ÄKTA*design*

Monitor pH/C-900

User Manual

18-1120-06

amersham pharmacia biotech

Important user information

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!

The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!

The Caution sign is used to call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note

The Note sign is used to indicate information important for trouble-free or optimal use of the product.

Should you have any comments on this instruction, we will be pleased to receive them at:

Amersham Pharmacia Biotech AB
SE–751 84 Uppsala
Sweden

Trademarks

Terms and Conditions of Sale

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Pharmacia Biotech group which supplies them. A copy of these terms and conditions of sale is available on request.

Addresses

Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Amersham Pharmacia Biotech UK Limited
Amersham Place
Little Chalfont
Bucks, HP7 9NA
England

Amersham Pharmacia Biotech Inc
800 Centennial Avenue
PO Box 1327
Piscataway, NJ 08855
USA

Amersham Pharmacia Biotech Europe GmbH
Postfach 5480
D-79021 Freiburg
Germany

Contents

Reference information

Short instructions on back page

About this manual

This manual comprises two parts; a practical part (sections 1 – 5) and a reference part (sections A – D).

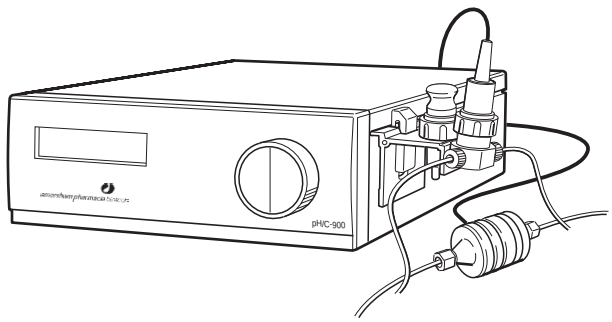
Sections 1 – 5 contain the necessary information for operating the instrument.

1 Introduction

1.1 General

Monitor pH/C-900 is a high precision on-line monitor for measurement of pH and conductivity in liquid chromatography. The pH/C-900 features:

- Fast response
- High accuracy and reproducibility
- Flow cells with low dead volume
- Accurate and reliable monitoring through self-test and self–calibration
- Flow cells can be connected close together, minimizing band broadening and time delay between detectors.



1.2 Safety

- The instrument is designed for indoor use only.
- Do not use in a dusty atmosphere or close to spraying water.
- Operate in accordance with local safety instructions.

WARNING! The instrument must not be opened by the user. It contains high voltage circuits which can be capable of delivering a lethal electric shock.

WARNING! Always disconnect the power supply before attempting to replace any item on the instrument during maintenance.

WARNING! The instrument must be connected to a grounded mains socket.

WARNING! When using hazardous chemicals, all suitable protective measures, such as protective glasses, must be taken.

2 Installation

2.1 Unpacking

Unpack the instrument and check the items against the supplied packing list. Inspect the items for obvious damage which may have occurred during transportation.

It is recommended that all packing materials should be retained if onward transport of the instrument is expected.

CAUTION! The following information should be read carefully to ensure that the instrument is installed correctly.

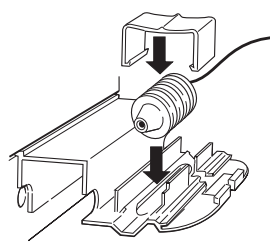
2.2 General precautions

The instrument should be located in a place of low temperature variations, away from heat sources, draughts and direct sunlight.

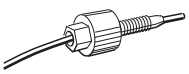
The instrument may be operated at normal ambient temperatures in the range +4 to 40 °C.

The instrument should be installed on a stable laboratory bench or in ÄKTA™ explorer or ÄKTA™ purifier. To ensure correct ventilation a free space of 0.1 m is required behind and in front of the instrument. Do not use any soft material under the instrument, to ensure that the ventilation inlet in the front is not blocked.

2.3 Installing the conductivity cell



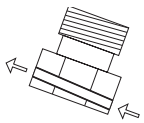
1 Place the conductivity cell in a suitable place, for example on the shelf of the Monitor UV-900 using the clip provided with the UV monitor. The cell can be placed up to 1.5 m from the monitor housing.



2 Connect the conductivity cell to the socket **Conductivity Flow Cell** on the rear panel of the instrument.

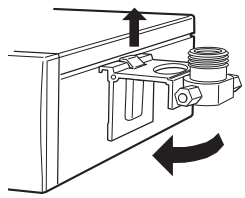
3 Connect the tubing with the “Fingertight” connectors. The flow cell itself does not have a recommended flow direction. In conjunction with the pH electrode, place the conductivity flow cell and select its flow direction so that the screw head end of the flow cell faces the pH flow cell (in ÄKTApurifier systems, the pH electrode is optional).

2.4 Installing the pH electrode (optional in ÄKTApurifier systems)


Flow direction

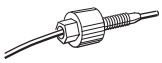
Mounting the flow cell holder

1 Hook the flow cell holder on the right hand side of the housing. Secure it with the slide clamp.



If the flow cell holder is not used, the flow cell must still be installed at an angle of 30° from the vertical with the outlet placed higher than the inlet to prevent air bubbles being trapped in the cell.

The flow direction is marked on the flow cell.



2 Connect the tubing with the "Fingertight" connectors.

Inserting the pH electrode

Note: *Handle the pH electrode with care.*

CAUTION! The tip of the pH electrode consists of a thin glass membrane. Protect it from breakage, contamination and drying out or the electrode will be destroyed. Always store the electrode with the end cover filled with a 1:1 mixture of pH 4 buffer and 1 M KNO_3. Do NOT store in water only.

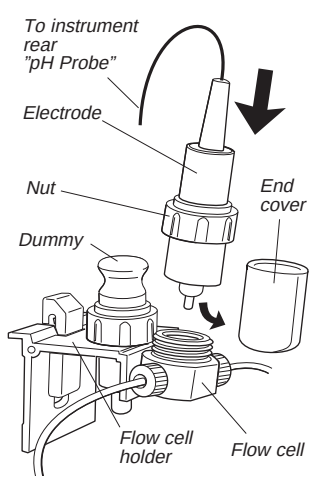
1 Unpack the pH electrode. Ensure that it is not broken or dry.

2 Prior to first using the electrode, remove the electrode end cover and immerse the glass bulb in buffer for 30 minutes.

3 Remove the dummy electrode from the flow cell and store it in the flow cell holder.

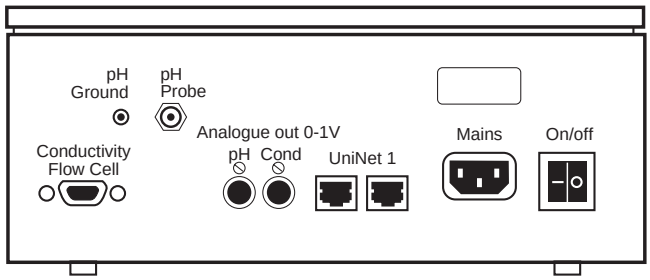
4 Carefully insert the electrode in the flow cell. Tighten the nut by hand to secure the electrode.
Note: *If the electrode is not fully inserted, the system will leak and a dead volume will occur in the holder.*

5 Connect the pH electrode cable to the rear of the instrument to the socket **pH Probe**.



2.5 Connecting electrical signal cables

The sockets for electrical signals are located on the rear panel.



Connecting to chart recorder (if used)

The external chart recorder outputs for pH and conductivity from the monitor are 0 – 1 V. There is also a 4 – 20 mA signal available at the same output connectors.

