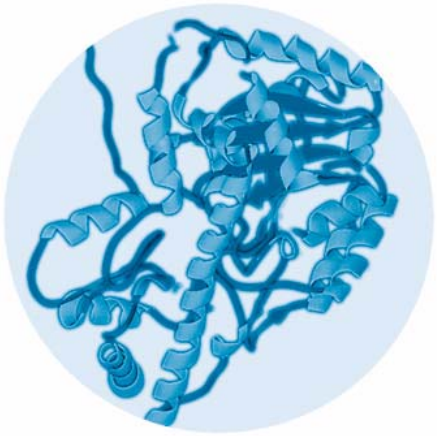

Agilent 3100 OFFGEL Fractionator

User Manual

Agilent Technologies

Notices

Manual Part Number

G3100-90001

Edition

04/2010

Printed in Germany

Agilent Technologies
Hewlett-Packard-Strasse 8
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Software Revision

This guide is valid for B.06.30 or higher revisions of the Agilent 3100 OFFGEL Fractionator Firmware, where the last two digits of the Firmware revision refer to minor revisions of the Firmware that do not affect the technical accuracy of this guide.

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CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide...

This book describes how to operate the Agilent 3100 OFFGEL Fractionator including the browser based user interface.

Chapter Overview

1 Product overview

This chapter gives an overview of the Agilent 3100 OFFGEL Fractionator. It describes the intended use and the fractionation principles of the instrument, highlights its features and outlines the different instrument components.

2 Site requirements and specifications

This chapter describes information about site requirements that have to be met so that the Agilent 3100 OFFGEL Fractionator performs within specifications.

3 PC, Browser and LAN Requirements

This chapter describes information about requirements for your PC, browser and LAN that have to be met for the control of the Agilent 3100 OFFGEL Fractionator.

4 Installation

This chapter describes how to install the Agilent 3100 OFFGEL Fractionator.

5 Operation

This chapter details how to operate the Agilent 3100 OFFGEL Fractionator. It familiarizes the user with all control functions from the internet browser interface. It describes the necessary steps to perform a fractionation and set up a LAN connection to a PC.

6 Maintenance

This chapter describes cleaning procedures that should be used to do maintenance on your instrument at regular intervals or before every use. In addition, it provides instructions on how update the firmware.

7 Troubleshooting

This chapter provides support for troubleshooting the instrument and the application.

8 Safety

This chapter contains safety information relevant to the Agilent 3100 OFFGEL Fractionator.

9 Appendix

This chapter contains details on non-condensing condition as well as ordering information for consumables and spare parts.

Related Documents

The Agilent 3100 OFFGEL Fractionator can be used with different consumable kits which each are supplied with a *Kit Manual*:

- 5969-1582 *Kit Manual*

Contents

1 Product overview

This chapter gives an overview of the Agilent 3100 OFFGEL Fractionator. It describes the intended use and the fractionation principles of the instrument, highlights its features and outlines the different instrument components.

Intended Use

The Agilent 3100 OFFGEL Fractionator system fractionates proteins or peptides based on their isoelectric points (pI) in liquid phase (OFFGEL mode). In addition, the system performs standard in-gel isoelectric focusing (IEF) using immobilized pH gradient gel (IPG) strips (in-gel mode). The system consists of an instrument that can be controlled via LAN by a User Interface based on a typical web browser. Earlier instruments were controlled by a handheld control module which can still be used in parallel. The web interface provides complete control and monitoring of the Agilent 3100 OFFGEL Fractionator. The system is capable of running a total of 16 samples in parallel (2×8).

OFFGEL pre-fractionation of complex protein and peptide sample provides additional sensitivity of downstream analysis tools such as LC-MS. In addition, the pI is a useful parameter that can be used for validation of peptide MS hits.

Fractionation Principles of the Agilent 3100 OFFGEL Fractionator

The Agilent 3100 OFFGEL Fractionator performs isoelectric focusing of proteins or peptides in immobilized pH gradient (IPG) gel strips. The 3100's OFFGEL electrophoresis differs from conventional gel electrophoresis in that the sample components do not remain in the gel. Instead, they are recovered from a buffer solution, making the recovery much easier than with conventional gels.

The three-part diagram in Figure 1 shows starting and ending states, as well as the OFFGEL fractionation process itself. After rehydration of gel strips, the immobilized pH-gradient (IPG) gel seals tightly against the compartment frame. Then, the diluted sample is distributed across all wells in the strip (Figure 1A). When a voltage is applied to the ends of the gel strip, the proteins or peptides move through the gel until the molecules reach a well covering a portion of the gel where the pH equals the pI of the molecule (Figure 1B). After fractionation, the liquid fractions containing pI-based separated proteins or peptides can easily be removed with a (multichannel-) pipette and processed for downstream experiments (Figure 1C).

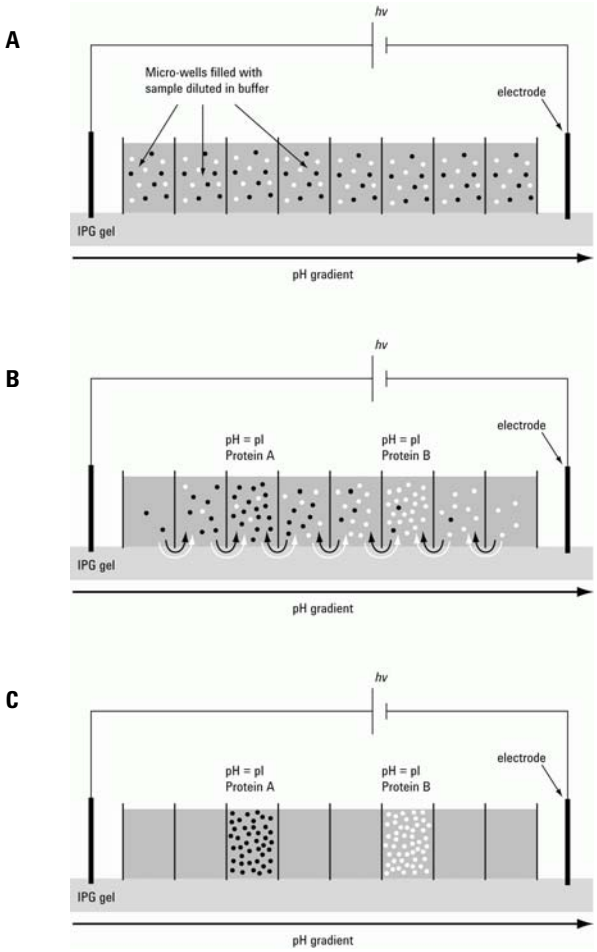


Figure 1 Fractionation principle of the Agilent 3100 OFFGEL Fractionator.

Features

- pI-based OFFGEL fractionation with in-solution recovery for easy transfer to downstream methods such as LC/MS.
- Reproducible pI-based OFFGEL fractionation down to 0.1 pH resolution depending on the consumable kit used.
- Preconfigured methods for OFFGEL and traditional in-gel IEF protein and peptide fractionation.
- Compatible with other upstream or downstream methods (immunodepletion, LC-MS, gel-based analysis)
- pI obtained acts as an additional validation parameter.
- No MS-incompatible additives used in fractionation buffer
- The instrument can also be operated in the traditional in-gel mode (IEF).
- Fractionation of up to 16 samples in parallel on 2 trays, accommodating 8 samples each.
- Broad loading capacity range from 50 µg sample load up to 5 mg sample load.
- Two independent power supplies allow two different methods to be run at the same time.
- Easy method setup and monitoring of important fractionation parameters and settings such as voltages and currents during a run.
- Online current control for each individual sample or gel-strip during fractionation.
- Viewing of up to three run parameters in an online plot.
- Easy export of important run parameters of a fractionation via LAN to the control PC or to a USB stick for further analysis
- Automated storage of up to 1200 data files, including important run parameters, on the compact flash card
- Monitoring of all operations and error events using the self-updating logbooks.

Instrument and Accessories

Figure 2 gives an overview of the complete Agilent 3100 OFFGEL Fractionator system:

Figure 2 Instrument overview

The instrument has two individual high voltage power supplies which provide voltages to the two trays. On the left side of the cooling platform is the anode connector; across the cooling platform are two cathode connectors (see Figure 3).

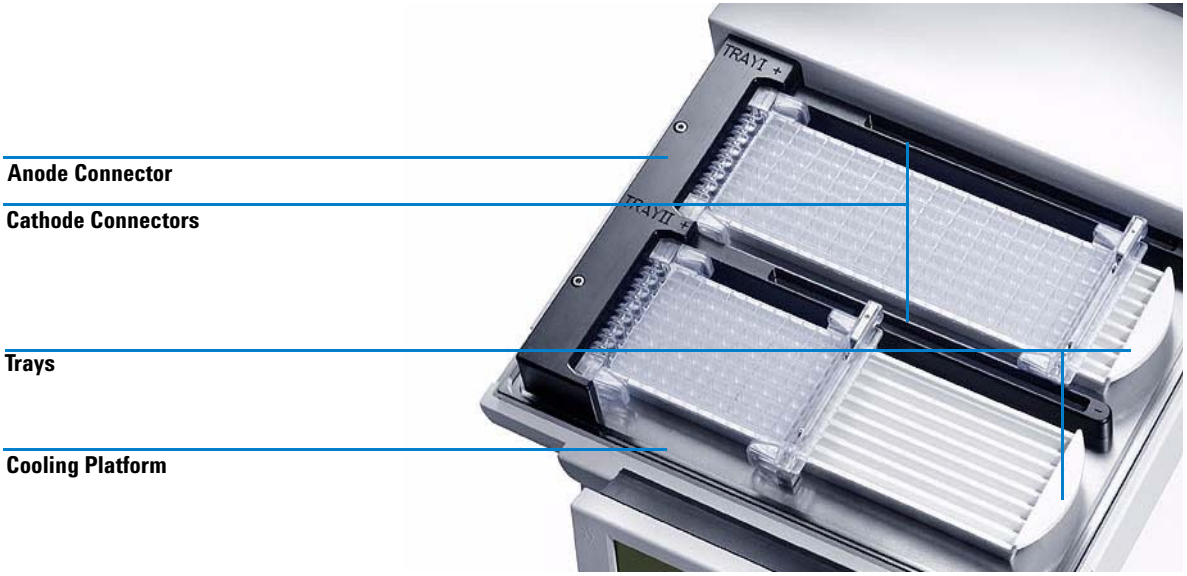


Figure 3 View inside the instrument.

Attached to each tray are a fixed electrode and a movable electrode that establish the connection to the anode and the cathode connectors (see Figure 4). Each tray accommodate up to eight samples. Each OFFGEL fractionation requires a frame to build up the well compartments as depicted schematically in Figure 1. There are two different frame lengths available, depending on the consumable kit and application chosen. The 12-well frame is typically used for fractionations requiring lower resolution, the 24-well frame is used for fractionations requiring higher resolution. Trays and electrodes can be ordered as spare parts if needed (for ordering details refer to Table 15 on page 156).

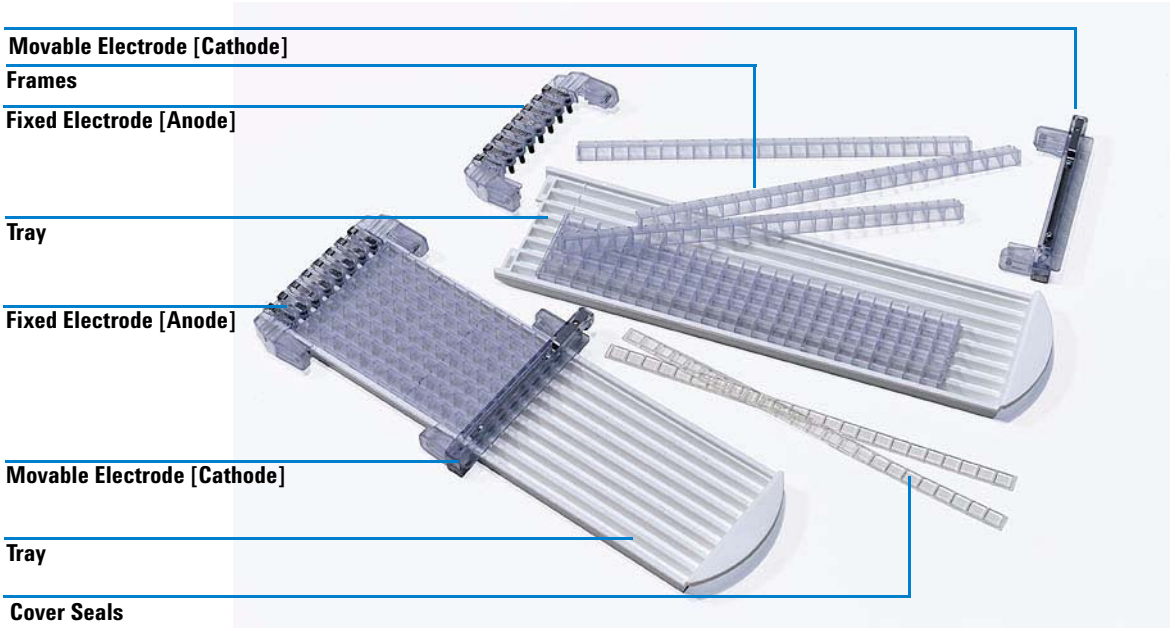


Figure 4 Electrodes, trays and frames

The frames are included in the consumable kits. The available consumable kits are listed in Table 1.

Table 1 Consumable kits

Product	Order Number
3100 OFFGEL, High Res Kit, pH 3-10	5188-6424
3100 OFFGEL, Low Res Kit, pH 3-10	5188-6425
3100 OFFGEL, High Res Kit, pH 4-7	5188-6426
3100 OFFGEL, Low Res Kit, pH 4-7	5188-6427
3100 OFFGEL, Starter Kit	5188-6444
OFFGEL, Protein Test Sample	5188-6428

2
Site requirements and specifications

This chapter describes information about site requirements that have to be met so that the Agilent 3100 OFFGEL Fractionator performs within specifications.

Site Requirements for the Agilent 3100 OFFGEL Fractionator

Power Considerations

The Agilent 3100 OFFGEL Fractionator power supply has wide ranging capabilities and accepts any line voltage in the range 100–240 V with a line frequency of 50–60 Hz (see also on page 25 for more details). Consequently, there is no voltage selector in the rear of the instrument.

CAUTION Shock hazard or damage to your Agilent 3100 OFFGEL Fractionator may result if the Agilent 3100 OFFGEL Fractionator is connected to a line voltage higher than specified.

Bench Space

The Agilent 3100 OFFGEL Fractionator requires sufficient desk or laboratory bench space; dimensions and weights are shown in Table on page 25. Approximately 15 cm (6 inches) of clear space at the rear of the instrument for electric connections is needed.

Environment

The Agilent 3100 OFFGEL Fractionator will work within the specifications of ambient temperatures and relative humidity described in Table 2 on page 25.

CAUTION Do not store, ship or use the Agilent 3100 OFFGEL Fractionator under conditions where temperature fluctuations could cause condensation within the Agilent 3100 OFFGEL Fractionator. Condensation will damage the electronics. If the Agilent 3100 OFFGEL Fractionator was shipped in cold weather, leave it in its box and allow it to warm up slowly to room temperature to avoid condensation.

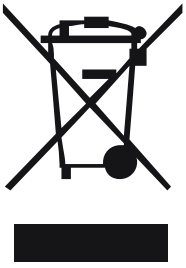
NOTE Put the Agilent 3100 OFFGEL Fractionator only on a flat and horizontal work top. Inclinations of more than 0.5% will cause the instrument to malfunction.

NOTE The Agilent 3100 OFFGEL Fractionator is designed to operate in a controlled electromagnetic environment (EN61326/A1) where RF transmitters, such as mobile phones, should not be used in close proximity.

The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC)

The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC), adopted by EU Commission on 13 February 2003, is introduced producer responsibility for all Electric and Electronic appliances from 13 August 2005.

NOTE



This product complies with the WEEE Directive (2002/96/EC) marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

Product Category:
With reference to the equipment types in the WEEE Directive Annex I, this product is classed as a "Monitoring and Control instrumentation" product.

Do not dispose of in domestic household waste

To return unwanted products, contact your local Agilent office, or see www.agilent.com for more information.

Sound Emission

This statement is provided to comply with the requirements of the German Sound Emission Directive of January 18th 1991.

This product has a sound pressure emission (at the operator position) < 70 dB (A).

- Sound Pressure Lp < 70 dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test).

Instruction for Use

The Agilent 3100 OFFGEL Fractionator is designed and tested as general purpose electrical equipment for laboratory use. It is designed and tested for research use only and not for use in diagnostic procedures.

CAUTION **If the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.**

Physical Specifications of the Agilent 3100 OFFGEL Fractionator

Table 2 Physical Specifications

Type	Specification	Comment
Weight	13 kg (29 lbs)	
Dimensions	157 x 355 x 427 mm	Height × width × depth
Line voltage	100–240 VAC	Wide-ranging capability
Line frequency	50–60 Hz	
Power consumption	140 VA	
Ambient operating temperature	5–40 °C (41–104 °F)	see also humidity specification
Ambient non-operating temperature	-40–70 °C (-40–158 °F)	Humidity 0 - 90%
Humidity	15 - 80 %, at 5–40 °C (41–104 °F) (non-condensing; e.g. max humidity is 55 % if the cooling platform is used 10 °C below ambient temperature)	see details in Appendix 9, "Non-condensing Conditions"
Cooling platform temperature	10 - 35 °C (50-95 °F) maximal 10 °C below ambient temperature	
High voltage power supply	500 V - 10 kV, maximal 150 µA per gel strip, maximal 1 W per gel strip	
Operating altitude	2000 m (6666 ft)	
Non-operating altitude	4500 m (15000 ft)	
Safety Standards: IEC, CSA, UL	Installation Category II Safety Class I, Pollution Degree 2	

3
PC, Browser and LAN Requirements

This chapter describes information about requirements for your PC, browser and LAN that have to be met for the control of the Agilent 3100 OFFGEL Fractionator.

