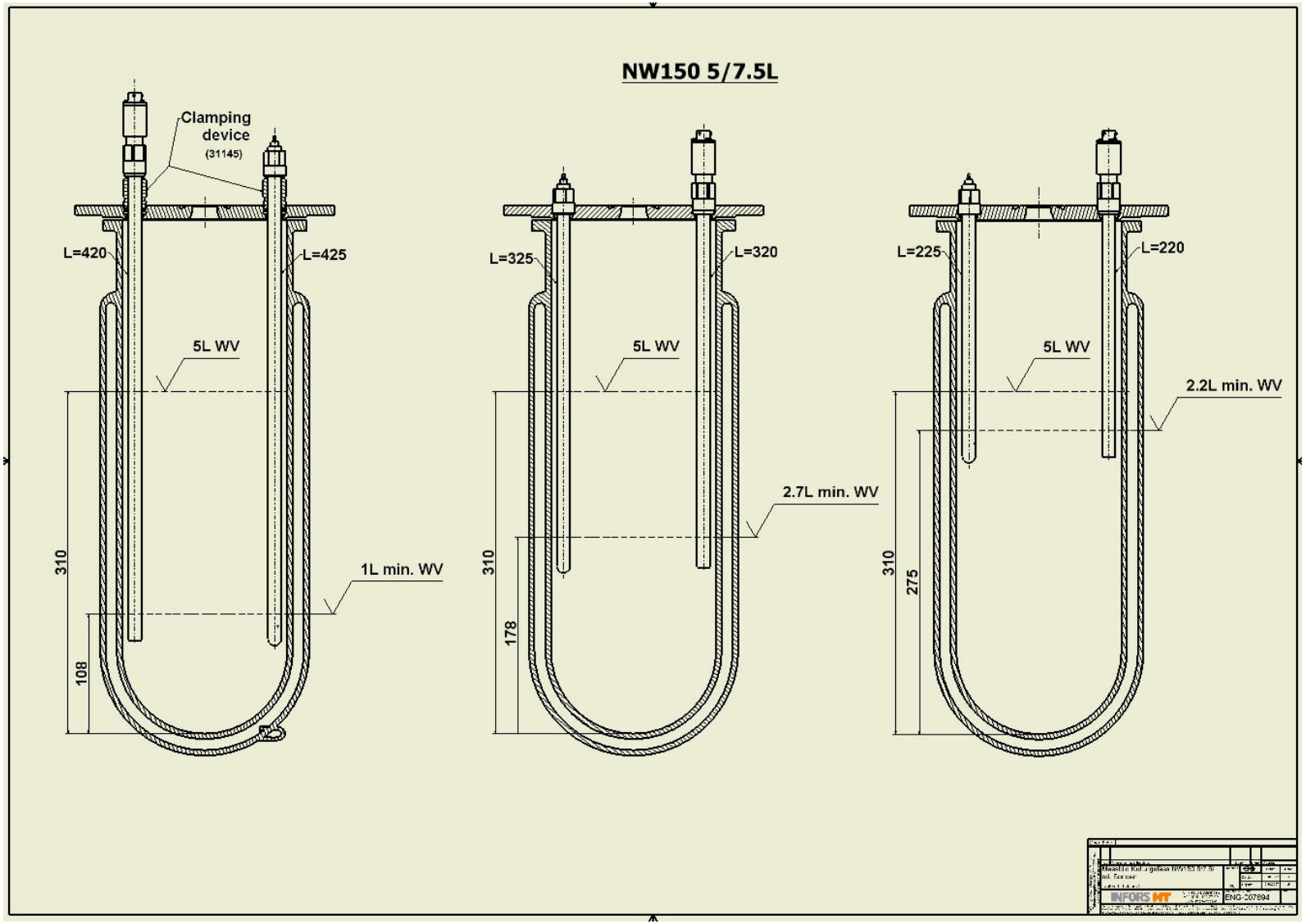
NW 200




NW115 1.2/2L
Clamping device
(31145)
L=220
L=225
1.2L WV
0.5L min WV
135
68
L=120
1L min. WV
1.2L WV
L=120
135
119
Oberfläche
Massbild Kulturgefäss NW115 1,2/2,0 L
mit Sonden
INFORS HT
ENG-007589