1 Connect the chart recorder to the DIN-socket pH or Cond using the cable supplied:
   a. For 0 - 1 V use wire 1 (+) and 2 (–). Use a recorder with floating inputs (i.e. none of the inputs connected to ground).
   b. For 4 - 20 mA use wire 5 (+) and wire 6 (–).
   Note: *The signal cable is delivered with protective covers on each wire. Do not remove the protective covers from unused connections as a short circuit may disturb the measurements.*

2 Set the recorder to 0–1 V or 4 - 20 mA input, full scale.

2.6 Connecting to communication link

The monitor can be used in ÄKTAexplorer and ÄKTApurifier, and can be controlled from a PC running UNICORN™ version 2.20 or higher, using *UniNet* cables.

Connect two *UniNet* cables to the *UniNet 1* connectors. The instrument can be connected in series anywhere between the PC and a termination plug. The *UniNet* 1 link connects, in series, the PC with Pump P-900, Monitor pH/C-900, Monitor UV-900 and the

> **CAUTION!** The mains power to ÄKTApurifier must be switched OFF before connecting the instrument to the *UniNet 1* link.

Frac-900. The termination plug is connected to the last instrument in the chain.

2.7 Connecting to supply voltage

1 Make sure the on/off switch is in the OFF-position (**O**).

2 Connect the supplied mains cable between the instrument and a grounded mains socket. Any voltage 100–240 V AC, 50–60 Hz can be used.

> WARNING! The instrument must be connected to a grounded mains socket.

The instrument contains no user replaceable fuse.

2.8 Preparing the instrument for use

Before the instrument is ready to use:

- Set the conductivity cell constant, see section *B.2.3*.
- Calibrate the temperature sensor, see section *B.2.6*.
- Calibrate the pH electrode (optional in ÄKTApurifier), see section *3.6*.

Note: *The conductivity cell constant is shown on the packaging. Retain the packaging in case the conductivity cell constant needs to be re-entered.*

Note: *The measured temperature is the temperature in the conductivity flow cell, which can differ from the ambient temperature, in ÄKTAexplorer systems.*

Note: *When running chromatography using organic solvents, it is recommended that the pH electrode is removed and the dummy electrode inserted in its place, as organic solvents will cause pH electrode degeneration.*

Before performing these procedures you are recommended to read sections 3.1–3.3.

3 Operation

3.1 On/off

```
Selftest
Please wait...
```

```
Monitor pH/C900
V1.00
```

```
Calibrating
Please wait...
```

```
Calibration OK
```

```
Cond   Temp    pH
 25.4% 22.9°C  11.50
```

Switch on the instrument at the mains switch on the rear panel.

At switch on, the instrument performs a selftest and then starts calibration. After approx. 30 seconds the display shows **Cond Temp pH** and the instrument is ready to use. All parameters are factory set to default values.

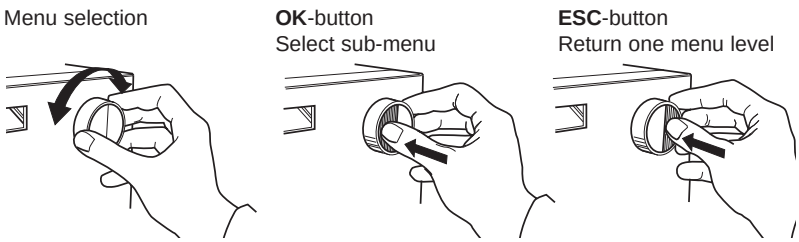
If the conductivity cell is not connected the **Temp** and **Cond** fields are blank. The **pH** field is blank if **Show pH** is set to off.

The monitor can be used immediately but the full specifications are not obtained until after a 1 hour warm–up.

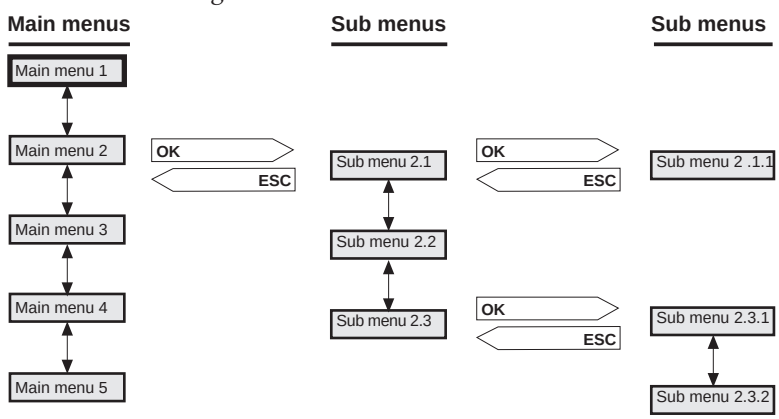
3.2 Menu selection and settings

Moving between menus

A specific menu is selected by turning the front selection dial clockwise or counterclockwise. When the required menu is visible the menu or selection is accepted by pressing the OK-button.



If a menu has sub levels, the sub menu is displayed by pressing the OK-button. Pressing the ESC-button moves back one menu level.

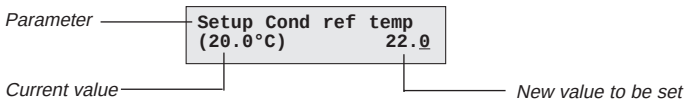


Return to main menu

Pressing **ESC** repeatedly, always returns to the **main menu 1** which is the main operating menu.

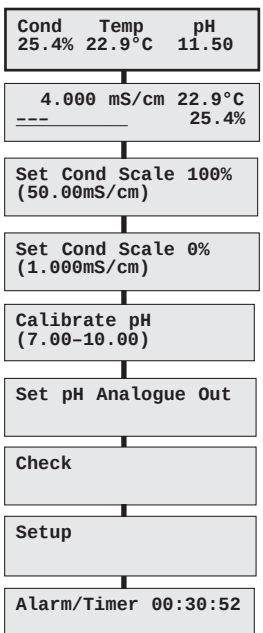
Select value

A cursor below a text or numerical value shows what is affected by the dial. To increase the value turn the dial clockwise. To decrease the value turn the dial counterclockwise. The value can be reset by turning the dial several clicks counterclockwise.



When setting numerical values the cursor moves up to the next digit if the dial is turned quickly in one direction (22.0), to simplify entering large values. The cursor moves back one place to the right every two seconds if the dial is not turned. The text or numerical value displayed is accepted by pressing the OK-button. To cancel, press the ESC-button.

3.3 Main menu overview



Main operating menu. The menu is accessed from all positions by pressing the ESC-button repeatedly.

Alternative display of conductivity with horizontal bar graph.

Setting conductivity full scale.

Setting conductivity zero point.

Calibration of pH monitor.

Setting the recorder output for the pH measurement.

Check internal operating values. See *Reference information section B.1.*

Setup conductivity, pH, temp., language, etc. See *Reference information section B.2.*

Set different timer options. See *Reference information section B.3.*

3.4 Reading pH and conductivity values

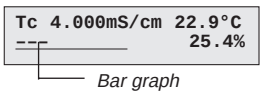
```
Cond    Temp    pH
25.4%   22.9°C  11.50
```

or

```
CondTc Temp    pH
25.4%  22.9°C  11.50
```

The main operating menu shows the conductivity as a percentage of full scale together with the current temperature in the flow cell, and the pH value. If the pH value is not stable or is changing, an asterisk is displayed after the value, e.g. 4.02*. The menu is reached from any other menu by pressing the ESC-button repeatedly.

If temperature compensation is switched-on, the display will show **CondTc** instead of **Cond**, see menu **Setup conductivity temperature compensation** in section *B.2.1* of *Reference information*.