PC, Browser and LAN Requirements

Browser Interface

The Agilent 3100 OFFGEL Fractionator can be controlled from an internet browser interface. The communication of browser application and the instrument is based on the TCP/IP network protocol as it is used for display of webpages from the world wide web. There is no specific control module on the instrument or dedicated software necessary on the computer. Table 3 lists browsers that were in principle capable for control of the instrument. There are no dedicated restrictions on the operating system.

Table 3 Examples of Compatible Browsers

Type
Internet Explorer IE 8.0 (Microsoft), recommended
Internet Explorer IE 7.0 (Microsoft)
Safari 4.0.4 (Apple)
Firefox 3.5 (Mozilla)

NOTE The Internet Explorer IE 6.0 (Microsoft) is not suitable for controlling the 3100 OFFGEL fractionator due to missing functionality in the browser.

LAN

The 3100 OFFGEL Fractionator comes with a default IP address (192.168.254.11) that cannot be changed. The instrument can also be assigned a user settable, static IP address. This custom IP address can be set after an initial connection via the default address. Configuration dip-switches at the instrument rear allow to select which of the two addresses are used by the 3100 OFFGEL Fractionator. This configuration is applied when the instrument is powered on the next time.

A detailed description on the steps for instrument setup is given under Chapter 4, “Installation,” starting on page 31.

NOTE The instrument is not enabled for DHCP (Dynamic Host Configuration Protocol). Therefore an automated assignment of network configurations from a server is not possible. For incorporation into a local network a static IP address is needed.

Tested Configurations

The configuration of the typical bundle equipment (Part Number G3100AA) of the system is described below. It was successfully tested and consists of

- a 3100 OFFGEL Fractionator
- a Netbook HP Mini 5102, Operating system Windows 7 Starter Edition, Internet Explorer 8.0
- a connection by a Cross-Over LAN cable

Another successfully tested configuration consisted of:

- a 3100 OFFGEL Fractionator with wired LAN connection to a local Network
- Apple iPod Touch 8G (FW Revision 3.1.2, including a Safari Browser) with wireless LAN access to the local Network.

PC, Browser and LAN Requirements

4
Installation

This chapter describes how to install the Agilent 3100 OFFGEL Fractionator and set up a LAN connection to a PC.

Unpacking the Agilent 3100 OFFGEL Fractionator

Before you begin the installation, ensure that the place you have chosen meets the site requirements as specified in "Site Requirements for the Agilent 3100 OFFGEL Fractionator" on page 22. The shipment consists of three boxes, one containing the computer (optional), one containing the instrument and one containing the power cable.

1 Unpack the computer.

   Please proceed with starting and configuring the computer as described the documentation associated with it. Make sure to retain the administrative rights until the system setup is complete.

2 Open the box in which the Agilent 3100 OFFGEL Fractionator was shipped. On the top you will find a box containing the accessories and a *Declaration of Conformity*.

Figure 5 Opened instrument shipment box.

3 Take out the accessory box and lift the instrument with its foam packing out of the outer box.

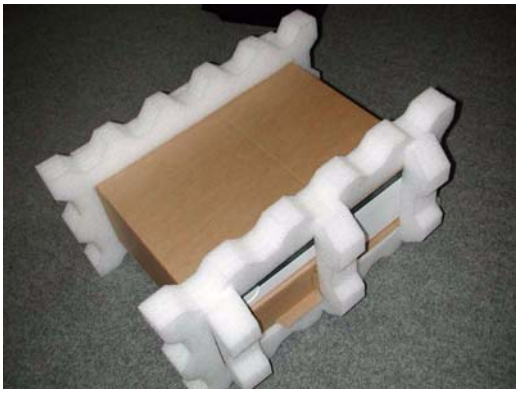


Figure 6 Instrument inner shipment box.

4 Remove the foam packing and the inner box and place the instrument in a suitable place.

5 Check that you have the complete set of accessories available using Table 4.

Table 4 Accessories

Description	Part Number
1 User Manual	G3100-90001
2 Quick Installation guide	G3100-90111
3 Hex key set 1 – 5 mm	8710-0641
4 Compact Flash Card	1819-0277*
5 Cross over LAN cable	2023-0203
6 USB-Memory Stick Kit	G4208-68700*

* Part numbers are of April 2010 and might be subject of changes.

Setting up the Agilent 3100 OFFGEL Fractionator

After you have unpacked the Agilent 3100 OFFGEL Fractionator and positioned it in a suitable place on your bench, you need to do the following:

- Install the Compact Flash Card
- Insert the USB memory stick
- Connect the power cord

The Agilent 3100 OFFGEL Fractionator has the following connectors at the rear:

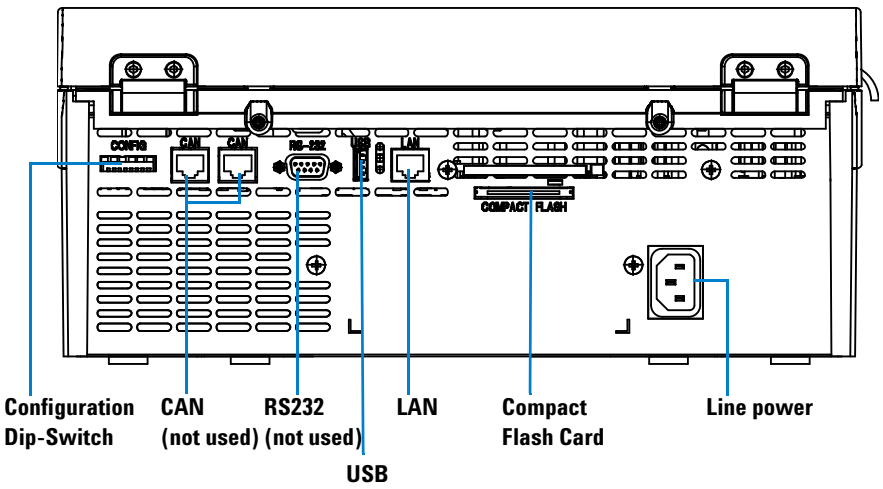


Figure 7 Connections at the rear of the instrument.

1 Insert the compact flash card into the dedicated position.

2 Connect the Cross-Over LAN cable to the LAN connector. Make sure the second end is available to be connected to the control PC at a later point in time.

NOTE Be sure to not to confuse the LAN connector with the CAN connector. The web browser access to the instrument will not work when connecting it to the CAN connector.

3 Connect the power cable to the instrument and to the line power.

4 Verify configuration dip-switches (Figure 8):
   - Switches N° 1 to 6 must be down
   - Switches N° 7 and 8 must be up

5 Switch on the instrument.

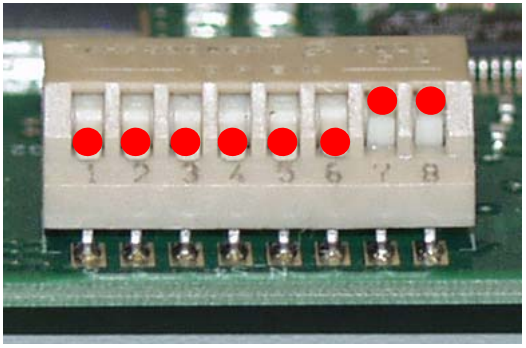


Figure 8 Configuration switches of the Agilent 3100 OFFGEL Fractionator

The instrument LED is usually yellow, which indicates that the cooling platform is cooling down to the specified temperature (typically 20°C). Once the temperature has been reached, the LED is switched off.

NOTE To test the instrument for proper functionality, it is recommended to perform the tests as specified in chapter "Troubleshooting the Instrument" on page 92.

Setting up the Control PC

To operate the control computer delivered with the system refer to its manual. You might request support from your IT department or administrator in case you do not feel comfortable with first time starting and configuring a computer. Make sure to retain the administrative rights until the system setup is complete. Make sure the browser Internet Explorer version 8.0 is installed.

The following description on necessary steps reflects a setup for computers with operating system Windows 7 Starter. This description reflects steps of the Quick Installation guide G3100-90111 which is part of the instrument shipment.

Assignment of an IP Address to the Control Computer

The Control PC and the the 3100 OFFGEL fractionator need to be enabled for LAN communication by assigning the PC a suitable IP address first. This IP address is associated with a certain subnet address. The 3100 OFFGEL fractionator has a similar default IP address from the same subnet. This default IP is hard coded in the instrument and enabled by configuration dip-switches on the backside of the instrument (Figure 7 on page 34).

For assignment of an IP address to the PC follow these steps:

1. Plug Cross-Over LAN cable into the LAN connector of the PC.
2. Make sure the second end of this Cross-Over LAN cable is accessible to be inserted to the 3100 OFFGEL fractionator.
3. Place the PC at a suitable location and power it on.
4. Log on with an user account that has administrator rights
5. Click on **Start** and select **Control panel**.
6. Go to the Section **Network and Internet** and select the item **View network status and tasks**.
7. Select and click with the right mouse button onto the item **Change adapter settings**.

8 At the item **Local Area Connection** click with the right mouse button onto **Properties**.

9 Within the Properties select **Internet Protocol Version 4**.

10 Select **Use the following IP address**.

11 Enter the following information:

- IP Adress: 192.168.254.10
- Subnet Mask: 255.255.255.0

12 Select **Ok** to finish.

13 Close remaining windows.

You may now proceed switching on the Agilent 3100 OFFGEL Fractionator and open the Internet Explorer on the control PC. Type the IP address 192.168.254.11 to view the web based user interface. See Chapter 5, “The User Interface,” starting on page 40 for the description of the interface.

NOTE A short description on how to set up the instrument and computer is given in a Quick Guide (**G3100-90111**) shipped with the instrument.

NOTE Do connect the PC only to the 3100 OFFGEL fractionator. Connecting the PC with this manually set IP address to your local area network will most likely create network issues.

Local Network Connection (optional)

For connecting the 3100 OFFGEL fractionator to a local network request a static IP address, subnet mask and gateway adress from your network administrator.

Although it is intended to use the 3100 OFFGEL fractionator in a local network at a later point in time you have to access the instrument first with the default IP address (192.168.254.11). The steps for this are given under “Assignment of an IP Address to the Control Computer” on page 36 and “Setting up the Agilent 3100 OFFGEL Fractionator” on page 34.

When a local LAN connection via Cross-Over LAN cable between control PC and 3100 OFFGEL fractionator is established you can set the provided values for static IP address, subnet mask and gateway address under **Config** of the

web-based User Interface. Please see the description of the web-based User Interface in Chapter 5, "The User Interface" starting on page 40 and find addition information on the Instrument Configuration on page 62.

After you have set the custom IP address, subnet mask and gateway address, do the following steps:

1 Power the instrument off.

2 Install a conventional LAN cable from the instrument to your local network.

3 Reconfigure the instrument to use the customer set IP address by changing the configuration dip-switches (Figure 7 on page 34).

   Put switch 7 up and all other configuration dip-switches down.

4 Power the instrument on again.

The instrument will be accessible now through your local network by typing the IP address into the address line of you browser.

NOTE Note down the initial Network configuration of the PC for your own reference.

NOTE Since the instrument is not enabled for DHCP the assigned custom IP address must be a static IP address.

5 Operation

This chapter details how to operate the Agilent 3100 OFFGEL Fractionator. It familiarizes the user with all control functions from the internet browser interface. It describes the necessary steps to perform a fractionation and to change the customizable IP address of the instrument.

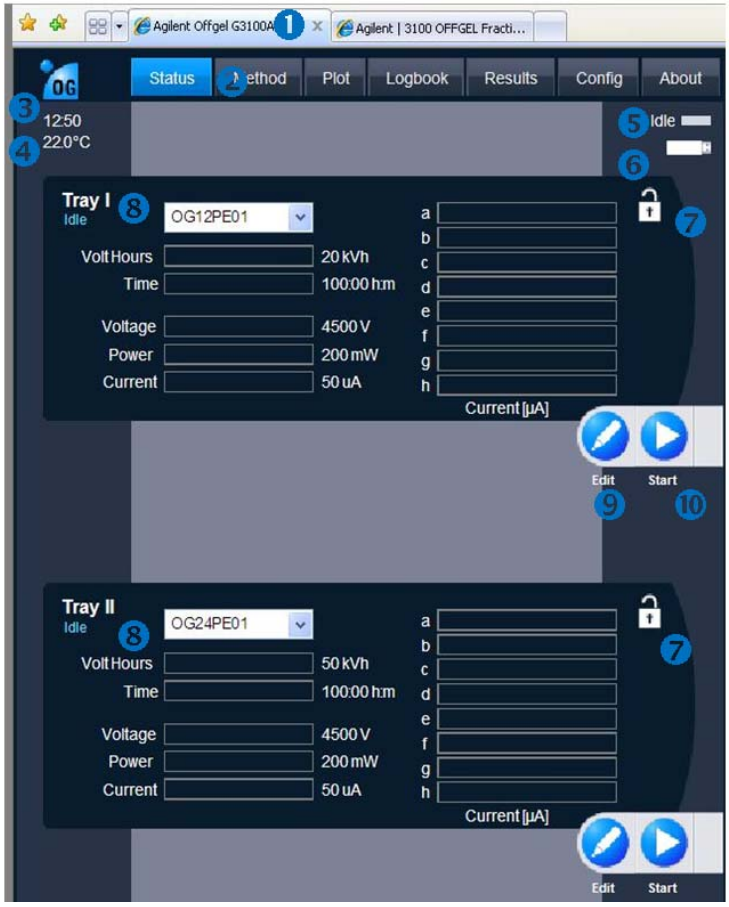
The User Interface

The Agilent 3100 OFFGEL Fractionator can be controlled from an internet browser interface. The communication of browser application and the instrument is based on the display of webpage content retrieved from the instrument. Please see Chapter 4 for establishing the LAN connection. Switch your instrument on and type its IP address (default IP address: 192.168.254.11) into the address line of your browser to display the user interface.

The user interface provides several tabs to control the instrument, see also (Figure 9 on page 41):

- **Status**, which is also the application start screen, shows the current state of both trays. You can select methods to be used, start and stop processing and modify used parameters interactively in this view.
- **Method** screen is used to view, modify and store methods. It shows all method parameters and a graphical view of the timetable settings.
- **Plot** screen allows to view actual over time in a XY plot. You can select up to 4 signals. The XY plot allows to zoom in and out and to pan the plot area.
- **Logbook** shows a time ordered list of the occurred events since last start of the instrument.
- **Result** view allows to download result files from fractionations using the browser download function and also to copy result files of a time span to a connected USB Memory stick inserted at the rear of the instrument.
- **Config** screen allows to setup/view LAN configuration. You can trigger module identification by flashing the control LED at the connected Agilent 3100 OFFGEL Fractionator.
- **About** page shows the revision information of the firmware and links to helpful Agilent web pages. Here you find copyright information of the Webinterface and the used open source Javascript libraries.

You can click on the tabs in order to see the different screens. When data, such as method parameters were not save they might disappear upon leaving the tab.



1 Web Browser Tab
2 OFFGEL interface tabs
3 Instrument clock
4 Platform temperature
5 Status indicator
6 USB indicator
7 Application Lock
8 Tray method
9 Method edit
10 Fractionation start

Figure 9 Overview on Agilent 3100 OFFGEL Fractionator interface.

General Functions

A few elements of the screen shown in Figure 9 on page 41 are common to many tabs. Besides the Status indicator and the USB indicator this are the **Instrument Clock** and the **Platform-Temperature**. You can adjust these parameters by clicking onto this area. The dialog shown in figure 5 appears and will allow you to set the date, time and the platform temperature.

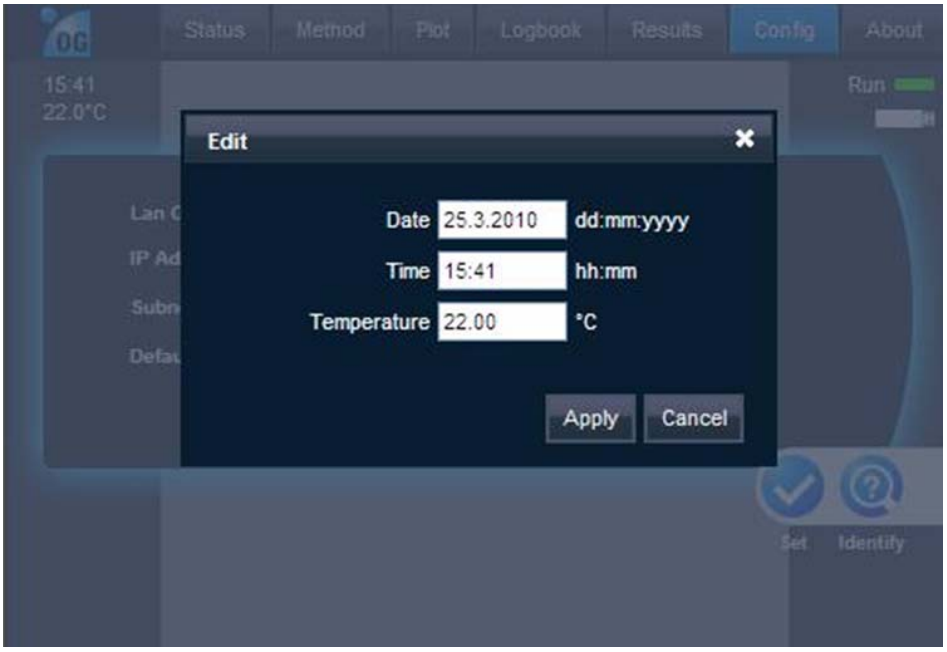
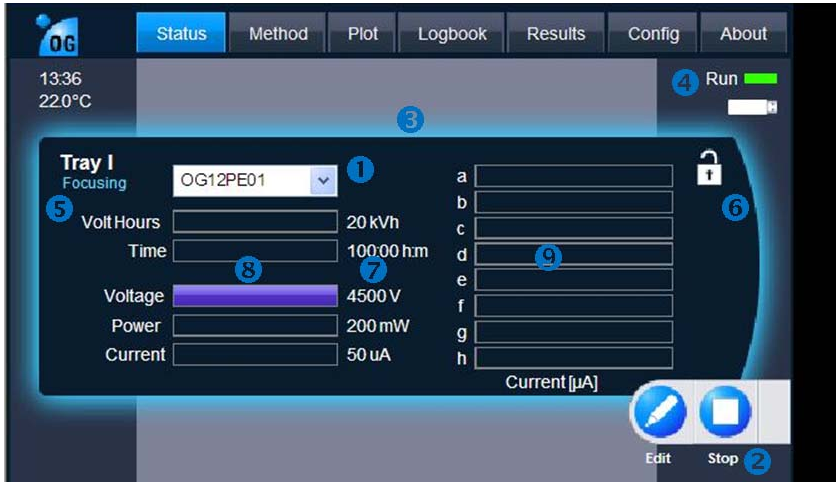


Figure 10 Tray temperature and system clock

NOTE The temperature applies to both trays.