NW115 2.3/3.6L
Clamping device
(31145)
L=425
L=420
2.3L WV
0.5L min WV
240
68
L=325
L=320
2.3L WV
0.6L min WV
240
78
L=225
L=220
2.3L WV
1.7L min WV
240
180
Massbild Kulturgefäss NW115 2.3/3.6L mit Sonden
INFORS HT
ENG-007615


NW150 2.3/3.6L
Clamping device (31145)
L=225
L=220
2.3L WV
1L min. WV
155
82
L=120
L=120
2.3L WV
2.2L min. WV
155
149
Massbild Kulturgefäss NW150 2.3/3.6L mit Sonden
INFORS HT
ENG-007672


NW150 5/7.5L
Clamping device
(31145)
L=420
L=425
5L WV
1L min. WV
310
108
L=325
L=320
5L WV
2.7L min. WV
310
178
L=225
L=220
5L WV
2.2L min. WV
310
275
INFORS HT
ENG-207894

15.7 Specifications

INFORS-HT Labfors 3

Fermentor Labfors-3 Specifications

		2 Liters	3.6 Liters	3.6 Liters	7.5 Liters	10 Liters	13 Liters
Fermentor	Dimensions (W /D/H)	475 x 475 x 880 mm	475 x 475 x 880 mm	475 x 475 x 880 mm	475 x 475 x 880 mm	475 x 475 x 880 mm	475 x 475 x 880 mm
	Weight (Base Unit)	~30 kg					
	Electrical	230V 10A single phase supply					
Integrated In-/ Outputs		4 x Analogue input 0-5V/ 0-10V/ 0-20mA (4-20mA); 6 x Analogue output 0-20mA (4-20mA); 2 x digital output open collector					
Heating / cooling		Electric heating up to 95°C (thermo cirqulation 70°C max) and tap water cooling, heat/ colling programmable +5° utility water to 70°C, (Chiller in option)					
Peristalltic Pum	Number of pumps	4 x Peristalltic pump					
	Tubing used	Marprene 64° shore D= 2.79 x 4.39 (biggest possible tube)					
	Speed	18 RPM fixed					
	Flow rates	8.9 ml/ min with 100%					
Parameters		Temperature, RPM (as options: pH, pO2 + AF, gas flow, level, pressure, weight, feed, analog pumps, ...Max 16 parameters)					
Gassing options		Mass flow controllers, Oxygene supplementation, CO2 supplementation					
Vessel	Total Capacity	2 Liters	3.6 Liters	3.6 Liters	7.5 Liters	10 Liters	13 Liters
	Working Volume Max.	1.3Liters	2.4 Liters	2.4 Liters	5 Liters	7 Liters	10 Liters
	Working Volume Min.	0.5 Liters	0.5 Liters	1.0 Liters	1.0 Liters	2.0 Liters	2.5 Liters
	Weight (empty)	double jacket ~9.7 kg	double jacket ~11.3 kg	double jacket ~14 kg	double jacket ~16 kg	single jacket ~20 kg	double jacket ~26 kg
	Dimensions	I.D.=115mm/ H=240mm	I.D.=115mm/ H=370mm	I.D.=150mm/ H=235mm	I.D.=150mm/ H=465mm	I.D.=200mm/ H=352mm	I.D.=200mm/ H=420mm
	Ratio H/D	2.1 : 1	3.2 : 1	1.6 : 1	3.1 : 1	1.75 : 1	2.1 : 1
	Material	Glass borosilicate					
	Geometry	Cylindrical with round bottom					
	Baffles	Removable support with 3 baffles					
Ports	Ø 12 mm	6 x PG 13.5 thread	6 x PG 13.5 thread	6 x PG 13.5 thread	6x12mm/ PG13.5 thread	3 x round & 2 x PG 13.5 thread	
	Ø 19 mm round thread	2	2	3	3	6	6
	Ø 2x4mm fix weldet	4	4	4	4	--	--
	Ø 10mm one Pt-100	2	2	2	2	1	1
Agitation	Drive	DC 0.5 Nm	DC 0.5 Nm	DC 0.5 Nm	DC 0.5 Nm	DC 2.5 Nm	DC 0.5 Nm/ Servomotor 1.4Nm
(with 2 Imp.)	Power	150 W	150 W	150 W	150 W	100 W	150 W/ 680W*
	Range (with 2 Imp.)	80-1500 (Cell 20-300)	80-1500 (Cell 20- 300)	80-1500 (Cell 20-300)	80-1400 (Cell 20-300)	Cell 20-300	80-700/ 10- 1250 (Cell 20-300)
	Bearing Bacterial	Bearing housing with mechanical seal					
	Bearing Cell	Guide and bearing in Vessel					
Impellers	Type	2 x Impeller (Optional 3) type Rushton with six blade					
	Outer Diameter	D= 46mm x 12 mm	D= 46mm x 12 mm	D= 46mm x 12 mm	D= 54mm x 12 mm	D= 70mm x 12 mm	D= 70mm x 12 mm
Temperature	Sensor	PT-100					
	Range	-10° to +150°C					
	Accuracy	+/- 0.2°C in range +10° to +60°C					
Air Flow	Inlet Filter	Sterilizable absolute 0.2 um filter					
	Outlet Filter	Sterilizable absolute 0.2 or 0.45 um filter					
	Control	1 x Rotameter (additional Rotameter, Mass flow controller in option)					
	Accuracy	Rotameter accuracy +/- 4 % in Pressure 1.2 bar absolute					
	Range (P=1.2 bar\ 20°C)	0.16- 1.6LPM	0.42- 4.2LPM	0.42- 4.2LPM	0.83- 8.3LPM	0.83- 8.3LPM	2 - 20LPM