By turning the dial one click clockwise, an alternative display of the conductivity is shown. This display shows the actual conductivity value in mS/cm together with the percentage value and as a horizontal bar graph with 10% resolution. If temperature compensation is switched on, **Tc** is shown in the display.

The display of pH and temperature can be disabled, see menu **Setup pH display** in section *B.2.5* and **Setup temperature display** in section *B.2.7* of *Reference information*.

3.5 Setting conductivity scale

The monitor measures conductivity over the complete working range. No range settings are required. However, to obtain a usable output signal for a recorder, or to view the conductivity as a percentage of buffer B, it is possible to set a 0 to 100% range. This can be done with reference to the buffers used or by selecting any fixed range between 0 µS/cm and 999.9 mS/cm.

To set the scale with reference to the buffers do as follows:

```
Set Cond Scale 100%
(50.00mS/cm)
```

1 Start the flow with the high conductivity buffer. Select main menu **Set Cond Scale 100%**, press OK.

2 When the conductivity level has stabilized, set the value which should correspond to **100%** by pressing OK. If required, the value can be changed with the setting dial.

```
Set Cond Scale 0%
(1.000mS/cm)
```

3 Change to the low conductivity buffer. Select main menu **Set Cond Scale 0%**, press OK.

4 When the conductivity level has stabilized, set the value which should correspond to **0%** by pressing OK. If required, the value can be changed with the setting dial.

The scale can be set without pumping buffer through the cell. Follow the same procedure, and simply set the the conductivity values directly at points 2 and 4, ignoring the measured conductivity values displayed. The difference between the value for **100%** and **0%** value (span) must be at least 1 mS/cm. The settings remain until they are changed. Values above 150% are shown as 150%, values below 0% as 0%.

3.6 Calibrating pH (optional in ÄKTApurifier systems)

A good laboratory routine is to calibrate the instrument once a day, when the electrode is replaced and if the ambient temperature is changed. The pH monitor is calibrated using standard buffer solutions in a two point calibration. The two buffer solutions can have any pH value as long as the difference between them is at least 1 pH unit. Calibration can also be performed from UNICORN. In UNICORN select **System Control:System:Calibrate**. Select the pH monitor. The calibration procedure can be done with the pH electrode either fitted in or removed from the flow cell.

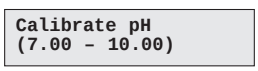
Calibrating with the electrode outside the flow cell

When calibrating the electrode out of the flow cell and changing from one buffer to another, rinse the electrode tip with distilled water and dab it carefully with a soft tissue to absorb the remaining water. Do NOT wipe the electrode as this may charge it and give unstable readings.

The steps below describe the procedure used with the electrode removed from the flow cell.

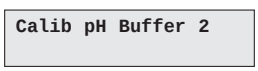
Note: *The Monitor must be unlocked if connected to a UNICORN control system.*

1 Remove the pH electrode from the flow cell and immerse the electrode in the 1:st standard buffer solution (normally pH 7.0).



2 Select main menu **Calibrate pH**. The display shows the current low and high calibrated pH value. Press OK.

3 Select sub menu **Calib pH Buffer 1**, press OK. When the pH value has stabilized, the **Please wait** message will disappear.



4 Adjust the pH value in the display using the dial, so that it corresponds to the known pH value of the 1:st buffer solution, press OK. The sub menu **Calib pH Buffer 2** is shown.

5 Rinse the electrode tip with distilled water and then immerse the electrode in the 2:nd standard buffer solution (e.g. 4.0 or 9.0), press OK.

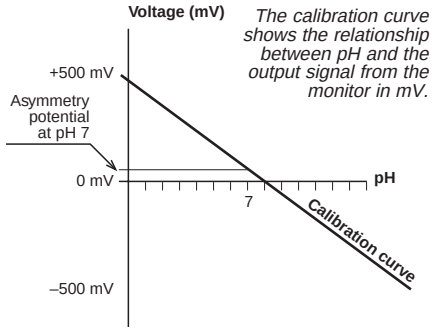
6 When the pH value has stabilized, the **Please wait** message will disappear.

7 Adjust the pH value in the display using the dial, so that it corresponds to the known pH value of the 2:nd buffer solution, press OK.

```
Calibrated Electrode
Slope 97.5%    8 mV
```

8 The sub menu **Calibrated Electrode Slope** shows the slope of the calibration curve where 100% corresponds to 59.16 mV per pH step at 25°C. The asymmetry potential at pH 7 is shown as a mV value. Press ESC to return to the main menu.

9 Before use, rinse the electrode using distilled water.



The calibration curve shows the relationship between pH and the output signal from the monitor in mV.

A new electrode has a slope of, typically, 95 - 102% and an asymmetry potential within ±30 mV. As the electrode ages the slope decreases and the asymmetry potential increases.

As a rule, when an electrode has an asymmetry potential outside of ±60 mV and a slope lower than 80%, and no improvement can be achieved by cleaning, it should be replaced.

An electrode is still usable at lower slopes and higher asymmetry potentials but the response will be slower and the accuracy diminished.

Calibrating with the electrode in the flow cell

When calibrating with the electrode fitted in the flow cell in ÄKTApurifier, follow the above procedure. Before adjusting the pH monitor, ensure that the pH has stabilized. Leave the pump running while calibrating. Switch to the other standard buffer solution and repeat the procedure. For a description of calibration from UNICORN with the electrode fitted in the flow cell, see section 6.6 in *UNICORN User Manual*.

3.7 Calibrating conductivity

The cell constant for the particular flow cell is written on the flow cell packaging. Refer to section *B.2.3* in *Reference information* for how to enter the cell constant.

Adjustment of the cell constant is only necessary when the monitor is to be used to determine specific conductivity with high accuracy. The procedure is described in *Reference information* section *B.2.2*. Calibration can also be performed from UNICORN.

3.8 Using an external chart recorder

The external chart recorder outputs for pH and conductivity from the monitor are 0 – 1 V. There is also a 4 – 20 mA signal available at the same output connectors.

For the conductivity signal, 0% represents 0 V and 100% represents 1 V as set under main menus **Set Cond scale 0%** and **Set Cond scale 100%.**

For the pH signal, the full scale and zero level has to be set according to below.

```
Set pH Analogue Out
```

1 Select main menu **Set pH Analogue Out,** press OK.

```
Set pH Full Scale
(pH12.00)    11.00
```

2 Select sub menu **Set pH Full Scale.** Set the range value, press OK. The range is the full scale pH range for the chart recorder (1 V).

```
Set pH Zero Level
(pH 2.00)       4.00
```

3 Select sub menu **Set pH Zero Level,** press OK.

4 Set the value, press OK. The zero level is the pH value corresponding to 0 V to the chart recorder. The difference between zero level and full scale must be at least 1 pH unit.

3.9 Storage and shut-down

CAUTION! Never leave the pH electrode in the flow cell for any period of time when the system is not used, since this may cause the glass membrane of the electrode to dry out. Dismount the pH electrode from the flow cell and fit the end cover filled with a 1:1 mixture of pH 4 buffer and 1 M KNO_3.

Do NOT store in water only.

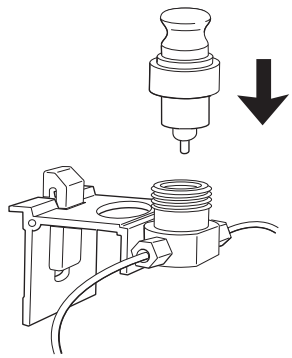
Storage of conductivity flow cell

Overnight: The conductivity cell can be left filled with a buffer.