Status tab

The Status tab shows the current state of both trays. Figure 11 shows Tray 1 in a run. The Status tab is also the main application screen which enables you to select and load methods, to start and stop them. The Status tab allows processing and modification of run parameters interactively of a method that is currently running.



1 Method selection
2 Start/Stop button
3 Tray corona
4 Status indicator
5 Method step
6 Instrument lock
7 Run parameters
8 Actual values
9 Actuals per lane

Figure 11 Status Screen for Tray I

In a pull down menu for **Method Selection** you can select methods that are present on the internal drive of the instrument or on the USB stick inserted at the rear of the instrument. These methods can either be default methods which were supplied with the instrument or user modified methods. See Chapter 5, page 65 for description of the default methods. Method modification and saving can be done from the Method-tab.

The fractionation can be started after assembly of the tray with the **start button**. This button changes subsequently to be the stop button. A fractionation will start only if the instrument is ready, for example if the platform temperature for the given method is reached. Readiness is given by the **status indicator**. An idle instrument will start immediately the

fractionation. Not ready indication on for example tray temperature will lead to a waiting step. An open lid will cause the not ready information and application of the voltage is done only if the lid is finally closed, see also Table 8 on page 92. The active Run status for each tray is additionally indicated by a blue **tray corona** around the respective tray-shaped interface.

An Agilent 3100 OFFGEL Fractionators method generally consist out of two **method steps** which are the focusing and the hold phase. Methods are described in more detail under "Working with Methods" on page 46. The run parameter shown in the status screen are the specified limits for volt hours, time, voltage, power and current of the loaded method. The bar graph on the left side visualizes the **actual values** online. The bar size is in relation to the set point; in addition, the numeric value is displayed by a tool tip if the mouse pointer is place over the respective bar. The bar graph labeled with letters from a to h on the right side visualizes the current measured as **actual per lane** for each strip online. Click into the lane area to swap from displaying Current in µA to Charge in mAh.

Modification of a currently loaded method can be done by clicking onto the **Edit** button or by clicking into the respective parameter area of a tray. A dialog will appear and allows changing the parameter of the current method step, to directly apply it to the ongoing fractionation or to cancel this attempt (see Figure 12 on page 44). Upon modification an asterisk (*) occurs next to the method name and denotes that the method was modified during the run.

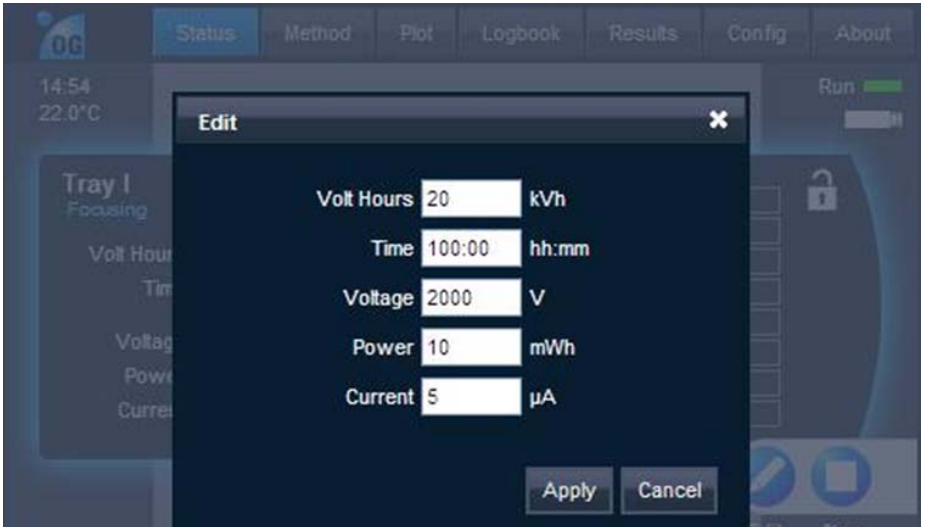


Figure 12 Edit a current method

The instrument is accessible from any computer in the local network. To protect it from unintended access an **Instrument lock** can be established. Click onto the lock icon and select a password. You might want to leave information for other users or a password hint for yourself in a free editable field. The password can be set individually per tray. Since it is transient it can be different from one locking event to another. In case of misplaced or forgotten passwords please power cycle the instrument after the current runs are finished to release the lock.

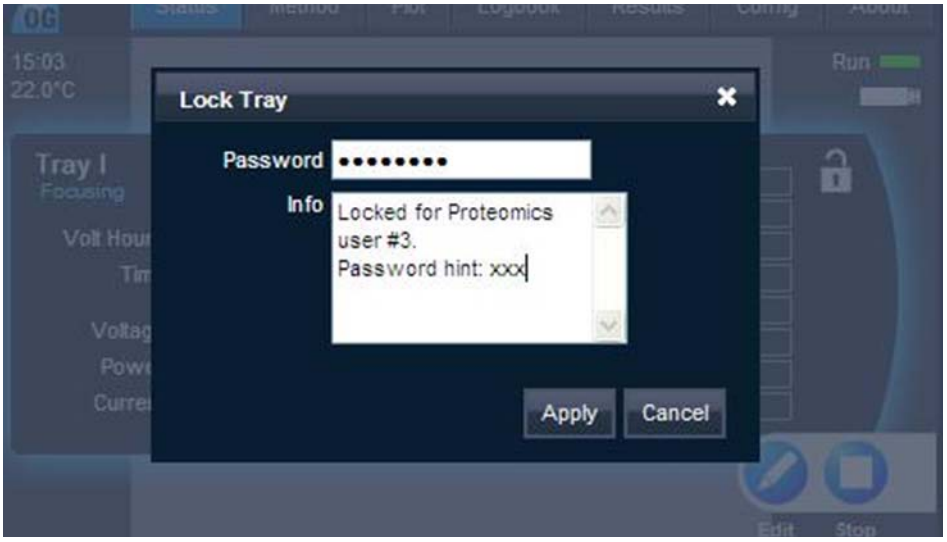


Figure 13 Locking a tray

Working with Methods

The Method tab (see Figure 14) is used to view, modify and store methods. It also shows all method parameters and a graphical view of the timetable settings. In opposite to the edit options from the status tab which reflects an online editing mode, the Method tab allows offline editing and storing. Changes that are made are not directly applied to a currently running fractionation. If you want to permanently change a method, you work in the method tab/offline mode and save a modified default method under a different file name.

1 Method selection
2 Parameter per step
3 Timtable graphic
4 Parameter in Timetable
5 Addition of Timetable lines
6 Save/Delete options

Figure 14 Editing Methods

In a pull down menu for **Method Selection** you can select methods that are present on the internal drive of the instrument or on the USB stick inserted at the rear of the instrument. These methods can either be default methods which were supplied with the instrument or user modified methods. A 3100 OFFGEL fractionators method generally consist out of two method steps which are the focusing and the hold phase. The methods do not contain the platform temperature as parameter. During the focusing step, the sample fractionation is taking place, typically at higher voltages, until a given Volt Hour value (kVh) is reached or a given Time (h:mm) has elapsed. The hold step is typically done at lower voltages after the fractionation just to maintain the achieved separation until the fractions are recovered from the wells.

The Volt Hour value (kVh) or Time (h:mm) are **limiting parameters** for the duration of the fractionation. The default methods have values that were found to be suitable for a broad range of applications and samples. The parameters Voltage, Current and Power are by Ohms Law in a correlation for a given fractionation in an individual strip/lane. The Method parameters Voltage, Current and Power are limiting factors during a run. One of the parameters, typically current, is at the limit.

NOTE Please save modifications before changing to another tab. Changes will be lost otherwise.

The focus step of a method can be sub-devided into different time based steps if desired. Create a time based program using the lower part of the method tab. A **Timetable** for the parameters Voltage, Current and Power is visualized in a **graphical** way with color coding per value. In case time table entries are done the parameters set above are used as starting values at time 0:00. Select the desired parameter, for example voltage, in the timetable pane from a drop down menu. Set the value in the adjacent field. Changing from one value setting to another one at a second timepoint can be done as one step or applied as a linear gradient. The point in time when this change should happen during the method execution can be given. Press the **Add** button to add more method steps. You also can edit any time table entry by highlighting it. By selecting one line, the corresponding values get populated in the parameter line. After modifying values you can apply them to the method with the **Set** button. The platform temperature is no method parameter and cannot be changed in the timetable. For changing platform temperature, please see page 42.

Printing of methods is typically possible and is based on the browser features. Select the print button of the browser or select the print dialog under the browser file menu. Selected elements of the OFFGEL method, such as the values for focussing and hold phase, the graphical display of the timetable and the time table will be printed.

NOTE Please note that Voltage setting beyond 4500 kV may lead to increased arcing under certain experimental conditions. Such conditions are dried pads which can cause burnt spots in the tray. Default methods are therefore limited to 4500 kV.

Functions in the method pane are:

- **Delete** : Removes the method from the instrument. Only user defined methods can be deleted.
- **Save** : Stores the shown setpoints in the currently selected method.
- **Save As:** Opens a file dialog that shows the currently existing methods and allows the user to specify a new method name to store the setpoints to.

Functions in the timetable pane are:

- **Set**: Takes over the set values for the currently selected point in the timetable graph.
- **Add**: Uses the set values to create a new point in the timetable.
- **Delete**: Removes the currently selected point from the time table.

NOTE Factory installed default methods cannot be changed or deleted. Please save changed method under a different name.

Default methods on the internal disk are part of the system and cannot be deleted.

NOTE A method that is currently loaded to one of the trays or is being executed cannot be deleted from the method list.

Displaying Data Graphically

The plot tab (Figure 15) allows viewing up to 4 signals which can be Volt, Volt Hours, Voltage Limit, Current Limit and Current per lane. The signals are available from the Signal Selection Menu. The scaling source of the Y-Axis is selectable by clicking onto the data series in the Graph area or reselecting it from the menu. The view area can be zoomed by clicking to the graph area of interest and using the mouse wheel. Clicking into the graph area and dragging allows moving the zoomed area to another section. Unzoom is available under Display Options. A stacked and overlay view onto the signal is possible as well as a switch between Tray I and Tray II.

1 Signal selection
2 Current Y-Axis
3 Signal/Units Y-Axis
4 Graph area
5 Display options

Figure 15 Plot of Signals

Zoom in Plots

Using a PC with a mouse connected, the mouse wheel can be used for zooming. Both axes scales can be changed independent from each other. The mouse wheel input will change Y-Scale as long as the mouse pointer is located nearby the Y-Axis inside the graph area. If the mouse pointer is anywhere else within the graph area the X-Scale will be changed.

Alternatively the arrow buttons in combination with control button from the control PC keyboard can be used for zooming in the plot area. Left arrow together with control button allows zooming in X-axis; right arrow together with control button allows zooming out X-axis; up arrow together with control button allows zooming in Y-axis; down arrow together with control button allows zooming out the Y-axis. The Y-axis is zoomed starting in the middle of the plot and the X-axis from the left of the plot. During zooming with arrows the cursor must be set over the graph area. The minimum time scale span in zoomed views is 20 minutes.

The **unzoom function** resets the plot scaling to the initial values which are defined by the button overlay/stacked for the Y-axis and a 2 hours time frame for the X-Axis.

NOTE Using the the iPod the pinch gesture is used to zoom the plot area.

Pan / Drag in Plots

On the PC the graph area is panned by pressing the left mouse button while the mouse is located in the plot area, moving the mouse and releasing it. Panning is performed separate for X and Y direction depending on which movement is larger.

NOTE Using the iPod the panning is performed by a two finger drag within the plot area.

Switching the Y-Scale in Plots

The source for the Y-Axis scaling can be changed from one to another signal by clicking onto the colored line of the signal in the graph area. The color of the y-Axis will change to reflect the color of the signal it is related to. Once a signal is loaded freshly the Y-axis will use this as source for scaling.

The stacked and overlay view in the plots specifies the arrangement of the signals on the plot pane. Stacked display allows visualization of signals with four individual offsets on the axis, while overlay has the same offset for all signals. Signals can mask each other when overlayed.

Printing of Plots

Printing of signal plots is typically possible and is based on the browser features. For printing of signal plots select the print button of the browser or select the Print dialog under the browser file menu. Selected elements of the OFFGEL interface, such as the graph of the selected signals, will be printed.

Instrument Logbook

The Logbook shows events occurred on the instrument. The list consists of all events since the last instrument start. It can be printed by selecting the **print** button or the print dialog of the browser.

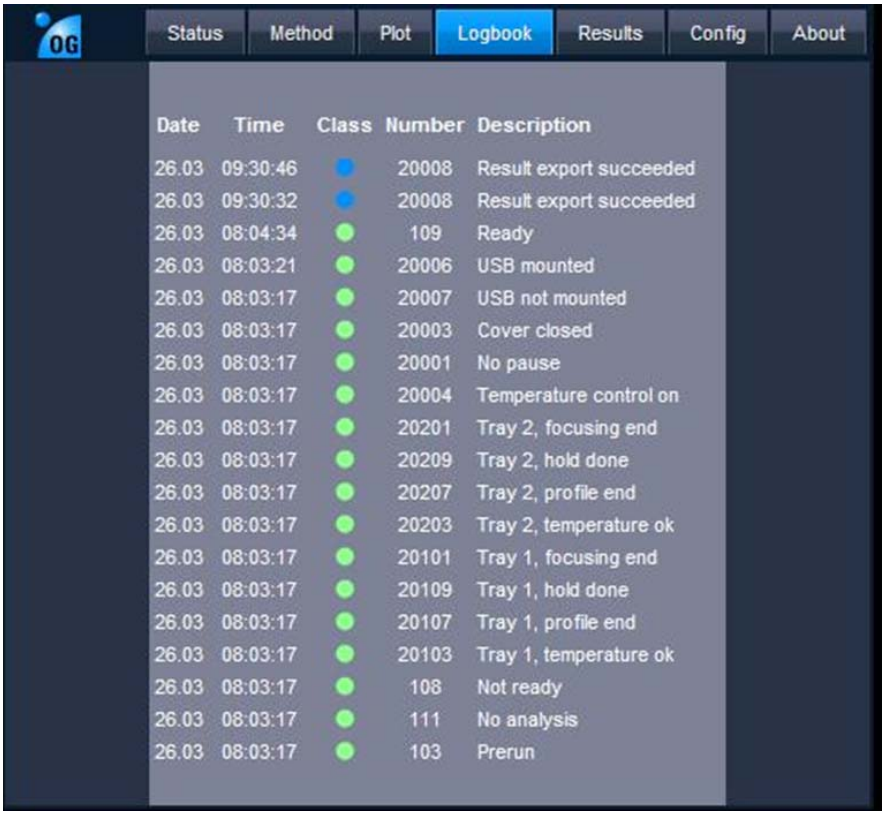


Figure 16 Instrument Logbook

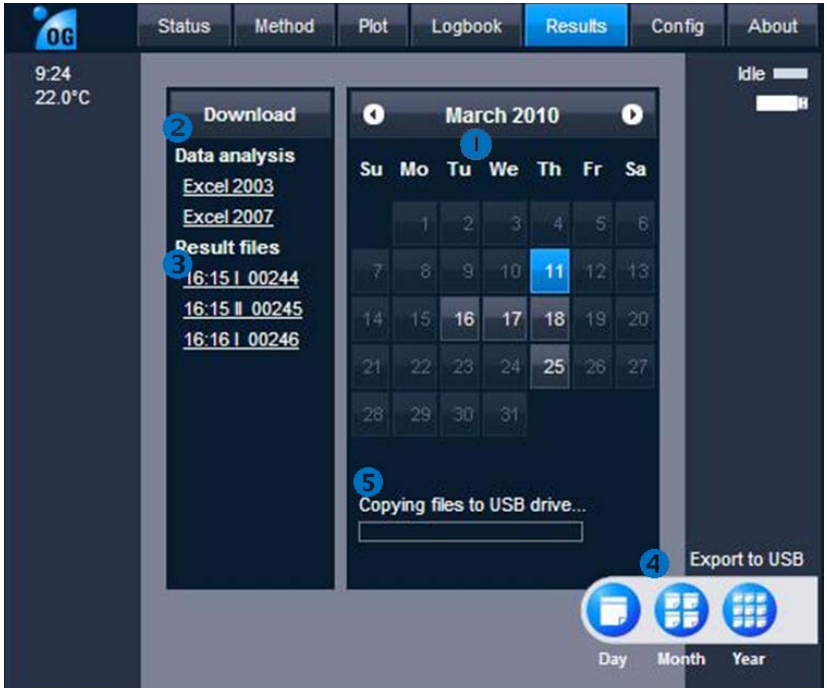
The log book entries consist of date and time for an event on the instrument. Each event is a ssigned a color-coded class:

- Green – change in status
- Blue – info event
- Red – error

An internal identification code serves as detailed information for the Agilent Service. A brief description of the detected events is given in the last column.