* depending on rotation speed

15.8 Utility Requirements

INFORS-HT Labfors 3

Utility requirements to Labfors 3 fermentor (Cell& Bacterial)

			2 Liters	3.6 Liters	7.5 Liters	10 Liters	13 Liters
Air in process air/ culture gazing	Flow	SLPM	up to 4	up to 7	up to 15	up to 20	up to 20
	Pressure	bar	2 bar (+/-0.5)	2 bar (+/-0.5)	2 bar (+/-0.5)	2 bar (+/-0.5)	2 bar (+/-0.5)
	Connection	Male adapter or h	NS 6mm (1/8")	NS 6mm (1/8")	NS 6mm (1/8")	NS 6mm (1/8")	NS 6mm (1/8")
Exit Air	Connection	Male adapter or h	Via disposable filter				
Water in circuit	Max flow at cooling	L/h	300	300	300	300	300
	Pressure	bar	2 bar (+/-1)	2 bar (+/-1)	2 bar (+/-1)	2 bar (+/-1)	2 bar (+/-1)
	Connection	Male adapter or h	NS 8mm	NS 8mm	NS 8mm	NS 8mm	NS 8mm
	Temperature	°C	10°C - 20°C	10°C - 20°C	10°C - 20°C	10°C - 20°C	10°C - 20°C
Drain	Pressure	bar	no back pressure!				
	Temperature	°C	up to 70	up to 70	up to 70	up to 70	up to 70
	Connection	Male adapter or h	It's on top of the Fermenter jacket; it requires a clear fall to the drain				
Electrical	Voltage	VAC	220-240	220-240	220-240	220-240	220-240
	Frequency	Hz	50/60	50/60	50/60	50/60	50/60
	Amperage	Amps	10	10	10	10	10

Gasing for Cell fermenter	pressure	max. bar	1.5 - 2.5	1.5 - 2.5	1.5 - 2.5	1.5 - 2.5	1.5 - 2.5
(CO2, air, N2, O2)	connection	Male adapter or h	NS 6mm (1/8")	NS 6mm (1/8")	NS 6mm (1/8")	NS 6mm (1/8")	NS 6mm (1/8")

Recommended maximum « soft » water (up to 1.5mmol/l CaCO3)

15.9 External I/O Pin configuration