Weekend or Long time storage: Flush the conductivity cell with water and fill with 20% ethanol.

Storage of pH electrode.

The pH electrode should **always** be stored in a 1:1 mixture of pH 4 buffer and 1 M KNO_3 when not in use. When the pH electrode is removed from the flow cell, the dummy electrode (supplied) can be inserted in the flow path.



Electrode regeneration: If the electrode has dried out, immerse the lower end of the electrode in buffer with a 1:1 mixture of pH 4 buffer and 1 M KNO_3 overnight.

3.10 Restart after power failure

If the power supply to the instrument is interrupted, the instrument automatically restarts itself and displays the main operating window. All set values are retained in the instrument.

4 Maintenance

WARNING! Disconnect the power supply before attempting to replace any item on the instrument during maintenance.

CAUTION! Only spare parts approved or supplied by Amersham Pharmacia Biotech may be used for maintaining and servicing the instrument.

4.1 Periodic maintenance

Interval	**Action** (see procedures below)
Every 6 month or more often if required	Change pH electrode
When required	Clean the conductivity cell Clean the pH electrode

4.2 Cleaning the flow cell

Remove the pH electrode and install the dummy electrode in the pH flow cell.

Pump a cleaning or sanitizing agent through the flow cells. The standard recommendation is to pump 1 M NaOH for 30 minutes and then wash out with buffer.

WARNING! NaOH is injurious to health. Avoid spillage.

4.3 Cleaning the pH electrode

Note: *The pH electrode has a limited life length and should be replaced every six months or when the response time is slow.*

Use one of the following procedures to clean the electrode to improve the response:

- **Salt deposits**: Dissolve the deposit by immersing the electrode first in 0.1 M HCl, then in 0.1 M NaOH, and again in 0.1 M HCl. Each immersion is for a 5 minute period. Rinse electrode tip in distilled water between each solution.
- **Oil or Grease Films**: Wash electrode tip in a liquid detergent and water. If film is known to be soluble in a particular organic solvent, wash with this solvent. Rinse electrode tip in distilled water.
- **Protein deposits:** Dissolve the deposit by immersing the electrode in a 1% pepsin solution, in 0.1 M HCl, for five minutes, followed by thorough rinsing with distilled water.

If these procedures fail to rejuvenate the electrode, the problem is most likely a clogged liquid junction.

1 Heat a 1 M KNO_3 solution to 60 - 80°C.

2 Place the electrode tip in the heated KNO_3 solution.

3 Allow the electrode to cool while immersed in the KNO_3 solution before re-testing.

If these steps fail to improve the electrode response, replace the electrode.

4.4 Changing pH electrode

See section *2.4 Installing the pH electrode*.

4.5 Cleaning the conductivity flow cell

If the conductivity measurements are not comparable to previous results, the electrodes in the flow cell may be contaminated and require cleaning. To clean the flow cell:

1 Pump 15 ml of 1 M NaOH at 1 ml/min through the flow cell either by using a pump or a syringe.

2 Leave it for 15 minutes.

3 Rinse thoroughly with 50 ml de-ionised water.

Note: *If the flow cell is totally blocked, the blockage can be broken using a thin needle or a piece of string with a diameter less than 0.8 mm.*

4.6 Changing conductivity flow cells

The flow cells can be changed when required. Make sure the instrument is switched off, before disconnecting/connecting the cells to the rear of the instrument housing.

1 If the cell is replaced with a new flow cell, the monitor must be calibrated with the new cell constant value which is written on the flow cell package. See sub menu **Setup conductivity** in section *B.2.3* in *Reference information*. If the cell constant is not known, it can be determined (see section *B.2.2* in *Reference information*).

4.7 Instrument housing

Wipe the instrument housing regularly with a damp cloth. Let the instrument dry completely before use.

5 Trouble shooting

5.1 General

```
Monitor pH/C900
V1.00
```

When contacting Amersham Pharmacia Biotech for support, state the program version of the instrument, which is shown for 2 seconds during switch-on.

WARNING! The instrument must not be opened by the user. It contains high voltage circuits which can be capable of delivering a lethal electric shock.

5.2 Faults and actions

If the suggested actions do not correct the fault, call Amersham Pharmacia Biotech.

pH measurement (optional in ÄKTApurifier systems)

Fault	*Action*
No response to pH changes	1 Check that the electrode cable is connected properly to the rear of the instrument. 2 The electrode glass membrane may be cracked. Replace the electrode.
Small response to pH changes	1 Clean the pH electrode according to section *4.3* and recalibrate. 2 If the problem remains, replace the pH electrode.
Slow pH response **or** **Calibration impossible**	1 Check the pH electrode tip. If it is contaminated clean the electrode following the instructions in section *4.3 Cleaning the pH electrode*. 2 If the membrane has dried out, the electrode may be restored by soaking it in buffer overnight. 3 Clogged liquid junction. Refer to section *4.3 Cleaning the pH electrode*.
Incorrect/ unstable pH reading	1 Check that the electrode cable is connected properly to the rear of the instrument. 2 Check that the pump and valves operates correctly. 3 Check that the electrode is correctly inserted in the flow cell and, if necessary, hand-tighten the nut. 4 If air in the flow cell is suspected, tap the flow cell carefully or tilt it to remove the air. Alternatively flush the flow cell with buffer at 8 ml/min for 1/2 min. 5 Check that the pH electrode is not broken. 6 Check that the pH electrode is calibrated.

Fault	*Action*
	7 Check the slope (see section *3.6 Calibrating pH*). If it is outside the range 80–105% or the asymmetry potential deviates more than 60 mV from 0 mV, clean the pH electrode. Recalibrate and if the problem persists, replace the pH electrode.
	8 Clean the pH electrode if required, see section *4.3 Cleaning the pH electrode.*
	9 Compare the response of the pH electrode with that of another pH electrode. If the response differ greatly, the electrode may require cleaning or replacement.
	10 There may be interference from other equipment. Connect the pH flow cell and the rear panel of the monitor using a standard laboratory 4 mm “banana plug” cable.
	11 Check that the pH electrode has been calibrated at the correct temperature.
	12 In organic solvents such as ethanol, methanol and acetonitrile, stable pH measurements are not possible since dehydration of the membrane will occur. It is recommended that the pH electrode is not used in applications using organic solvents. Mount the dummy electrode instead.
	13 Clogged liquid junction. Refer to section *4.3 Cleaning the pH electrode*.
pH values vary with varied back-pressure	1 Replace the pH electrode.

Conductivity measurement

Fault	*Action*
Incorrect or unstable reading	1 Check that the conductivity flow cell cable is connected properly to the rear of the instrument.
	2 Check that the pump and valves operate correctly.
	3 If temperature compensation is being used, check that the temperature sensor is calibrated, and that the correct temperature compensation factor is in use.
	4 Check that the column is equilibrated. If necessary clean the column.
	5 Check the operation of the mixer.
Baseline drift or noisy signal	1 There may be air in the flow cell. Use a flow restrictor after the flow cell.
	2 Check for leaking tubing connections.
	3 Check the column is equilibrated. If necessary clean the column.
	4 Check the operation of the mixer and the pump.
	5 Clean the flow cell according to procedure in section *4.5 Cleaning the conductivity flow cell.*

Fault	*Action*
Conductivity measurement with the same buffer appears to decrease over time	1 Clean the flow cell according to procedure in section *4.5 Cleaning the conductivity flow cell*. 2 The ambient temperature may have decreased. Use a temperature compensation factor, see section *B 2.1 Setting up conductivity temperature compensation*, in *Reference information*.
Absolute conductivity value wrong	1 Turn the flow cell so the end with the screws is facing the pH flow cell. 2 Recalibrate the conductivity cell. 3 Calibration solution, 1.00 M NaCl, not correctly prepared. Prepare a new calibration solution and recalibrate the conductivity cell.
Ghost peaks appear in the gradient profile	1 A charged sample has been detected (e.g. protein). 2 Air bubbles are passing through the flow cell. Check for loose tubing connections. If necessary use a flow restrictor after the conductivity flow cell.