Exporting Results

The Result tab allows downloading result files from fractionation runs stored in the internal memory. This can be done per individual file by using the browser download function on the results tab. Also a copy of result files from a time span to a USB Memory stick inserted at the rear of the instrument can be done.



1 Results Calendar
2 Macro Download
3 Result files Download
4 USB export selection
5 USB export progress

Figure 17 Results from fractionations

In the **Results Calendar** you can select the month and day when results were created. Clicking onto a date populates the files available for this selection. You can download the Agilent 3100 OFFGEL Fractionator plotter for data analysis by Microsoft Excel at any date containing data files. Under Data

analysis is a link for **Macro Download** of the two versions for Microsoft Office 2003 or 2007 available. Next to this you find the links for a **Result file download**.

Under operating system Windows 7 with Internet Explorer 8 a download of result file is done best by a right-click onto the respective download link and selection of the option "Save Target As... ".

A double click will open the result file, which is comma separated values for electrical data in an Internet Explorer window. It is advantageous to save a file first, open and analyze the result file by using the macro from within Microsoft Excel (see Chapter 7 on page 105).

NOTE The download mechanism from the browser Internet Explorer is not available on the iPod.

A different way to access and analyze the result files is to copy them to a connected USB stick. To use this function select a day, month or year by clicking with the mouse to a day within the desired period. Click for example on "Day" at the **USB Export selection**. This will export or copy all files of the selected day to the USB stick. Pressing "Month" copies all of the currently selected month and "Year" all files of the selected year. The progress of copying is shown in the **USB progress bar**.

The compact flash card which is inserted at the rear of the instrument has a limited capacity. It can store results from a period up to approximately 3 years. The storage capacity is dependent on the file size and is thus method dependent. When the compact flash card is full, the oldest data file is overwritten automatically. Therefore old files might become unavailable. It is recommended to archive result files on a different location.

NOTE Since USB memory sticks may vary from vendor to vendor or from type to type, incompatibilities can occur. In general, USB-memory sticks from Sandisk and Kingston were used with success. The USB- memory sticks must be FAT-16 formatted and without encryption, maximum size is 2 GB. See "Firmware Change via USB Stick" on page 88.

A compatible USB stick is included with the shipment.

NOTE

In case the instrument detects an USB-memory stick which is incompatible due to the formatting it will try to format memory to FAT-16. Please be aware that any information present on the stick will be lost.

Reviewing Result Files

Result files are either transferred to your control PC by direct download using the link from the result tab and the browser function “Save Target as...” or they were transferred to the USB stick mounted to the 3100 OFFGEL fractionators. In order to review them it is reasonable to store them in a typical location of the Computer used for reviewing them such as under “MyDocuments”.

How to find the exported files on the USB stick

When exporting results or transferring methods to an USB stick, the following directories are generated:

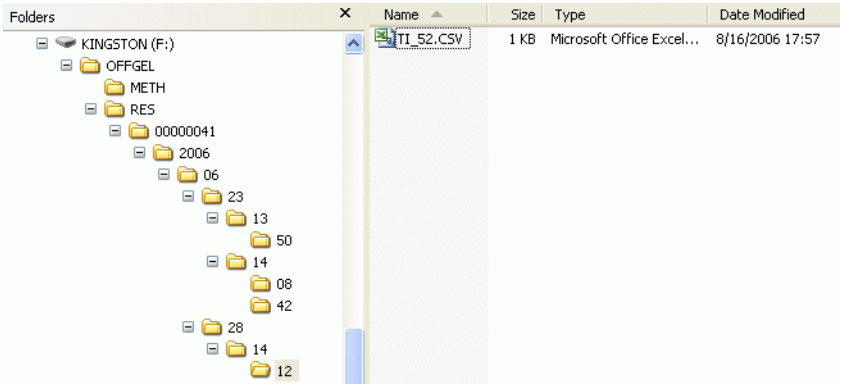


Figure 18 Folder directories on the USB stick

The METH folder contains all exported methods. The RES folder contains all exported results. The subdirectory of the RES folder starts with an instrument folder in the name of the instrument serial number. The following directories follow the structure of the export menu: year, month, day, hour, minute (Figure 17 on page 54). The .csv file in the minute folder finally contains all run parameters of a fractionation.

How to Display Exported Results

The results of all run parameters can be exported as comma separated value (.csv) file and thus can be visualized with Microsoft Excel. Agilent Technologies has designed the Agilent 3100 OFFGEL fractionator plotter which is an Excel add-on and facilitates the analysis of the run parameters. The tool is downloadable from the instrument in revisions for Microsoft Excel 2003 and 2007. The tool is also provided via the Agilent Technologies web page http://www.agilent.com (Life Sciences and Chemical Analysis Solutions - Technical Support - Downloads & Utilities) or direct via http://www.chem.agilent.com/scripts/DownloadsUtilities.asp and can be downloaded free of charge.

NOTE Note that the tool was designed for Microsoft Excel 2003 and 2007 with US-American system local settings only and has not been tested with other versions of Microsoft Excel. However, it is expected that the tool also runs under international versions and international system local settings.

1. In Microsoft Excel 2003, open the downloaded file **Agilent 3100 OFFGEL Fractionator.xla**. You may also want to copy the file in the XLSTART folder (for office 2003 it can be C:\Program Files\Microsoft Office\OFFICE11\XLSTART) so that the program is loaded automatically when Excel is started.
2. Click **Enable Macros** when the security warning window pops up.

NOTE If you cannot run the program, check that Excel security settings under tools>macro>security allow macros to be run.

The following button is generated in your Microsoft Excel 2003 tool bar or is available under Add-Ins/Custom Toolbars for Microsoft Excel 2007:

Figure 19 Agilent 3100 OFFGEL Fractionator toolbar button.

3 Click on the Agilent 3100 OFFGEL Fractionator toolbar button to activate the following user interface:

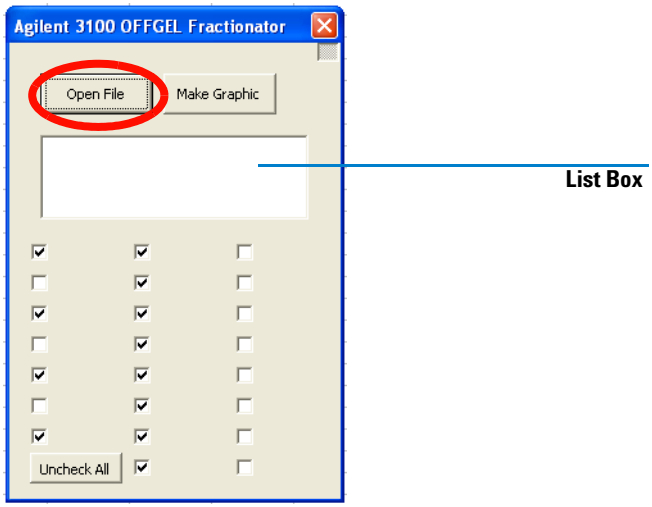


Figure 20 Agilent 3100 OFFGEL Fractionator user interface.

NOTE You can close the Agilent 3100 OFFGEL Fractionator user interface by clicking the close window button. You can reopen it by clicking the Agilent 3100 OFFGEL Fractionator toolbar button.

4 Click **Open File**, select an exported file with the browser and open the file.

NOTE The file must be an unchanged Agilent 3100 OFFGEL Fractionator .csv file.

5 The file name of the loaded file appears in the Agilent 3100 OFFGEL Fractionator list box (Figure 21)

NOTE Multiple files can be loaded simultaneously. You can toggle between them by clicking on the file name.

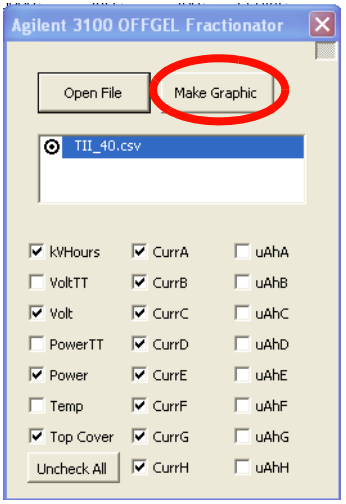


Figure 21 Agilent 3100 OFFGEL Fractionator user interface - result file opened.

6 Select all run parameters which should be plotted.

7 Click **Make Graphic**. The plot is displayed on a separate worksheet

File name/Run at / Instrument Serial Number with Tray

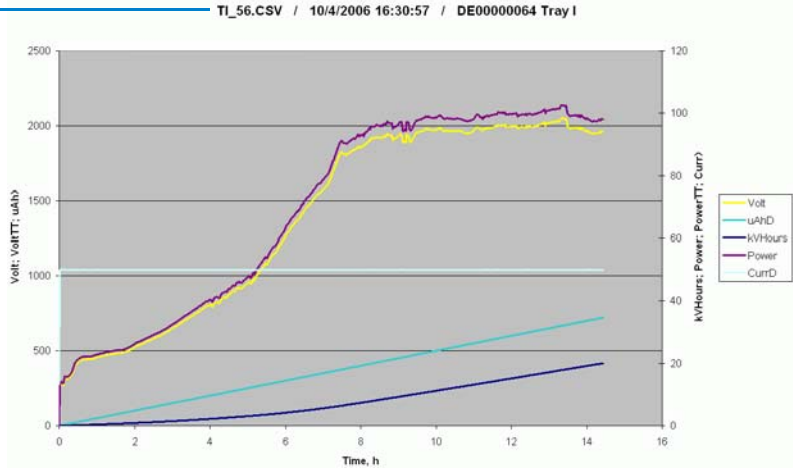


Figure 22 Plotted graph of run parameters (y-axes) versus time [h].

NOTE If a worksheet is manually deleted or the data file is reloaded, the corresponding graph is deleted. If a plot to a certain worksheet is repeatedly generated, the previous plot version is deleted.

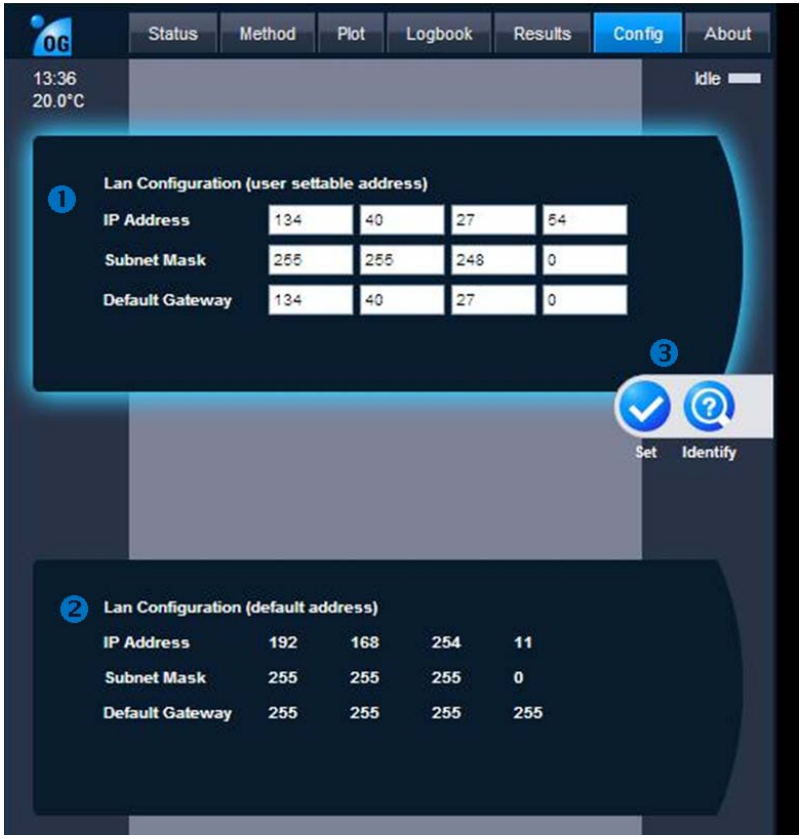
Table 5 gives details of the different run parameters:

Table 5 Run parameters

Run Parameter	Description
VoltTT, PowerTT	Fixed settings of Volt and Power of the method that was used.
kVHours, Volt, Power	kVHours, Volt, Power measured during the fractionation for the complete tray.
Temp	Temperature of the cooling platform during the fractionation.
Top Cover	The events or time intervals when the top cover was opened during a fractionation are marked with the red diamond symbol on the X-axis. If there are no Top Cover events registered, the Top Cover check box is automatically unchecked.
CurrA, CurrB, CurrC, ...	Currents measured for individual tray lanes (A-H).
µAhA, µAhB, µAhC, ...	Measured µA-hours acquired for individual tray lanes (A-H).

Instrument Configuration

The Configuration tab (Figure 23) allows to setup and to review LAN configuration of the instrument. The active IP address that is currently in use is highlighted by a blue corona. On this tab you can also trigger module identification.



1 User LAN Configuration
2 Default LAN configuration
3 Set/Identify

Figure 23 LAN Configuration

The Agilent 3100 OFFGEL Fractionator has two LAN configurations stored. They can be used alternatively. The selection which of the stored LAN configurations is used upon the next instrument start is done by configuration dip-switches at the rear of the instrument. Any change in the LAN configuration is applied only **after** the instrument has been restarted.

Configuration dip-switches 7 and 8 up, while all other switches are down, configures the instrument to start with the default IP address 192.168.254.11.

Only configuration dip-switches 7 up, while all other switches are down, configures the instrument to start with the user settable static IP address.

A **User LAN Configuration** with a settable address is visible in the upper panel (Figure10). The user defined address must be a static IP address. DHCP (Dynamic Host Configuration Protocol) is not supported. The user settable address can be edited by clicking into the fields adjacent to the IP Address, Subnet Mask and Default Gateway. A click onto the button **Set** stores the entered values to the connected Agilent 3100 OFFGEL Fractionator.

A **Default LAN Configuration** with default address settings, which cannot be changed by the user, is present in the lower panel of the configuration tab.

NOTE

Please see instructions for first time connection of the Agilent 3100 OFFGEL Fractionators to an instrument on page 31, Chapter 4.

Please note that any configuration change will be active only after an instrument restart.

On the Configuration tab you can also trigger module identification. A click onto the button **Identify** sends a command to the connected Agilent 3100 OFFGEL Fractionator to let the control LED flash for a few seconds in all three colors.

Additional Information

The About page provides information on the revision of the currently active firmware of the connected instrument. It provides links to helpful Agilent web pages such as the Agilent 3100 OFFGEL Fractionator for latest news on the instrument or to a download location for the firmware update tool. Here you find copyright information of the Webinterface and the used open source Javascript libraries. For service purposes the diagnosis buffer can be downloaded here. Provide the diagnosis buffer together with result files to you local Agilent customer care center in case of serious issues.

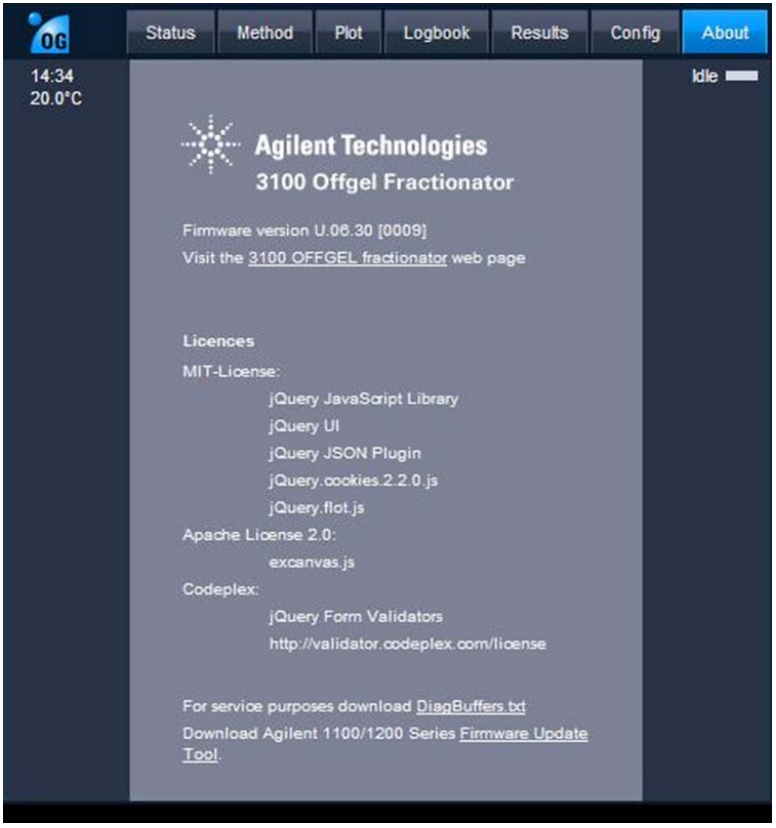


Figure 24 About the Agilent 3100 OFFGEL Fractionator

Performing a Fractionation

Fractionations using the Agilent 3100 OFFGEL Fractionator can be performed in the OFFGEL mode or in the standard IPG IEF mode (in-gel mode).