Other problems

Fault	*Action*
Error in external chart recorder	1 Check the chart recorder in accordance with its manual. 2 Test the recorder function and input voltage which should be 1 V full scale. 3 Check the conductivity scaling and pH scaling, see menus **Set cond scale 100%** (section *3.5*)**, Set cond scale 0%** (section *3.5*) and **Using an external chart recorder** (section *3.8*).
No text on the front display	1 Check that the mains cable is connected and the power switch is in ON-position.

5.3 Error messages

If the suggested actions do not correct the fault, call Amersham Pharmacia Biotech.

Message	*Action*
`Cell constant is out of range`	1 Wrong solution used during calibration. Use 1.00 M NaCl Recalibrate. 2 Air in the conductivity cell during calibration. Flush the flow cell with calibration solution and recalibrate. 3 Dirty conductivity flow cell. Clean the cell according to section 4.5. Recalibrate the cell 4 Bad cell. Replace.
`pH diff is too small`	The difference between the zero level and full scale must be at least 1 pH unit.

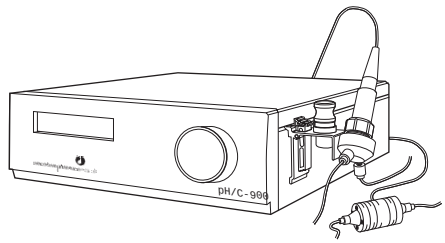
Message	*Action*
Bad pH cell or not connected	1 Check that the pH electrode is connected. 2 Replace the pH electrode.
Temp cell is bad or not connected	1 Check that the conductivity cell is connected. Recalibrate. 2 The calibration value differs from the predefined calibration value by more than ±5 °C. Recalibrate. If the message is still displayed, replace the conductivity cell.
Too close between buffer 1&2	The difference between the pH of the buffers used during calibration must be at least 1 pH unit.
Cal failed	Very bad slope, <10% or >199%. Recalibrate. If the message is still displayed, replace the pH electrode.
Bad Slope	Slope is <70% or >110%. Clean the pH electrode and recalibrate. If the message is still displayed, replace the pH electrode.
Check temp&cond condition	Check that the conductivity cell is connected.
Diff cond 0% & 100% is too small	The difference between 0 and 100% must be at least 0.1 mS/cm. Recalibrate.
Only allowed in "stand-alone"	Unit number cannot be changed when the instrument is connected in *ÄKTAexplorer* or *ÄKTApurifier*.
ERROR 88-89, 96	Call for Service.
ERROR key(OK) **ERROR key (Esc)** **ERROR key (OK+Esc)** **ERROR 100** **ERROR 109-113**	1 Switch off the instrument. 2 Check all connections. 3 Switch on the instrument.
ERROR 120	Call for Service.
ERROR 121	Calibration range not within limits. Recalibrate with new values.
ERROR 106-108 **ERROR 118**	1 Switch off the instrument. 2 Check all UniNet1 connections. 3 Switch on the instrument.
Exc x/y in ab.c **Exc DIV/O in ab.c** **Exc instr in ab.c** **Exc address in ab.c**	1 Switch off the instrument. 2 Check all connections. 3 Switch on the instrument.

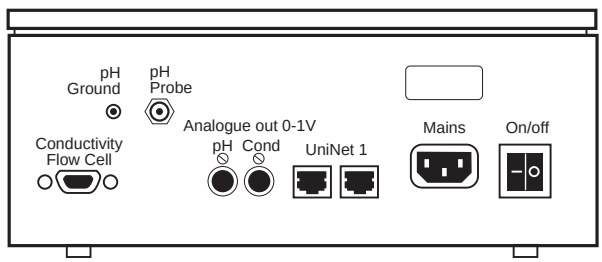
Reference information

A Description

A.1 Instrument

Monitor pH/C-900 is an on-line monitor for measurement of pH (optional in ÄKTApurifier)and conductivity. The monitor can work with standard glass pH electrodes with a built in liquid-filled reference electrode and BNC connector.

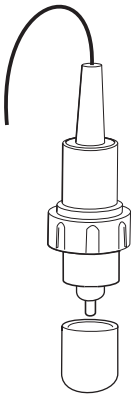




The conductivity monitor has a very large dynamic range from 1 µS to 999.9 mS/cm and is therefore suitable for a wide range of applications.

Connector/switch	Function
pH Ground	Signal ground
pH Probe	Connection to pH electrode, standard BNC socket
Conductivity, Flow Cell	Connection to conductivity flow cell, 9 pole D-sub connector.
Analog out 0–1V	2 separate recorder outputs for pH and conductivity. Recorder output 0–1 V
UniNet 1	Computer network
Mains	Supply voltage, grounded
On/off	Instrument on/off switch

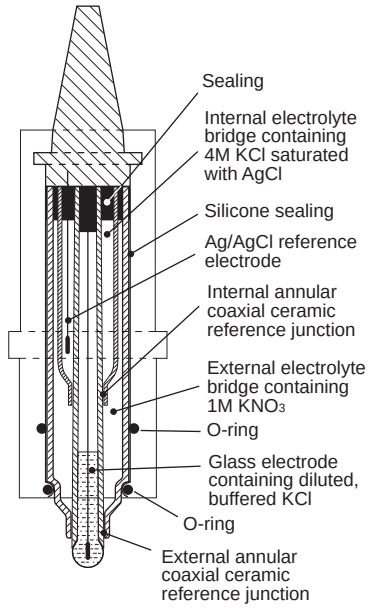
The instrument contains no internal user replaceable items.



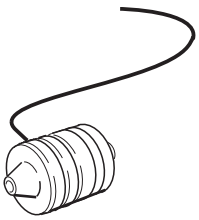
A.2 pH electrode

The pH electrode is of the sealed combination double junction type. It contains a sealed Ag/AgCl reference which cannot be refilled, an internal electrolyte bridge of 4M KCl saturated with Ag/AgCl, an outer electrolyte bridge of 1M KNO_3, an annular ceramic reference junction and a low profile pH membrane. The pH electrode is delivered with a transparent cover.

The flow cell should not be used with any other pH electrode.



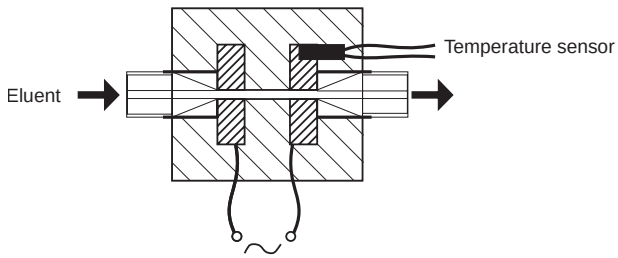
A.3 Conductivity cell



The flow cell has two cylindrical titanium electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the eluent. The monitor controls the AC frequency and increases it with increasing conductivity between 50 Hz and 50 kHz giving maximum linearity and true conductivity values.

The conductivity is automatically calculated by multiplying the measured conductance by the flow cell's cell constant. The cell constant is pre-calibrated on delivery but can be measured with a separate calibration procedure. This procedure is described in *Reference information* section *B.2*.