The table lists the default methods delivered with the system and ready to use with a cooling platform temperature of 20°C

Table 6 Default methods

Method name	recommended use
OG24PR01	OFFGEL default method for proteins for use with 3100 OFFGEL High Res Kit, pH 3-10, 24 well frames OR for use with 3100 OFFGEL High Res Kit, pH 4-7, 24 well frame
OG24PE01	OFFGEL default method for peptides for use with 3100 OFFGEL High Res Kit, pH 3-10, 24 well frames OR for use with 3100 OFFGEL High Res Kit, pH 4-7, 24 well frame
OG12PR01	OFFGEL default method for proteins for use with 3100 OFFGEL Low Res Kit, pH 3-10, 12 well frames OR for use with 3100 OFFGEL Low Res Kit, pH 4-7, 12 well frame
OG12PE01	OFFGEL default method for peptides for use with 3100 OFFGEL Low Res Kit, pH 3-10, 12 well frames OR for use with 3100 OFFGEL Low Res Kit, pH 4-7, 12 well frame
IG_24_00	IPG-IEF in-gel default method for protein and peptides for use with 24 cm IPG-strips pH 3-10 OR pH 4-7.
IG_13_00	IPG-IEF in-gel default method for protein and peptides for use with 13 cm IPG-strips pH 3-10 OR pH 4-7.
DIAGNOSE	use for hardware diagnosis as described in "Checking High Voltage, Electrodes and Electrode Connectors" on page 97.

NOTE Please note that latest OFFGEL methods do have a voltage limit of 4500 V. This voltage limit reduces the risk of arcing and damage to the tray in case of dried out electrode pads.

Preparing a Tray for the OFFGEL Mode

To prepare a tray for the OFFGEL mode, refer to the *Kit Manual* (5969-1582) for detailed instructions. The manual for OFFGEL fractionation is included in every consumable kit (see Table 1 on page 19).

Preparing a Tray for the Standard IPG IEF Mode

You can use any IPG-strip for fractionations in the standard IPG IEF mode (in-gel mode). Follow the recommendations of the IPG-strip supplier about rehydration loading of the sample to the IPG-strip. The tray of the Agilent 3100 OFFGEL Fractionator system can be used as a rehydration tray.

The following workflow uses an IPG-strip already loaded with sample and describes an example of the tray preparation for one IPG strip.

1. Place the IPG-strip in a new tray lane with the gel side up. The low pH side (Anode), marked with a “+”, must be at the left, where the fixed electrode will be attached.
2. Take two electrode pads with tweezers, wet them with rehydration buffer, and place one at each end of the IPG-strip so that it connects to the swelled gel.
3. Attach the electrodes to the tray exactly as described in the OFFGEL *Kit Manual* (5969-1582). If necessary, reposition the IPG-strip with the electrode pads so that the fixed electrode connects to the left electrode pad.
4. Place the tray into the instrument.
5. Cover the complete tray lane with mineral oil using 1.5 ml mineral oil per IPG-strip.

NOTE Distribute the mineral oil evenly across the whole tray lane by pipetting droplets across the lane. Make sure that no mineral oil is spilled out of the tray.

6 Start the run as described in "Starting a Run" on page 68.

Starting a Run

1 To start the fractionation, make sure you have configured the cooling platform according to your needs. For details, refer to “General Functions” on page 42.

2 From the Status screen of the connected instrument select the Method in the drop down menu of the tray you will use. The run parameters of the loaded method are visible on status screen.

3 Press the Start button to start the fractionation.

NOTE

You will be able to monitor the voltage applied and the current flowing through the individual lanes from the status page. It is recommended to monitor the startup phase in order to correct for example for missing connectivity.

You can protect the instrument from an unwanted access of a third user by the lock function (see **Instrument lock** on page 45).

NOTE

A blue light below the anode connector of the respective tray indicates that the instrument is running. A blinking blue light indicates that the fractionation is finished.

Both trays can be used independently. If the top cover is opened, the fractionation pauses until the top cover is closed again.

4 Upon finished fractionation you can stop the run and retrieve the fractions.

6 Maintenance

This chapter describes cleaning procedures that should be used to do maintenance on your instrument at regular intervals or before every use. In addition, it provides instructions on how to update the firmware. Only perform maintenance on the Agilent 3100 OFFGEL Fractionator as specified in this chapter. Other maintenance and repairs must be done by Agilent Technologies trained personnel. Unauthorized maintenance can be dangerous and damage is not covered by warranty.

Cleaning the Agilent 3100 OFFGEL Fractionator

Materials used in the instrument that may come in contact with liquid reagents are resistant to chemicals typically used for isoelectric focusing (IEF).

WARNING

Always turn off the instrument and unplug the power cord before doing any cleaning procedures.

Always wear powder-free gloves when handling parts that are in contact with sample or mineral oil.

CAUTION

Do not use ketones, such as acetone, or strong acids to clean the instrument

Cleaning the Electrodes

Electrodes can be used multiple times. They are designed to be robust but might suffer from permanent coating of the surface and from arcing events during fractionations.

Regular Cleaning Procedure

Both the fixed and the movable electrodes have to be cleaned before every use.

1. Wipe the electrode pins with water using a lint-free tissue.
2. Wipe the electrode pins with isopropanol using a lint-free tissue to remove the mineral oil.
3. Thoroughly air dry the electrodes or dry well with a lint-free tissue prior to use.

Cleaning Procedure for Heavily Contaminated Electrodes

If for any reason not only the electrode pins but the complete electrodes are contaminated use the following cleaning procedure:

1 Rinse the complete electrodes with hand-warm tap water. Do not exceed a water temperature of 50°C.
2 Use a mild detergent such as dishwashing detergent or a 10% SDS solution and a brush to clean the electrodes.
3 Carefully rinse the electrodes with hand-warm tap water to remove the detergent.
4 Carefully rinse the electrodes with distilled water to remove remaining salts.
5 Thoroughly air dry the electrodes prior to use to avoid any electrical short circuit.

CAUTION Do not use heat such as a hair dryer to dry the electrodes since the plastic parts of the electrodes may be deformed.

CAUTION Do not use a dishwasher to clean the electrodes since the plastic parts of the electrodes may be deformed or corrosion due to alkaline conditions might occure.

NOTE Additional electrodes are also available as spare parts see Table 15 on page 156

Cleaning the Trays

Trays have to be cleaned before every use to avoid cross-contamination of protein or peptides from one sample to the other. This is especially necessary when the instrument is used in the IPG IEF mode (in-gel mode).

1 Rinse the complete tray with hand-warm tap water. Do not exceed a water temperature of 50°C.
2 Use a mild detergent or 10% SDS solution and a brush to clean the tray.
3 Carefully rinse the tray with hand-warm tap water to remove the detergent.
4 Carefully rinse the tray with distilled water to remove remaining salts.
5 Thoroughly air dry the tray or dry well with a lint-free tissue prior to use.

CAUTION Do not use heat such as a hair dryer or dish washer to clean and dry the trays since the material may be deformed.

NOTE Additional trays are also available as spare parts see Table 15 on page 156.

Cleaning the Cooling Platform

If for any reason the cooling platform is contaminated, use the following cleaning procedure:

1. Wipe the cooling platform with water using a lab tissue or paper towel.
2. Wipe the cooling platform with isopropanol using a lab tissue or paper towel to remove the mineral oil.
3. Thoroughly air dry the cooling platform or dry well with a lab tissue or paper towel prior to the next fractionation.

NOTE A leak sensor is installed on the left side of the rear of the cooling platform. The sensor and its surroundings has to be completely dry before it is possible to start the next fractionation.

Cleaning the Anode Connector

If you see heavy contamination of the fixed electrode, such as salts or mineral oil, it is strongly recommended to also detach and clean the anode connector.

1 Loosen the 4 screws on top of the anode connector with a hex key.

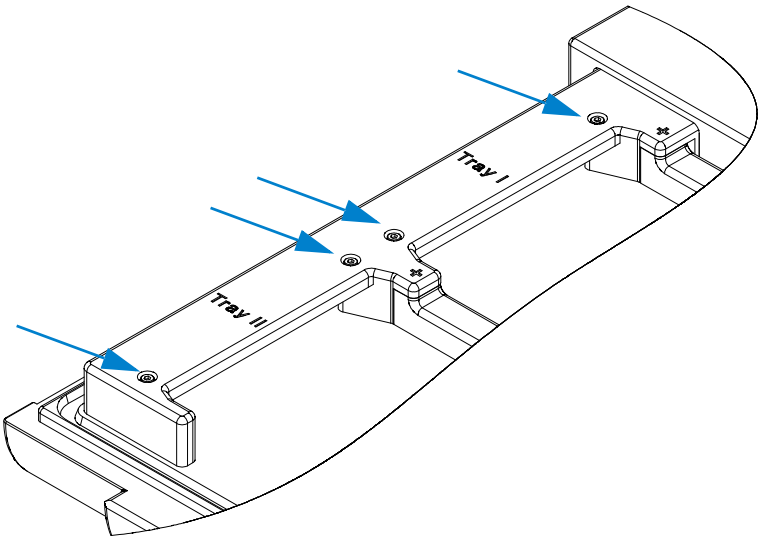


Figure 25 Disassembling the anode connector.

2 Carefully lift the anode connector from the front (Figure 26). Take care to not damage the cables at the rear of the anode connector.

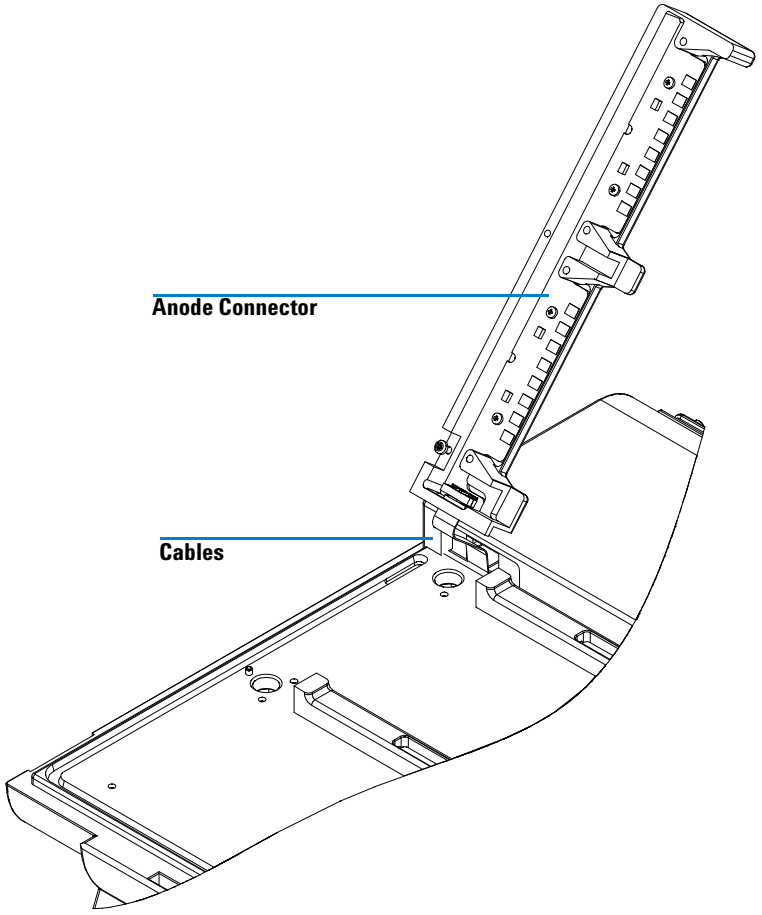


Figure 26 Detached anode connector.

3 Wipe the anode connector with water using a lab tissue or paper towel.

4 Wipe the anode connector with isopropanol using a lab tissue or paper towel to remove the mineral oil.

5 Thoroughly air dry the anode connector or dry well with a lab tissue or paper towel prior to the next fractionation.

6 Return the anode connector to its original position and fasten the screws with a hex key.

Cleaning the Top Cover and the Housing of the Instrument

The top cover and the housing can be cleaned with a damp cloth either using water or a mild detergent.

CAUTION Do not use alcohols or ketones for the top cover, since the material is not resistant to these solvents.

Firmware Update

At regular intervals, Agilent Technologies releases new firmware revisions of its products to provide new features or bug fixes. These revisions and the Firmware Update Tools are provided via the Agilent Technologies web page http://www.agilent.com (Life Science and Chemical Analysis Solutions - Technical Support - Downloads & Utilities) or direct via http://www.chem.agilent.com/scripts/DownloadsUtilities.asp.

A link to this page is provided on the About page of the connected instrument (see Figure 24 on page 64).

This section describes how you can update the firmware of the Agilent 3100 OFFGEL Fractionator instrument. It requires

- installation of the Firmware Update Tool,
- installation of the firmware onto a PC,
- connection of the PC to the instrument using a LAN cable.

NOTE

Alternatively a connection can be done by a 9-to-9 pin (female) connector RS-232 cable (Agilent part number G1530-60600 or RS232-61601). Such a cable was included in the accessory kit (see Table 4 on page 33) that was delivered with earlier systems that included a handheld controler.

This is a so-called “Null Modem Cable” with full handshaking, where the connection are made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.

Besides the Firmware Update Tool you can use for upgrading future revisions of instrument Firmware also the convenient Update-via-USB. This requires the Main Firmware B.06.30 or higher to be already installed on the instrument.

Installation of the Firmware Update Tool

1 Download the Firmware Update Tool from the Agilent web site (http://www.chem.agilent.com/scripts/DownloadsUtilities.asp) onto your PC.

NOTE Do not use older versions of the Firmware Update Tools as provided via the Agilent web. Versions below 2.3 [001] (introduced 07/2006) should no longer be used.

2 Double-click the exe-file to install the program files. Follow the instructions. The program is installed into a folder (default is *C:\Program Files\Agilent\LC Firmware Update Tool*).
You may change this according your needs.

A Firmware Update Tool icon is installed onto your desktop, and a program folder is installed under Start - Programs (see Figure 27).

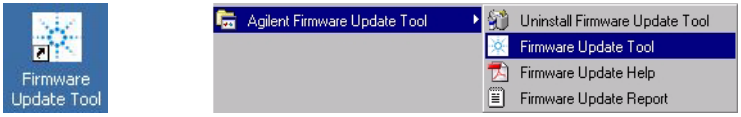


Figure 27 Icon on your desktop and the Program Folder

How to un-install the Firmware Update Tool

The Firmware Update Tool has an un-install routine. If required, remove the Firmware Update Tool via the "**Uninstall Firmware Update Tool**" function within the program folder, see Figure 27.

Installation of the Firmware on your PC

1 Download the Agilent 3100 OFFGEL Fractionator firmware from the Agilent web site

(http://www.chem.agilent.com/scripts/DownloadsUtilities.asp) onto your PC.

2 Double-click the exe-file to extract the content into a folder (default is *c:\Program Files\Agilent\LC Firmware Update Tool*.

NOTE The Firmware Update Tool can be customized to use different folders as firmware location. Please change the path under **Options/Configure Directories** from within the Firmware Update Tool.

Connect your PC to the Agilent 3100 OFFGEL Fractionator

Your PC has to be connected by a Cross-Over LAN cable or an normal LAN cable to the Agilent 3100 OFFGEL Fractionator (Please see instructions for Installation in Chapter 4). In case you would like to use the alternative approach to connect the PC with the Firmware update tool by RS232 see a description of connections at the rear of the instrument see Figure 7 on page 34.

When using a LAN connection to the instrument the established parameters for TCP/IP communication are used. Typically speed and duplex mode are determined by auto-negotiation.

When using the RS 232 cable the Firmware Update Tool uses the following port settings when performing the update:

- Baudrate 19200 (overrides the PC settings)
- No Parity
- 8 Data Bits
- 1 Stop Bit
- COM1, COM2 etc.

The settings are set automatically for the time of the firmware update.

NOTE Note that the upgrade process with LAN will take proximately 5 minutes. An update with RS 232 cable connection can take up to 2 hours. No fractionations can be performed during this time.

Setup of the Firmware Update Tool

1 Start the Firmware Update Tool via the shortcut (see Figure 27). The following initial screen is displayed:

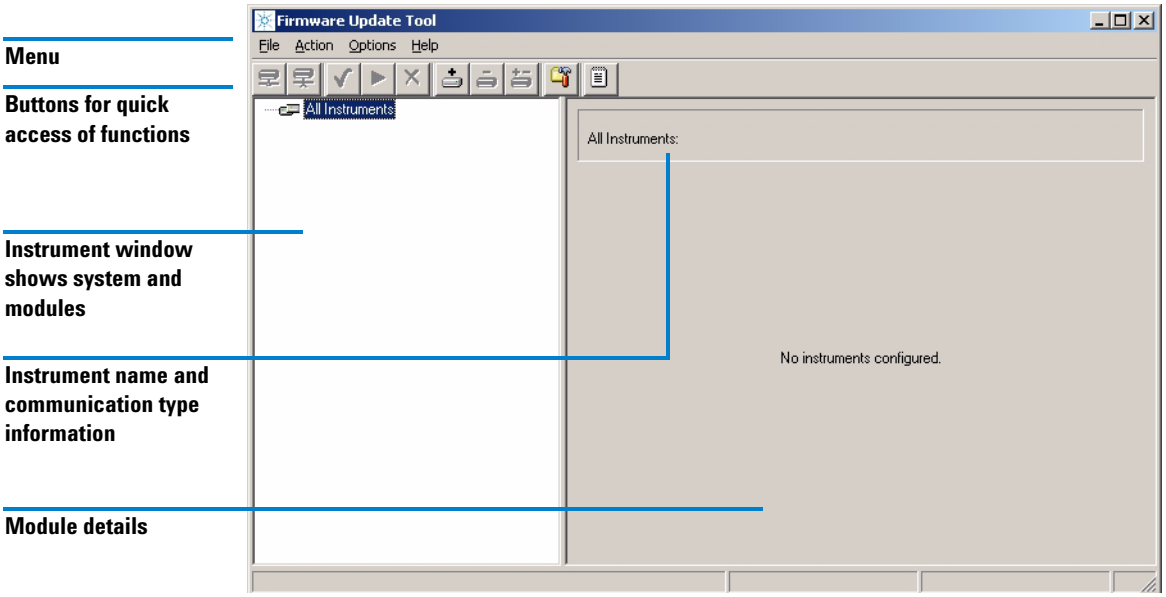


Figure 28 Start-up screen

When you move with the cursor over a information is given about its function. Alternatively, you may use the menu items.

File	Open Protocol File - Exit
Action	Connect - Disconnect - Select for update - Identify module - Start/Cancel update

Options	Configure directories - Add/Delete/Modify Instrument - Support G1323B (active/not active)
Help	Help - About (revision of program)

Table 7 Functions of Buttons

Button	Description
	Connect to module/system
	Disconnect from module/system
	Select for update of all modules of a system
	Start the update for all selected modules
	Add instrument to the list
	Delete instrument from the list
	Modify instrument (name or the connection can be changed)
	Configure directories of the firmware files and report
	Open protocol file (logbook with details of the actions made during the update)

Configure the Firmware Update Tool

1 Select the button **Configure Directories** and browse for an alternative location of the firmware files (e.g. E:\Fwupgrad\LC). You can move a firmware file

as well to the default location (C:\Program Files\Agilent\LC Firmware Update Tool).

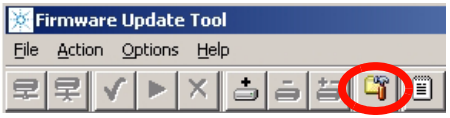


Figure 29 Select the button Configure Directories

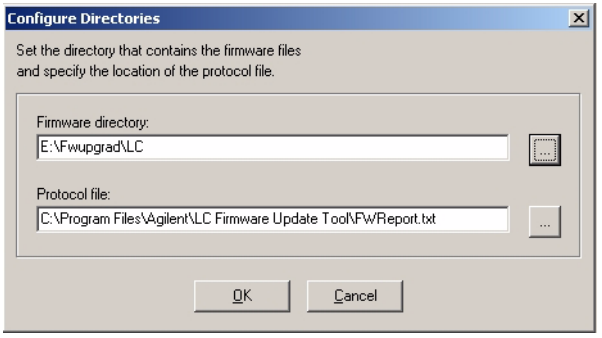


Figure 30 Configure Directories

The Protocol file is a text file that gives the complete status of the firmware update process. This file is useful as a compliance document to prove that the module firmware files were updated successfully or as diagnostics information in the case of problems, see "The Log-file" on page 87. Therefore, name the protocol file appropriately.

2 Press **OK**.

3 Select the **Add Instrument** button.

Figure 31 Select the Add Instrument button.

4 Enter a name for the system (e.g. `System 1`)

5 Enter the TCP/IP address information in the instrument address field, e.g. 168.192.254.11 for the default address of the instrument.

- Set here the static IP address of your instrument in case you connected it to a network.
- In case you connect alternatively with RS232 cable enter the valid COM port connection information in the instrument address field, e.g. COM1.

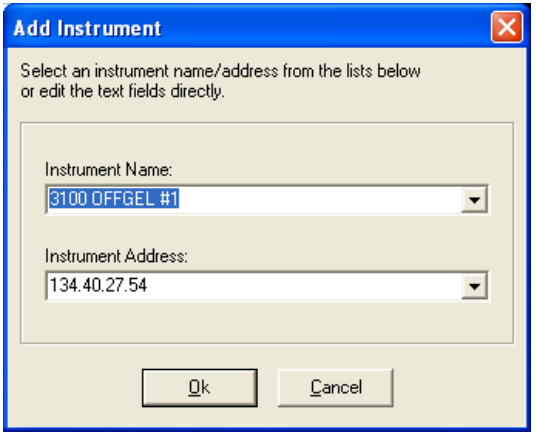


Figure 32 Add instrument screen

6 Press **OK**.

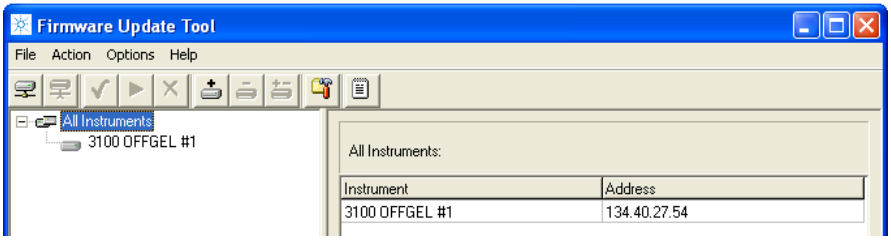


Figure 33 Add instrument screen

7 Double-click on **All Instruments** to expand the view.

NOTE

If you have a control module G3101A connected in parallel and want to update the Control Module, select **Options** and check **Support Control Module**. The control module is optional and might come from earlier instrument configurations.

8 Select the instruments name e.g. OFFGEL #1 and click on the **Connect to Instrument** button.

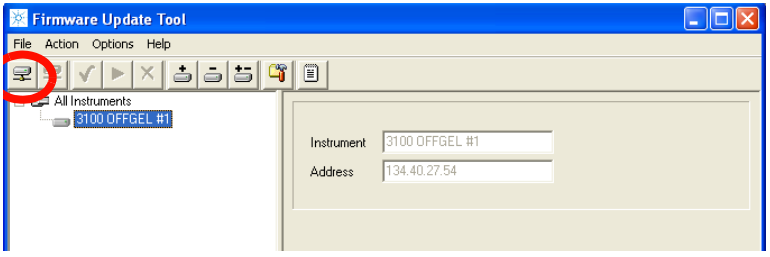


Figure 34 Select "3100 OFFGEL #1"

All modules within OFFGEL #1 are listed with serial number, firmware revision and build number. G3100A refers to the Agilent 3100 OFFGEL Fractionator instrument. In case of an additionally connected control module this will show up as G3101A.

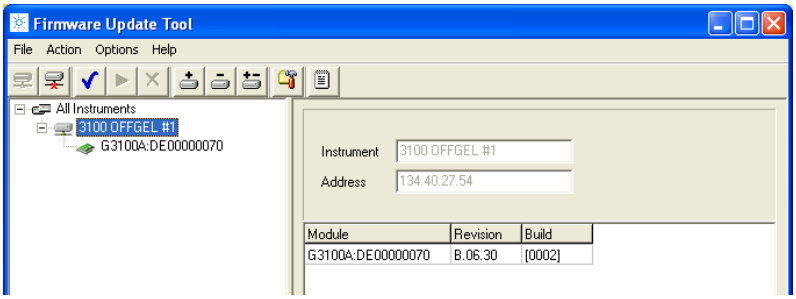


Figure 35 Modules of "3100 OFFGEL #1" are listed

Doing the Firmware Update

The following describes the general workflow for a firmware update of the 3100 OFFGEL fractionator instrument.

1 Select a module.

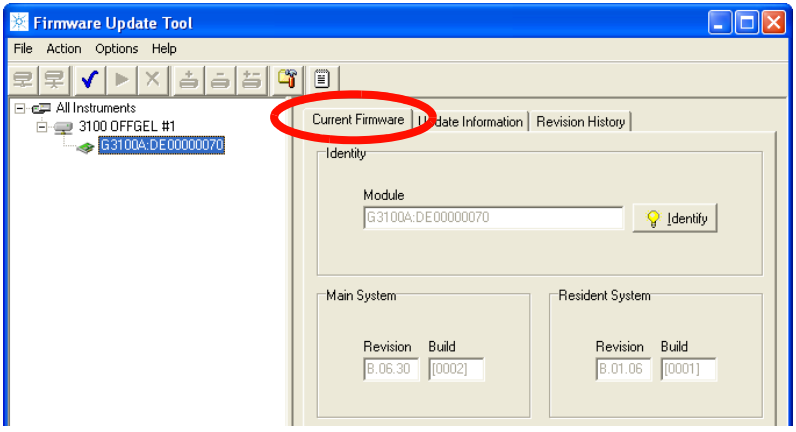


Figure 36 Current Firmware Information screen

2 Above screen shows the current firmware information

3 Select the **Revision History** tab. This window shows details of the used main board and the update history in the module, see Figure 37.

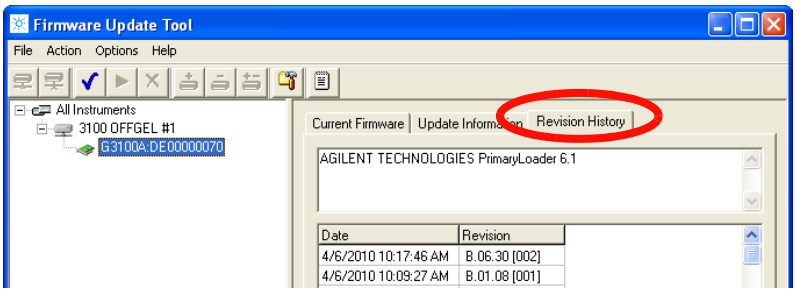


Figure 37 Revision History Information screen

4 Select the **Update Information** tab. In this window, you can select the firmware for the main and resident part in the module.

NOTE If no firmware is listed when you click on the file selection button, correct the firmware folder locations, see Figure 30 on page 81.

5 Activate the check boxes for the firmware. This firmware part is added to the update list.

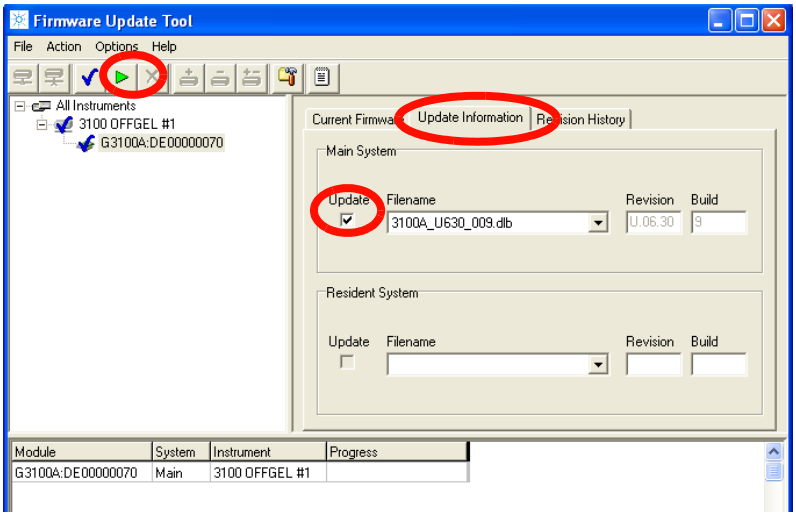


Figure 38 Update Information screen

6 Select the **Start Update** button. All selected modules are updated. The update status is shown at the bottom of the screen. In case you also update the resident Firmware this will be done first followed by the main firmware.

NOTE When the main firmware is being updated, the Status LED of the instrument is red and blinking.

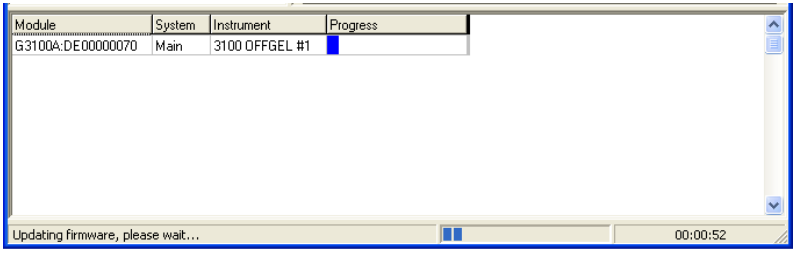


Figure 39 Update is running.

NOTE If you cancel an update, the module remains in the current mode, resident or main, depending when the process was stopped. It cannot be used until the update process has been restarted.

At the end of the update, a report is shown. The update status is either PASS (successful) or FAILED (not successful), see Figure 40. If the update failed, the Protocol file contains more information, see "The Log-file" on page 87.

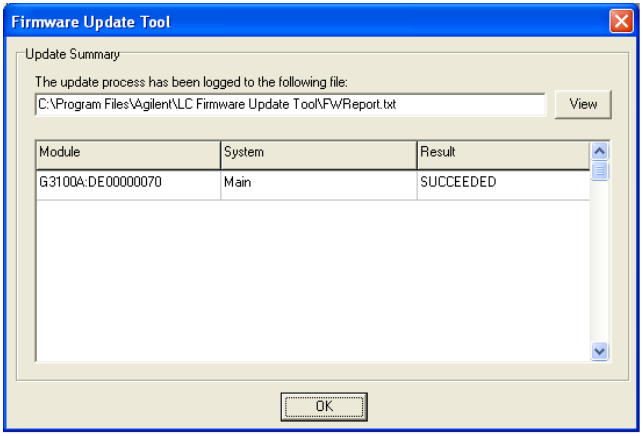


Figure 40 Report Screen

7 If desired, open and print the Protocol file.

8 Press **OK** to continue.

9 Perform a power cycle of the instrument.

The Log-file

This file is the verification that the firmware update was completed successfully or, in the case of problems, the log-file may give some specific information.

Press the button **Open Protocol File** to open the report. Information can be copied/pasted into an e-mail for further help from Agilent support.

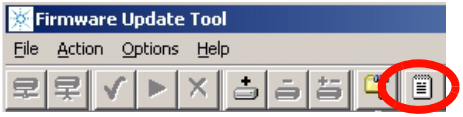


Figure 41 Select the button Open Protocol File

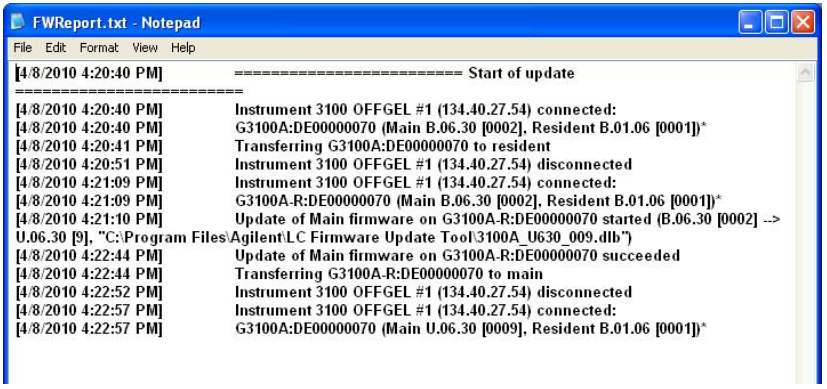


Figure 42 Example of log-file with successful update

NOTE

If you encounter any problems with the firmware update, feel free to send an e-mail with your findings to our technical support specialists (http://www.chem.agilent.com/scripts/cag_techsupport.asp) or contact your local Agilent Technologies representative. Attach your log-file and details of your system, revision and errors.

Firmware Change via USB Stick

A convenient alternative to the Firmware Update Tool is the instrument Firmware update via USB stick. Prerequisite is the Main Firmware on the instrument is revision B.06.30 or higher. Please check in the About screen of the web interface of your instrument (see Figure 24 on page 64) if this is installed.

The USB-memory stick used must be FAT-16 formatted and without encryption, maximum size is 2 GB. See “List of Spare Parts” on page 156. In general the suitability of an USB stick can be check by inserting it simply to

the port at the rear of instrument. Upon recognition the browser interface will show the USB stick symbol in the status screen. In case the USB-memory stick is FAT-32 formatted the instrument will attempt to format it to FAT-16. The file browser of your computer operating system also allows you to format USB sticks. FAT-16 is typically noted simply as FAT.

Steps for Firmware Change via USB

1. Download the Agilent 3100 OFFGEL Fractionator firmware from the Agilent web site (http://www.chem.agilent.com/scripts/DownloadsUtilities.asp) onto your PC.
2. Double-click the exe-file to extract the content into any folder on your hard disc drive. The Main Firmware file has a name starting with the instrument number, revision description and the suffix .dlb. An example for a typical Main Firmware file name is: 3100A_B630_002.dlb.
3. Create a folder on the USB stick with exactly this path: X:\OFFGEL\UPDATE. Please use capital letters for the path. X: represents the drive letter of your USB stick and is a variable.
4. Move the extracted firmware file to the new folder on the USB stick. Only exactly this file should be present in the folder.
5. Power the 3100 OFFGEL Fractionator off.
6. Insert the prepared USB stick to the USB port of the instrument.
7. Power the Agilent 3100 OFFGEL Fractionator on, during the restart the instrument will check the USB stick folders for relevant files and perform an update in case the installed firmware has a lower revision.
8. Allow the instrument to perform the Firmware update. A typical time for this is 10 minutes. During this time the red status LED will blink.

   After a successful update (or downgrade) of main or resident firmware, the 3100 OFFGEL fractionator restarts automatically. The instrument restart is indicated by a toggling of the three LED colors. In case main and resident firmware are changed in one attempt wait until the second cycle of red blinking LED and a toggling of the three LED colors is complete.

NOTE Do not disturb the firmware process. Any interruption will lead to incomplete firmware programming. Instruments in such status are not functional.

9 Populate the web site with the instrument user interface in your browser again. Confirm the changed main firmware in the About screen.

10 Remove the USB stick from instrument.

To perform an update of the Resident part of the Firmware create a folder X:\OFFGEL\RESIUP on the USB stick, provide the firmware file and follow steps 6 - 11. An example for a typical Resident Firmware file name is: res_B623_001.dlb. There is no way to check the resident firmware from the web interface. Please use the Firmware Update Tool to confirm the success.