One of the electrodes has a small temperature sensor for measuring the temperature of the eluent in the flow cell. Temperature variations influence the conductivity and in some applications, when highly precise conductivity values are required, it is possible to program a temperature compensation factor that recalculates the conductivity to a set reference temperature.



B Menus

B.1 Check menu

B.1.1 Check Monitor Cell and Electrode

```
Check Monitor Cell
and Electrode
```

A check of the conductivity cell and pH electrode can be performed.

1 Select main menu **Check**, press OK.

2 Select sub menu **CheckMonitor cell and Electrode**, press OK.

3 The text **Passed** will appear for 2 seconds if the cell and electrode are indicating reasonable values. If there are any problems an error message will appear.

B.1.2 Check Service Mode

Service information relevant to the instrument can be checked. Information may not be available in all menus.

```
Check Service Mode
```

1 Select main menu **Check**, press OK.

2 Select sub menu **Check Service Mode**, press OK.

```
Telephone Service
012345678901
```

3 The service telephone number is displayed, press OK.

```
Contract Number
012345678901
```

4 The service contract number is displayed, press OK.

```
Serial Number
01234567 YM 012345
```

5 The instrument serial number is displayed, press OK.

```
Monitor pH/C-900
V1.00
```

6 The instrument name and software version are displayed, press OK.

```
Date of Maintenance
      ?
```

7 The date of the last service is displayed, press OK.

8 A test of the instrument buzzer is performed, press OK.

```
Buzzer Test
```

B.2 Setup menu

B.2.1 Setting up conductivity temperature compensation

The conductivity in a buffer is temperature dependent. To relate conductivity to concentration and/or compare conductivity values, temperature compensation should be used. The compensation consists of a compensation factor together with a reference temperature. All conductivity values will then automatically be converted to the set reference temperature.

```
Setup Conductivity
```

1 Select main menu **Setup**, press OK.

```
Setup Cond Temp Comp
(2.0%/°C)        2.0
```

2 Select sub menu **Setup Conductivity**, press OK.

3 Select sub menu **Setup Cond Temp Comp**, press OK.

```
Setup Cond Ref Temp
(22.3°C)
```

4 Set a temperature compensation factor, press OK.

The factor is expressed in percentage increase of conductivity per °C increase in temperature. If the temperature compensation factor is unknown, a general approximate value of 2% can be set for many common salt buffers.

Set the value to 0 for no temperature compensation.

5 Select sub menu **Setup Cond Ref Temp**, press OK. Select the reference temperature to which the measured conductivity values will be converted (normally 20 or 25°C), press OK.

B.2.2 Calibrating the conductivity cell

Normally it is not necessary to adjust the cell constant as the flow cell is pre-calibrated on delivery. Adjustment is only necessary when replacing the conductivity flow cell with a flow cell whose cell constant is unknown. It is also recommended that the conductivity flow cell is recalibrated after cleaning. When adjusting the cell constant from UNICORN select **System Control:System:Calibrate** and then select **CondCalib**.

Note: *The conductivity temperature compensation must not be used when adjusting the cell constant. Set the* ***Setup Cond temp comp*** *to 0 (see section B.2.1). The temperature sensor must be calibrated before adjusting the cell constant (see section B.2.6).*

1 Prepare a calibration solution of 1.00 M NaCl, 58.44 g/l. Let the solution stand until it is at room temperature. This is important for exact measurements.

2 Fill the flow cell completely with the calibration solution, by pumping at least 15 ml through the cell with a syringe.

3 Stop the flow and wait 15 minutes, until the temperature is constant in the range 20–30°C.

4 Read the conductivity value displayed and compare it with the theoretical value from the graph (on opposite page), at the temperature of the calibration solution. If the displayed value and the theoretical value correspond, no further action is required.

If the values differ, proceed with actions 5–8.

5 Select main menu **Setup,** press OK.

```
Setup Conductivity
```

6 Select sub menu **Setup Conductivity,** press OK.

```
Setup Adjust Cond
(83.53mS/cm)
```

7 Select sub menu **Setup Adjust Cond,** press OK.

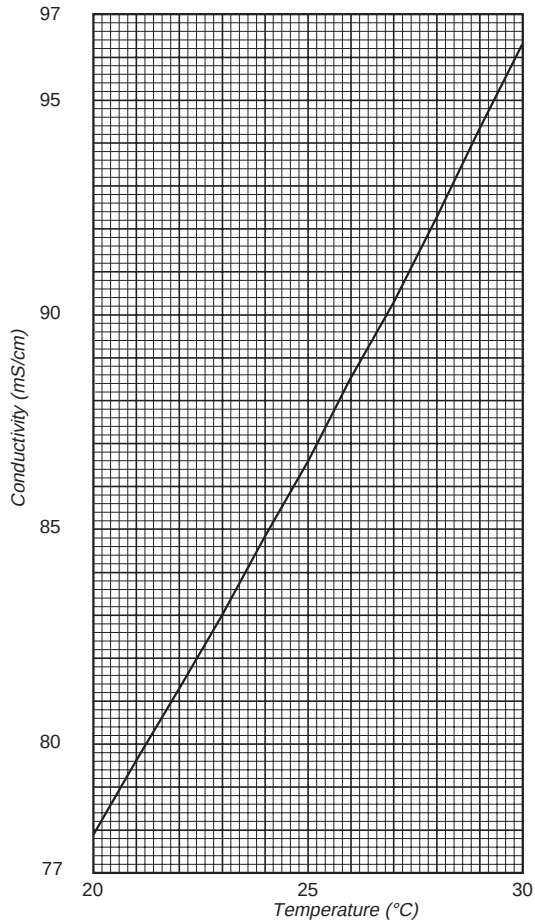
```
Warning! This will
change cell calib
```

8 A warning message will be displayed, press OK.

```
Setup Adjust Cond
(83.53mS/cm)   86.60
```

9 Enter the theoretical conductivity value according to the graph, press OK. The new cell constant is automatically calculated and updated.

Conductivity of 1.00 M NaCl at 20–30°C



B.2.3 Entering the conductivity cell constant

After replacing the flow cell, the cell constant has to be set. (The cell constant is shown on the packaging).

1 Select main menu **Setup,** press OK.

```
Setup Conductivity
```

2 Select sub menu **Setup Conductivity,** press OK.

```
Setup Adj Cell Const
(34.4/cm)
```

3 Select sub menu **Setup Adjust Cell Constant,** press OK.

```
Warning! This will
change cell calib
```

4 A warning message will be displayed, press OK.

```
Setup Adj Cell Const
(34.4/cm)      35.5
```

5 Enter the cell constant, press OK. The new cell constant is updated.

When entering the cell constant from UNICORN select **System Control:System:Calibrate** and select **Cond_Cell**.

B.2.4 Setting up pH temperature compensation

The relationship between pH and the output signal from the pH electrode is temperature dependent. For more accurate measurements during temperature changes, the pH measurement can be temperature compensated. In normal applications, if the temperature of the buffers and calibration buffers are identical, temperature compensation does not need to be on.

When using temperature compensation it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.

1 Select main menu **Setup**, press OK.

```
Setup pH
```

2 Select menu **Setup pH**, press OK.

3 Select sub menu **Setup pH Temp Comp**, press OK.

```
Setup pH Temp Comp
(Off)        On Off
```

4 Set the temperature compensation on or off, press OK.

B.2.5 Setting up the pH display

Normally the pH is displayed in the main operating menu or its alternative (see section *3.4 Reading pH and conductivity values*). If not required the pH display can be set to off.