Main firmware is automatically downgraded when the USB stick is mounted on startup and the directory X:\OFFGEL\UPDATE does not exist or is empty and exactly one .dlb file for the Agilent 3100 OFFGEL Fractionator with a firmware version older than the current version is present in the directory X:\OFFGEL\DOWNDATE.

Resident firmware is automatically downgraded when the USB stick is mounted on startup and the directory X:\OFFGEL\RESIUP does not exist or is empty and exactly one .dlb file containing a resident firmware version older than the current version is present in the directory X:\OFFGEL\RESIDOWN.

If the USB stick contains both, a folder for resident and a main system, the device will first update (or downgrade) the resident system, then restart. After the restart it will update (or downgrade) the main system and restart again.

Please delete the folders for update or downgrading the firmware if you wish to leave the USB stick in the instrument. The presence of these folders decreases the boot-up speed of the instrument at future starts.

7 Troubleshooting

This chapter provides support for troubleshooting the instrument and the application.

Troubleshooting the Instrument

Status Indicators

Status LED

The instrument has a status LED on the front top right position (see Figure 2 on page 17). With the power switched on, the LED can have the following states:

Table 8 LED status overview

LED Status	Description	Comment
Green	The instrument is performing a fractionation.	
Yellow	The instrument is currently not ready. Possible reasons: • the set temperature of the cooling platform has not been reached • top cover is open	A running fractionation will be paused if the top cover is opened.
Red	The instrument has detected an error.	A running fractionation will be aborted. see Table on page 94
Off	The instrument is not performing a fractionation. The set temperature of the cooling platform has been reached.	

NOTE While the main firmware is downloaded to the instrument, the status indicator LED on the front of the instrument front will blink in red.

The status LED will blink red in case the instrument uses the resident firmware only. This might happen if the main firmware installed to the instrument encountered an error or is corrupt. In such case only limited functionality is available. Please restart the instrument. If the blinking LED is still present after an instrument restart, connect to the instrument with the

Firmware download Tool and update to the latest firmware revision. The resident section of the firmware is identical in many Agilent devices and modules. Its properties include:

- The complete communication capabilities (CAN, LAN and RS-232C)
- Memory management
- Ability to update the firmware of the “main system”

Status Bars

The Agilent 3100 OFFGEL interface displays several status bars (see “General Functions” on page 42).

Table 9 Status bar title overview - LED is yellow

Status bar title	Description	Comment
Cover open	The top cover of the instrument is open.	A running fractionation will be paused if the top cover is opened
Temp	The set temperature of the cooling platform has not been reached.	You can start a fractionation; however, the status bar will switch to Wait Temp I/II.
Wait Temp I or Wait Temp II	The set temperature of the cooling platform has not been reached; however, a fractionation was already started on one of the trays.	Once the cooling platform temperature is reached the fractionation will be started automatically.

Error Messages

Table 10 Error messages due to wrong operation

Error message	Solution
USB drive full. Exchange USB drive or delete files.	Exchange the USB drive or delete files on the USB drive.
File export failed. The selected files cannot get copied to USB drive. Check if drive is full.	Exchange the USB drive or delete files on the USB drive.

Table 10 Error messages due to wrong operation (continued)

Error message	Solution
Result Storage Empty. No result data to copy. CF-Card is empty or not present	Insert a compact flash card (for details refer to Figure 7 on page 34)
No USB drive. USB drive not present or incompatible drive format.	Insert a USB drive. Make sure the format is compatible (for details refer to page 54).
Cannot overwrite default method. A default method cannot be overwritten. Change method name.	Change the method name to save modification of default methods.
The method file <name> on <drive> cannot be overwritten. It is currently running on <tray>.	Change the method name to save modification to this method or save the method once the fractionation is finished.
Delete Denied. The method <name> from <drive> is currently in use on <tray>.	Wait until the fractionation is finished to delete this method.

Table 11 Error messages hardware related - LED is red

Error message	Comment	Action
Cooling platform temperature error (EE 20302).	The cooling platform temperature has not been reached.	• Make sure the ambient temperature and the set cooling platform temperature are within instrument specifications. • A Peltier element might be defective. Call Agilent Technologies.
A leak was detected in the module. Use the logbook to determine date and time the fault occurred (EE 00064)	A leak has been detected by the leak sensor.	• Check if there is liquid spillage or condensation on the cooling platform and near the leak sensor and wipe it away. For details refer to "Cleaning the Cooling Platform" on page 72. • Acknowledge the error in the logbook to reset the red LED status.

Table 11 Error messages hardware related - LED is red (continued)

Error message	Comment	Action
Cover sensor failed (EE 20300).	Opening and closing the top cover is no longer recognized.	• Check that the top cover closes tightly. • The cover sensor might be defective. Call Agilent Technologies.
Temperature sensor failed (EE 20301).		• The temperature sensor is defective. Call Agilent Technologies.
Fan failed (EE 00068).		• The fan might be defective. Call Agilent Technologies.
Leak (EE 00080, EE 00081, EE 00082 or EE 00083).		• The leak sensor is defective. Call Agilent Technologies.
High voltage (EE 20305).	The voltage did not reach the set point.	• Check for condensation or dirt on anode connector, electrodes or tray. Clean the the parts as described in "Cleaning the Agilent 3100 OFFGEL Fractionator" on page 70. • The high voltage module might be defective. Call Agilent Technologies.
Peltier Error (EE 20304).	The voltage or currents of the Peltier elements are out of limits.	• A Peltier element might be defective. Call Agilent Technologies.

NOTE

In case you wish to call Agilent Service for support on the 3100 OFFGEL fractionator provide a detailed error description, exported results from your latest sample runs, diagnosis buffers, content of the instruments log book and firmware revision.

Hardware Diagnosis

With the following few steps, you can verify if your instrument is performing to its specifications. Note that a meaningful hardware diagnosis requires a fully functional communication to the instrument.

Checking the LED and the cover sensor

1 Switch on or restart the instrument and watch the Status LED during startup.

The Status LED **must** show all three LED colors for approximately one second during startup in the following order: yellow-green-red.

If this is not the case, a LED is broken and needs repair. Call Agilent Technologies for further help. Otherwise, proceed with step 2.

2 Set the cooling platform temperature to 20 °C and wait until the cooling platform temperature is reached and the LED is turned off.

3 Open the top cover.

The LED **must** turn yellow. The interface must show **Cover Open** in the status bar. This confirms that the cover sensor is working properly. A failed cover sensor would be recognized by the instrument and result in an error, see Table on page 94.

Checking Temperature Control of the Cooling Platform

NOTE For this test, the ambient temperature must be within specification (maximum 30°C if the cooling platform is set to 20 °C) and conditions should be non-condensing.

1 Set the cooling platform temperature to 20 °C and wait until the cooling platform temperature is reached and the LED is turned off.

2 Set the cooling platform temperature to 30 °C and measure the time until the LED is turned off.

The temperature ramp up should not take longer than 5 minutes.

3 Set the cooling platform temperature back to 20 °C and measure the time until the LED is turned off.

The temperature ramp down should not take longer than 15 minutes.

Checking High Voltage, Electrodes and Electrode Connectors

1 Make sure your two electrode sets and trays are completely dry and clean.

2 Assemble both trays with the electrodes (without frames) and insert them to the instrument. Attach the movable electrodes at the most right position.

3 Load the DIAGNOSE method to both trays and press **Start** on both trays to start the runs.

4 Check that the actual voltage values reach approximately 800 V after one minute and that the currents of all lanes are < 1 µA.

If this is not the case, check once again that the tray and the electrodes are completely dry and clean. Inspect the cathode connector for contamination and clean it if necessary. Make sure that no sparking occurs. If you cannot solve this problem call Agilent Technologies for further help. Otherwise, proceed with step 5.

5 Press **Stop** to stop both runs.

6 Open the top cover and pipette 4 ml deionized water (18 MOhm/m) into lanes A, C, E and G on each tray.

7 Close the top cover.

NOTE Make sure the water is evenly distributed and the electrode pins are in contact with the water. However, avoid spillage of water across the electrodes into neighboring lanes and the electrode connectors. This might result in sparking.

8 Press **Start** on both trays to start the DIAGNOSE method again.

9 Look at both tray status screens. You should see a regular current pattern as shown in Figure 43.

Figure 43 Typical current pattern on tray I when every second lane is filled with water.

No currents should flow in the empty tray lanes (<1.0 µA). If this is the case proceed with step 10. Otherwise stop here because you may have contaminated electrodes, electrode connectors or trays. Clean and carefully dry the electrodes, trays and the electrode connectors as described in "Cleaning the Agilent 3100 OFFGEL Fractionator" on page 70 and start this test again at step 6.

Typical values for currents are between 20 µA and 50 µA and one lane has to be at maximum current of 50 µA (representing the current limit) if the voltage is just at 800 V or below 800 V. In case the voltage is constantly at 800 V (representing the voltage limit) a current lower than 50 µA might be present in all lanes. Differences in current from lane to lane are natural and might be due to variation in the resistance of the electrode surface. The voltage has to be greater than 500 V.

NOTE

The absolute value for the voltage is dependent on the conductivity of the deionized water used and is not a criterion for the proper function of the instrument. The more salt ions the water contains, the lower is the voltage value.

10 Open the top cover and pipette 4 ml deionized water (18 MOhm/m) into lanes B, D, F and H on each tray.

11 Close the top cover.

12 You should now see the a typical current pattern for all eight lanes (Figure 44).

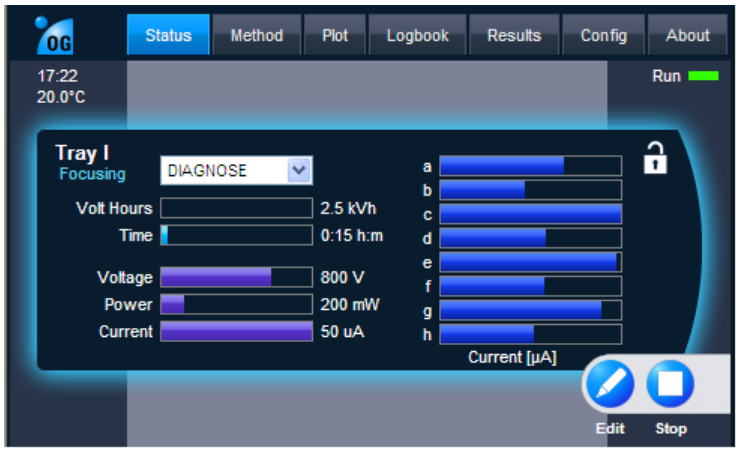


Figure 44 Typical current pattern on tray I when every lane is filled with water.

13 Press **Stop** on both trays to stop the runs.

NOTE

If you require further assistance, call Agilent Technologies and send the corresponding results file of this diagnose test to the customer call center together with a detailed description, exported results from your latest sample runs, diagnosis buffers, content of the instruments log book and firmware revision.

Troubleshooting the Electrodes

Electrodes can be used multiple times. They are designed to be robust but they must still be regarded as consumable. They might suffer from permanent coating of the surface and from residues formed by arcing events during fractionations.

Coatings of the surface may derive from substances in sample buffers that are not hold back by the gel system and can modify the electrodes. Arcing during fractionation might happen if an insufficient amount of liquid is applied to pads in the assembly of the experimental setup or if these pads dry out during the fractionation.

Checking Fixed and Movable Electrodes

1 Make sure your two electrode sets and trays are completely dry and clean.

2 For movable electrodes measure the resistance from the tip of the electrode (8 individual pins) which contact to the pad to the tip of the anode connector electrode (one pins).

3 For movable electrodes measure the resistance from the tip of the electrode (8 individual pins) which contact to the gel strip at the cathode side to the metal spring of the movable electrode (8 individual metal springs).

The resistance for each of this measurement should have a value below 100 kOhm. In case the measured resistance is higher, exchange the respective electrode. See page 156 for product numbers for spare parts.

Troubleshooting the LAN Connection

If your web interface to the Agilent 3100 OFFGEL Fractionator does not work correctly check for disconnected LAN cables at the rear of the instrument.

NOTE Make sure you are using the LAN connector and not the CAN connector.

If the problem still remains, then:

- Power down the Agilent 3100 OFFGEL Fractionator and wait 1 minute and then restart.
- Check the positions at the rear of the instruments according to the installation instruction in chapter 3.

If the problem still remains and you were had the instrument in a local network, then:

- Set up the instrument in a direct LAN connection to a PC. Install a cross over LAN cable and change the configuration dip-switches 7 and 8 in the rear of the instrument to On. Follow the instruction in Chapter 4. Once the connection is reestablished review and correct the IP address in the Config tab of the web interface.

Call Agilent Technologies if the problem cannot be solved.

Troubleshooting the Application

Impaired focusing results can have many different root causes. In addition to hardware errors, the impaired focusing results can be sample and/or handling related. Therefore, always make sure that you set up your focusing experiment exactly as described in the kit manual. The sample concentration should be between 50 µg/ml and 5 mg/ml and the salt content of your sample buffer should be at most 10 mM. Run only samples similar in protein content and buffer composition on the same tray, otherwise it is highly recommended that you use separate trays.

With every focusing, a results file containing all electrical parameters during a run are stored to the compact flash card and can be exported. The traces also provide useful information for troubleshooting (see "How to Display Exported Results" on page 58), and some examples are discussed in this chapter (see "Looking at Run Parameters" on page 105)

The following table gives an overview of symptoms you might experience when performing OFFGEL experiments, their possible causes and actions required for remedy.

Table 12 Troubleshooting the OFFGEL application

Symptom	Possible Cause	Action
No current or very low current (less than 20% of the current limit) during the first 5 minutes after start of a run in one lane compared to other lanes.	**1** No electrical contact or impaired contact.	• check if there is any damage to the electrodes . If so, exchange the electrodes. • check if there is any damage to the anode connector. If so, call Agilent Technologies. • Make sure that the electrodes are in place correctly and the electrode pins are connecting to the electrode pads. • Make sure that the electrode pads are damp and provide a good contact between the electrode pins and the gel ends of the IPG-strip.
	2 IPG strip is improperly rehydrated.	• Make sure that the IPG-strip was rehydrated completely and that all wells in the frame are filled with rehydration buffer during rehydration.
One lane shows very high current signal compared to other lanes on the same tray. This might impair the focusing result of all other lanes on that tray.	**1** the corresponding lane is leaking.	• inspect the corresponding frame for any damage. If damage is present, exchange the frame. • Make sure that the IPG-strip is inserted with the gel side up. • Make sure that the corresponding frame is snapped into the tray lane as described in the kit manual.
	2 the corresponding IPG-strip was inserted in the opposite direction.	• Make sure that the “+” on the IPG-strip is in the direction of the fixed electrode/anode connector.

Table 12 Troubleshooting the OFFGEL application (continued)

Symptom	Possible Cause	Action
	3 the corresponding sample has higher salt concentration or higher protein/peptide content than the other samples.	• Run only samples similar in protein content and buffer composition on the same tray.
After fractionation has finished, the external wells of the frame have reduced liquid or are empty.	**1** During IEF, a water transport is observed due to migrating ions that drag the water in their hydration shell to the electrodes. Furthermore, water is dragged from wells at the basic strip end (cathodic end of strips pH 3-10) to wells in the neutral pH range due to the ionization of DTT. Therefore, after OFFGEL electrophoresis the wells close to the electrodes are sometimes filled with less liquid than the inner wells or are even empty.	• If low recovery in the extreme wells is a problem, refill the corresponding wells with diluted OFFGEL Stock Solution *without ampholytes* (4 parts OFFGEL Stock Solution without ampholytes plus one part water) one hour prior to the end of the focusing phase.
Sparking at the electrodes.	**1** short circuits at the electrodes.	• make sure that the electrodes and the electrode connectors are completely dry.
After fractionation has finished, the cooling platform is contaminated with mineral oil.	**1** Mineral oil tends to creep across the electrodes and tray wells once they are moistened. However, if the electrodes do not dry out during fractionation this does not impair the focusing results.	• carefully pipette the mineral oil and avoid spillages. • when inserting the tray into the instrument, make sure that no oil is spilled out of tray. • Insert the tray before adding the mineral oil to the instrument.

Looking at Run Parameters

The results of all run parameters can be exported as a .csv file and thus can be visualized with Microsoft Excel. Agilent Technologies has designed the *Agilent 3100 OFFGEL fractionator plotter* which is a Excel add-on and facilitates the analysis of the run parameters (see "How to Display Exported Results" on page 58). The following examples show typical results for good runs as well as some impaired results where the root cause of the problem is known.

Example 1. Typical Results of the Protein Test Sample

The fractionation of the protein test sample is a way to check that the Agilent 3100 OFFGEL Fractionator in combination with the consumables kit is working in your hands, and functions as a system checkout test. You find detailed information on how to run the protein test sample in the kit manual.

Figure 45 shows a typical result for the electrical run parameters of the protein test sample using the 3100 OFFGEL Low Res Kit, pH 3-10. The method used for this fractionation was the default method OG12PR00, which has a current limit of 50 µA, a voltage limit of 8000 V, a power limit of 200 mW and a volt hour limit of 20 kVh. With this setup the fractionation is running in current limiting mode. Therefore, the current signal in Figure 45 is stable and at 50 µA.

NOTE Please note that latest OFFGEL methods do have a voltage limit of 4500 V. This is reduce the risk of arcing and damage to the tray in case of dried out electrode pads.