1 Select main menu **Setup**, press OK.

2 Select menu **Setup pH**, press OK.

```
Setup Show pH
(On)
```

3 Select sub menu **Setup Show pH**, press OK.

4 Set the pH display on or off, press OK.

B.2.6 Calibrating the temperature sensor

Calibration of the temperature sensor in the conductivity flow cell is only necessary if the monitor is used in high accuracy measurement or the conductivity flow cell is replaced.

1 Place the flow cell together with a precision thermometer inside a box or empty beaker to ensure that they are not exposed to draft. Leave them for 15 minutes to let the temperature stabilise.

2 Read the temperature on the thermometer.

3 Select main menu **Setup**, press OK.

```
Setup Temperature
```

4 Select sub menu **Setup Temperature**, press OK.

```
Setup Adjust Temp
(23.5°C)
```

5 Select sub menu **Setup Adjust Temp**, press OK.

6 A warning message will be displayed, press OK.

```
Warning! This will
change temp calib
```

7 Enter the temperature shown on the thermometer, press OK.

```
Setup Adjust Temp
(23.5°C)       25.0
```

B.2.7 Setting up the temperature display

Display of the temperature in the conductivity flow cell, in the main operating menu, can be enabled or disabled.

1 Select main menu **Setup**, press OK.

```
Setup Temperature
```

2 Select sub menu **Setup Temperature**, press OK.

3 Select sub menu **Setup Show Temp**, press OK.

```
Setup Show Temp
(On)          On Off
```

4 Set the temperature display on or off, press OK.

B.2.8 Selecting language

The language used on the display can be changed.

1 Select main menu **Setup**, press OK.

```
Setup Language
(GB) GB D F E I
```

2 Select sub menu **Setup Language** press OK.

3 Select the desired language.
GB = British English
D = German
F = French
E = Spanish
I = Italian

B.2.9 Setup unit number

The unit number is the identification the Monitor pH/C-900 has on the UniNet-bus. It should correspond to the number set in UNICORN for the Monitor pH/C-900. The number should be set to 0 if one pH/C-900 is used. If more than one pH/C-900 monitor is used they must all have different numbers.

1 Select main menu **Setup**, press OK.

```
Setup Unit Number
(0)
```

2 Select sub menu **Setup Unit Number**, press OK.

3 Select unit number (0–25).

B.2.10 Setup display angle

The display angle can be set to compensate for different viewing heights.

1 Select main menu **Setup**, press OK.

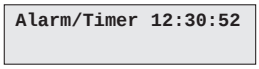
```
Set Display Angle
( ->|) ->\ ->| ->/
```

2 Select sub menu **Set Display Angle**, press OK.

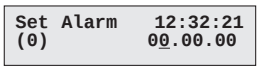
3 Select viewing angle (`->\` Up, `->|` Mid or `->/` Down).

B.3 Setting and using the alarm timer

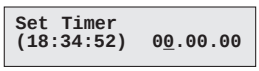
You can set the alarm function to either a fixed alarm time or using a count-down timer. The default or previous value is shown in parentheses.



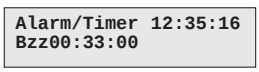
1 Select main menu **Alarm/Timer**, press OK. The display shows the current time.



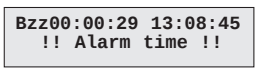
2 Select sub menu **Set Alarm**, if you want to set an alarm at a fixed time. Press OK to enter the time value in the form **HH.MM.SS**, pressing the OK button after entering each time unit.



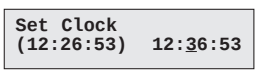
3 If you want to set a count-down timer, turn the dial to select sub menu **Set Timer**. Press OK to enter the countdown value in the form **HH.MM.SS**, pressing the OK button after entering each time unit. An alarm time and a count-down timer cannot both be set.



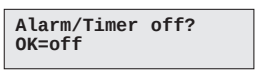
4 Press ESC button to return to the **Alarm/Timer** menu which now shows the set alarm time or count-down time as **BzzHH:MM:SS**.



5 When the alarm time is due or the count-down timer reaches 00:00:00, an alert display is shown and the instrument beeps, until the OK button is pressed. The display shows the time elapsed since the alarm, and the current time.



The alarm timer is based on the internal instrument clock which can be set in the **Set Clock** menu placed after the **Alarm/Timer** menu. The clock is reset when the power is turned off.

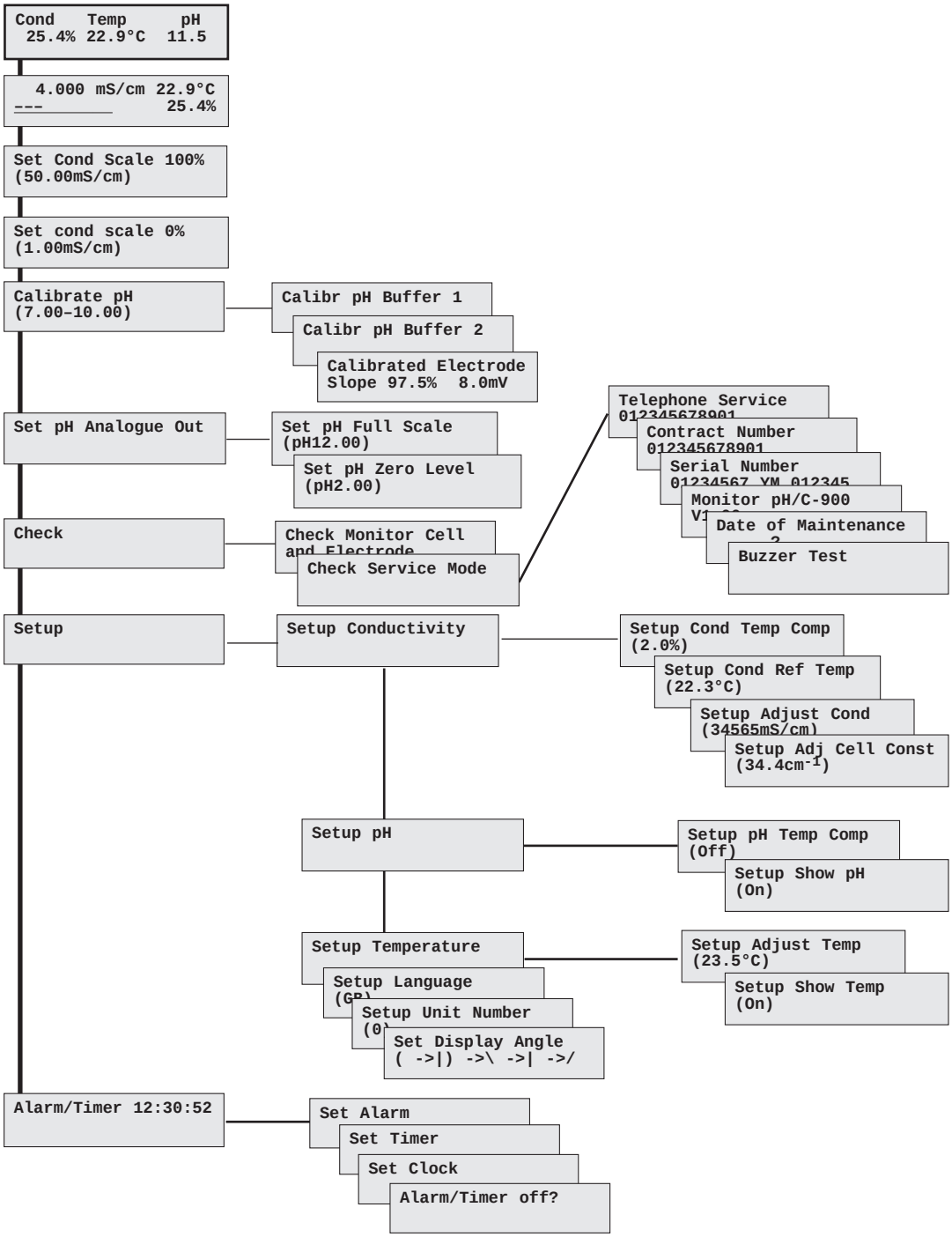


A set alarm/timer function can be reset by pressing OK in the menu **Alarm/Timer off?**.

B.4 Service displays

The instrument has service displays for use by authorised service personnel. If the service display **Insert Access Code:** is accidentally selected, press the ESC-button to exit to the normal operation display.

B.5 Menu overview



C Technical specifications

The full specifications apply only after at least 1 hour warm-up.

Operating data

pH measurement

pH range	0 to 14 (spec. valid between 2 and 12)
Accuracy	
temperature compensated	±0.1 pH within +4 to +40°C
not temperature compensated	±0.2 pH within +15 to +25°C ±0.5 pH within +4 to +15°C and +25 to +40°C
Response time	Max. 10 s (0 - 95% of step)
Long term drift	Max. 0.02 pH/h (measured at pH 4.0)
Flow rate sensitivity	Max. 0.1 pH units within 0–10 ml/min

Conductivity measurement

Conductivity range	1 µS/cm to 999.9 mS/cm
Deviation from theoretical conductivity	Max. ±2% of full scale calibrated range or ±10 µS/cm whichever is greater in the range 1 µS/cm to 300 mS/cm
Reproducibility	
short term	Max. ±1% or ±5 µS/cm
long term	Max. ±3% or ±15 µS/cm
Noise	Max. ±0.5% of full scale calibrated range
Response time	Max. 3 s (0 - 95% of step)
Temperature sensor	
Accuracy	±2.0°C
Drift	±0.5°C per 10 h
Flow rate sensitivity	±1% within 0–100 ml/min

Environment	+4 to +40 °C 20-95% relative humidity 84-106 kPa (840-1060 mbar) atmospheric pressure

Flow cells

pH cell (optional in ÄKTApurifier)

Max Flow rate	100 ml/min
Max Pressure	0.5 MPa (5 bar, 72 psi)
Back pressure	Max. 0.02 MPa (0.2 bar, 2.9 psi)
Internal volume	88 µl
Wetted materials	pH electrode and flow cell: Glass, FFKM (perfluororubber), titanium Dummy electrode: PTFE (polytetrafluoroethylene)

Chemical resistance	The wetted parts are resistant to organic solvents and salt buffers commonly used in chromatography of biomolecules, except 100% Ethyl acetate, 100% Hexane, and 100 % Tetrahydrofuran (THF).

Conductivity cell

Max Flow rate	100 ml/min
Max Pressure	5 MPa (50 bar, 725 psi)
Back pressure	Max. 0.01 MPa (0.1 bar, 1.5 psi)
Internal volume	14 µl
Wetted materials	Titanium, CTFE
pH stability range	1–13, 1–14 (<1 days exposure)
Chemical resistance	The wetted parts are resistant to organic solvents and salt buffers commonly used in chromatography of biomolecules, except 100% Ethylacetate, 100% Hexane, and 100 % Tetrahydrofuran (THF).

Physical data

Control	Stand alone or from a PC with UNICORN version 2.20 or higher, through UniNet 1 connection.
Degree of protection	
Housing	IP 20
Flow cells	IP 44
Power requirements	100–240 V AC, 50–60 Hz
Power consumption	25 VA
Functions	Languages selectable; English, German, Spanish, French, Italian
pH electrode cable length	1.5 m, BNC connector
Cond. cell cable length	1.5 m, D-sub 9 pole connector
Inlet- and outlet tubing	UNF 10-32 2B ”Fingertights” with capillary tubing 1/16” outer diameter
Analogue outputs	0–1 V and 4–20 mA full scale, overrange function (see section 2. *Installation* for pin configuration)
Display	2 rows with 20 characters each
Dimensions, H x W x D	100 x 260 x 370 mm
Weight	8.5 kg

EMC standards	This product meets the requirement of the EMC Directive 89/336/EEC through the harmonized standards EN 50081-1 (emission) and EN 50082-1 (immunity) **Note:** The declaration of conformity is valid for the instrument when it is • used in laboratory locations • used in the same state as it was delivered from Amersham Pharmacia Biotech except for alterations described in the user manual • used as “stand alone” unit or connected to other CE labelled Amersham Pharmacia Biotech instruments or other products as recommended.
S*afety standards*	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standard EN 61010-1.

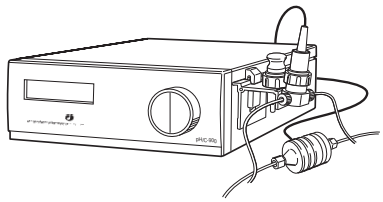
D Accessories and spare parts

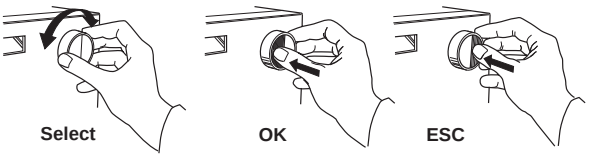
Item	*Quantity per pack*	*Code no.*
Monitor pH/C-900 without pH electrode and flow cells	1	18-1107-76
pH electrode, round tip	1	18-1111-26
pH electrode with flow cell and holder, round tip	1	18-1134-84
pH flow cell, incl. dummy electrode	1	18-1112-92
Dummy electrode, round tip	1	18-1111-03
Conductivity flow cell	1	18-1111-05
Signal cable, mini-DIN, open	1	18-1110-64
Teflon tubing, i.d. 1/8", o.d. 3/16"	3 m	18-1112-47
Tubing connector for 3/16" o.d. tubing	10	18-1112-49
Ferrule for 3/16" tubing	10	18-1112-48
Stop plug, 5/16"	5	18-1112-50
Stop plug, 1/16"	5	18-1112-52
Union Luer female/1/16" male	2	18-1112-51
Union 1/16" female/M6 male	6	18-1112-57
Union M6 female/1/16" male	8	18-1112-58
PEEK tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-53
Teflon tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-54
PEEK tubing, i.d. 1.0 mm, o.d. 1/16"	2 m	18-1115-83
Fingertight connector 1/16"	10	18-1112-55

April 1999

Short instructions

The following short instructions are intended as a guide to users who are fully familiar with safety precautions and operating instructions described in this manual. The instructions assumes that the instrument is installed according to the installation instructions.





1 **Switch on the instrument** by means of the mains switch on the rear panel.

```
Calibrating
     Please wait...
```

2 The **main operating menu** (RUN-menu) is shown.

```
Cond    Temp      pH
 25.4% 22.9C    11.5
```

3 **Calibrate** the pH electrode before use and/or daily by using 2 buffers with known pH values.

```
Calibrate pH
(7.00 – 10.00)
```

4 The **conductivity flow cell** does not normally need to be calibrated.

5 Set the **conductivity scaling** for 0 and 100%.

```
Set Cond Scale 100 %
(50.00 mS/cm)
```

6 Always store the **pH electrode** in 1:1 mixture of pH 4 buffer and 1 M KNO_3 when not in use.

TC information ab, Uppsala. Printed in Sweden by TK i Uppsala AB

amersham pharmacia biotech