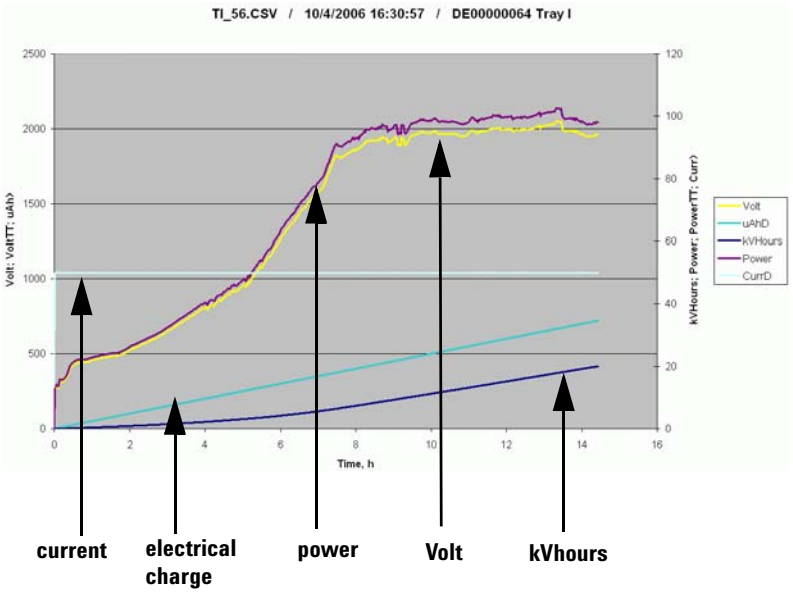


Figure 45 Example 1- typical results for the Protein test sample using the 3100 OFFGEL Low Res Kit, pH 3-10.

The run stopped when the volt hour limit of 20 kVh was reached. Both volt signal and power signal are increasing throughout the run, since the sample is depleted of ions and thus the resistance is increasing. After fractionation, the protein test samples should be focused in fraction 3 (see Figure 46).

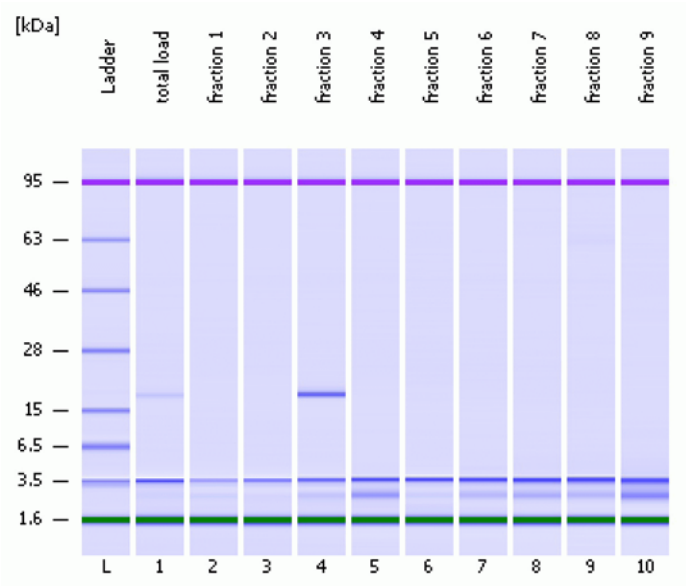


Figure 46 Beta-lactoglobulin Protein Test Sample in well 3 of a 12 cm pH 3-10 IPG strip. (Gel-like image from an Agilent 2100 bioanalyzer analysis using the Protein 80 kit of wells 1-9.)

Example 2 . Current Drop During Fractionation

Figure 47 shows an example where the current (see arrow) dropped close to zero after approximately 30 minutes of the run. In addition, the volt and power signal is highly fluctuating.

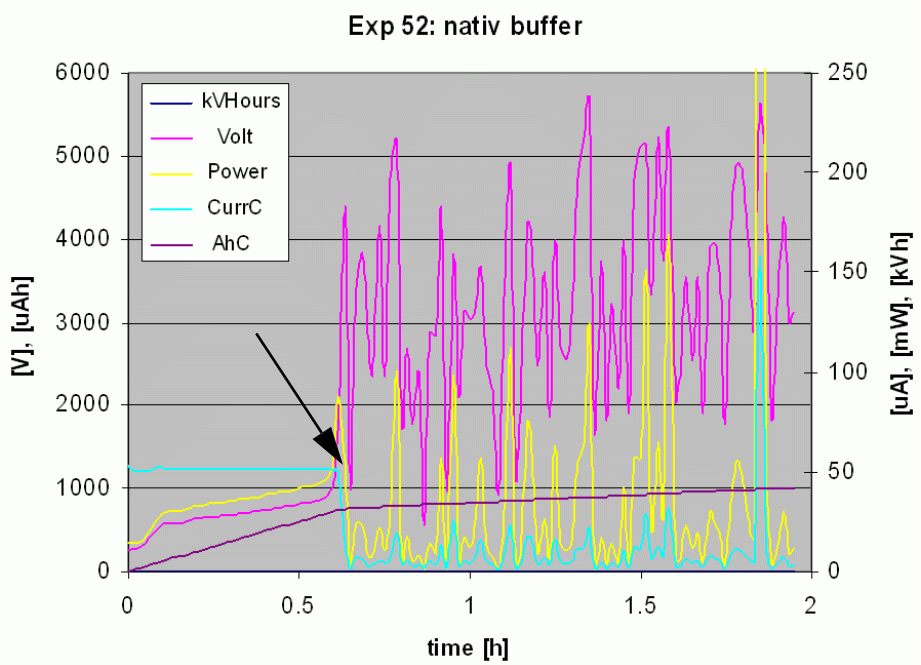


Figure 47 Example 2- current drop during fractionation.

The root cause of this focusing failure was that the IPG strip was dried out at one position so that the conductivity was lost.

Example 3. Reduced Electrical Charge in One Lane

Figure 48 shows an example of a fractionation of eight samples in parallel and just highlights the different electrical charges (in µAh) that were measured throughout the focusing for each lane. The signal of electrical charge for lane F (see arrow) is clearly lower than for the other lanes. Indeed, it could be verified in a follow-up experiment that the focusing of lane F was impaired.

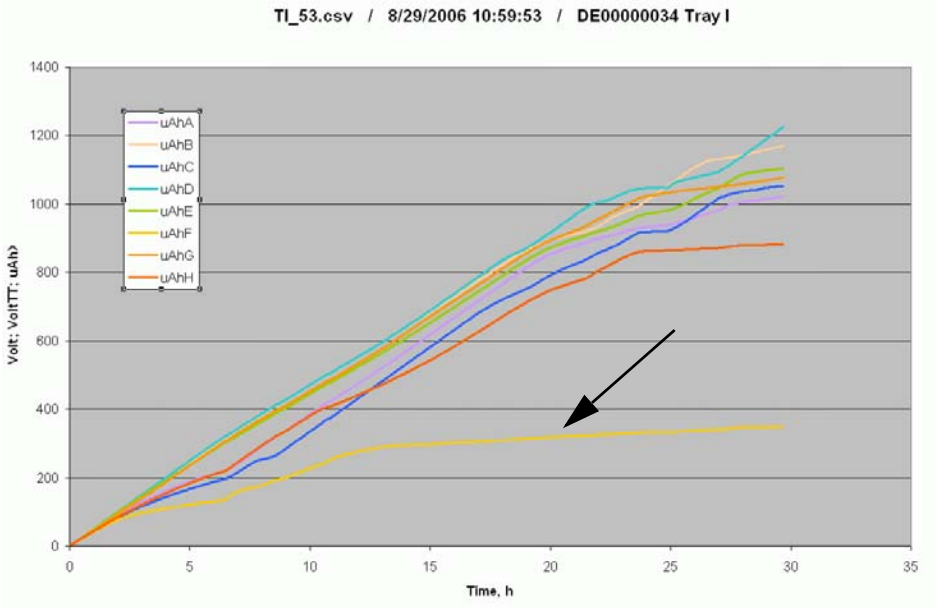


Figure 48 Example 3- one lane shows reduced electrical charge.

The root cause of this failure was a conductivity problem in lane F.

8 Safety

This chapter contains safety information relevant to the Agilent 3100 OFFGEL Fractionator.

Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of the Agilent 3100 OFFGEL Fractionator.

All safety instructions should be read and understood before installation, operation and maintenance of the instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture and intended use of the instrument.

Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING **A warning alerts you to a situation that could cause physical injury or damage to the equipment. Do not proceed beyond a warning until you have fully understood and met the indicated conditions.**

CAUTION A caution alerts you to situations that could impair Agilent 3100 OFFGEL Fractionator functionality or performance and lead to loss of data. Do not proceed beyond a caution until you have fully addressed the indicated conditions.

NOTE **Instrument Safety**
This is a Safety Class I instrument (provided with a terminal for protective grounding) and has been manufactured and tested according to international safety standards.

Power Cords

Different power cords are offered as options with the Agilent 3100 OFFGEL Fractionator. The female end of all power cords is identical. It plugs into the power-input socket at the rear of the instrument. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

WARNING **Never operate your instrument from a power outlet that has no ground connection. Never use a power cord other than the Agilent Technologies power cord designed for your region.**

WARNING **Never use cables other than those supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

Operation

Before applying power, follow the installation instructions. Additionally the following must be observed.

Do not remove instrument covers when operating. Before the instrument is switched on, all protective ground terminals, extension cords, auto-transformers, and devices connected to it must be connected to a protective ground socket. Any interruption of the protective grounding will cause a potential shock hazard that could result in serious personal injury. Whenever it is likely that the protection has been impaired, the instrument must be made inoperative and be secured against any intended operation.

Any maintenance of the instrument under voltage should be avoided.

Do not replace components of the instruments with power turned on.

Do not operate the instrument in the presence of flammable gases or fumes. Operation of any electrical instrument in such an environment constitutes a definite safety hazard.

Do not install substitute parts or make any unauthorized modification to the instrument.

Safety Symbols

Table 13 Safety Symbols used on Instruments and in Manuals

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the *User Manual* in order to prevent risk of harm to the operator and to protect the apparatus against damage.

Chemical and Biological Safety

WARNING **When working with solvents please observe appropriate safety procedures (for example goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

WARNING

If pathogenic, toxic, or radioactive samples are intended to be used in this instrument, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions and practices are adhered to accordingly. This includes also the handling of genetically modified organisms. Ask your laboratory safety officer to advise you about the level of containment required for your application and about proper decontamination or sterilization procedures to follow if fluids escape from containers.

Observe all cautionary information printed on the original solution containers prior to their use.

Because leaks, spills, or loss of sample may generate aerosols, observe proper safety precautions.

Agilent 3100 OFFGEL Fractionator covers have not been designed as bioseals for aerosol or liquid containment.

Handle body fluids with care because they can transmit disease. No known test offers complete assurance that they are free of micro-organisms. Some of the most virulent – Hepatitis (B and C) and HIV (I-V) viruses, atypical mycobacteria, and certain systemic fungi – further emphasize the need for aerosol protection.

Always follow local state and federal biohazard handling regulation when disposing of biohazardous waste material e.g. contaminated compartment frames.

Handle all infectious samples according to good laboratory procedures and methods to prevent spread of disease.

Dispose of all waste solutions and products according to appropriate environmental health and safety guidelines.

安全性

この章では、Agilent 3100 OFFGEL フラクショネーターに関する重要な安全性情報を紹介します。

安全性について

Agilent 3100 OFFGEL フラクショネーターの操作、保守、修理を行う際には、以下の安全性に関する一般的な注意事項を必ずお守りください。

装置の取り付け、操作、メンテナンスを行う前に、安全性に関する指示をお読みになり、理解する必要があります。これらの諸注意、あるいは本書の他の箇所に記載されている特定の警告に従わなかった場合には、装置の設計、製造、および使用目的の安全基準に違反します。

当社は、顧客がこれらの条件や指示に従わなかった場合、それによって生じるいかなる損失に対しても、一切その責任を負いません。

警告

警告は、人身事故や装置にダメージを与える可能性がある状況を警告します。示された条件を完全に理解し、その条件に適合するまで、この警告より先に進まないでください。

注意

注意は、Agilent 3100 OFFGEL フラクショネーター の機能や性能に障害を与え、データの紛失を引き起こす可能性がある状況を警告します。示された条件に完全に対応できるまで、この注意より先に進まないでください。

注

装置の安全性
本器は、Safety Class I の装置です（感電防止用アース端子を備えています）。本器の製造およびテストは、国際的な安全基準に基づいて行われています。

電源コード

Agilent 3100 OFFGEL フラクショネーターでは、オプションとして様々な電源コードが提供されます。電源コードの雌側はすべて同一の形状です。装置の背面に位置する電源入力ソケットへ電源コードを差し込みます。電源コードの雄側の形状は、特定の国や地域の壁面の差込口に適合する設計になっています。

警告

アース接続のない電源の差込口へプラグをつないだまま装置を操作しないようにしてください。地域の基準に合うよう設計された Agilent Technologies 社の電源コード以外は使用しないでください。

警告

安全基準や EMC 規則に準拠して正しく機能させるため、Agilent Technologies 社が提供するケーブル以外は使用しないでください

操作

電気を供給する前に、取り付けの指示に従ってください。装置を安全に操作するためです。

操作中は装置のカバーを外さないでください。

装置をオンにする前に、すべての感電防止用アース端子、延長コード、自動変圧器、および装置に接続されたデバイスを、感電防止用アース・ソケットに接続する必要があります。感電防止用アース線を断線させると、人体に危険の及ぶおそれがある感電事故を引き起こす可能性があります。感電防止機能が損なわれていると思われる場合は、装置の使用を中止し、手を触れないようにしてください。

電圧をかけた状態での装置のメンテナンスは避けてください。

電源を入れたまま、装置のコンポーネントを交換しないでください。

本装置を可燃性のガスや煙のある場所で使用しないでください。電気機器をこうした環境で操作すると、安全性が損なわれます。

代替部品を取り付けたり、装置を許可無く改造しないでください。

安全記号

表 13　装置およびマニュアルで使用される安全記号

記号	説明
⚠	装置上にこのマークが表示されている箇所では、ユーザは必ずユーザマニュアルを参照してください。理解していないとオペレーターに危害があったり、装置が損壊したりするおそれがあります。

化学および生物的な安全性

警告

溶剤を扱うときには（特に、毒性または危険性のある溶剤を使用するときには）、溶剤メーカーが作成した材料の取り扱いおよび安全に関するデータ・シートの説明に従い、適切な安全対策（ゴーグル、安全手袋、防護服の着用など）を講じてください。

警告

本装置で病原性サンプル、毒性サンプル、または放射性サンプルの使用を予定している場合は、必要とされるすべての安全に関する規制、ガイドライン、予防措置、慣例をユーザが責任を持って厳守する必要があります。これには、遺伝子変異生物も含まれます。アプリケーションで要求される遮蔽（封じ込め）のレベル、および液体が容器から漏れ出した場合にとる適切な除染または殺菌手順については、所属する実験室の安全管理責任者におたずねください。

使用前に、溶液の元の容器に印刷されているすべての注意事項を遵守してください。

サンプルが漏れたり、こぼれたり、流出すると、エアロゾールが生成される可能性があるため、適切な安全対策を講じてください。

Agilent 3100 OFFGEL フラクショネーターのカバーは、エアロゾールや液体を遮蔽するためのバイオシールとして設計されていません。

体液は病気の感染源となるおそれがあるため、体液の取り扱いには注意してください。これまでのところ、微生物が存在しないことを完全に保証するテストはありません。B 型および C 型肝炎ウィルス、HIV（I-V）ウィルス、異型マイコバクテリア、ある種の浸透性のかびなど、非常に有毒な微生物に対しては、さらにエアロゾール対策を強化する必要があります。

汚染されたコンパートメントフレームなど、生物学的に危険な廃棄物を処分するときには、常に、政府および各自治体の定める生物学的危険処理規制に従ってください。

病気が広がらないように、感染性のサンプルはすべて、実験室の手順やメソッドに従って処理してください。

残った溶液や製品はすべて、該当する環境衛生および安全性のガイドラインに従って廃棄してください。

安全

本章介绍了与 Agilent 3100 OFFGEL 分馏器相关的重要安全说明。

安全说明

在操作、维护和检修 Agilent 3100 OFFGEL 分馏器的过程中必须遵守以下常规安全操作规程。

在对设备进行安装、操作和维修保养之前务必认真阅读和理解全部安全说明。不遵从操作规程或无视手册中提出的特别警告将会违反仪器的设计、制造和使用安全标准。

安捷伦科技对由于使用者违反操作规程所导致出现的问题不承担任何责任。

警告

警告 （WARNING）为危险提示。提醒您遵守操作规范，如果操作不当可能会导致人身伤害或损坏设备。在完全理解并满足所示条件之前不要进行警告提示之后的操作。

小心

小心 （CAUTION）为危险提示。提醒您遵守操作规范，如果操作不当可能会削弱 Agilent 3100 OFFGEL 分馏器的功能或性能和导致数据丢失。在完全理解并满足所示条件之前不要进行小心提示下面的操作。

注意

设备安全

本设备的安全级别为 I （配置有一个地线端口），根据国际安全标准制造和检验。

电源线

Agilent 3100 OFFGEL 分馏器提供不同的电源线选件。所有电源线与仪器连接的一头都是一样的，插在仪器后面的电源插槽里。电源线与电源连接的插头各不相同，与不同国家和地区的墙上插座相匹配。

警告 **电源插座没有接地时不得操作仪器。只能使用为所在地区专门设计的安捷伦科技公司的电源线。**

警告 **为确保正常功能，并符合安全或 EMC 规范的要求，不得使用非安捷伦科技公司的电缆。**

操作

接通电源之前，首先应阅读安装说明。另外，还要遵守以下规定。

为了安全地操作仪器，操作过程中不要取下仪器盖。在仪器开启之前，要检查全部接地端、电缆、变压器和与仪器相连的设备是否都与地线插座相连。接地保护中的任何一点故障，将有引起电击的可能，造成严重的人身伤害。如果有任何现象表明接地保护环节上存在有不安全的可能性，请立即停止仪器运行并对已有的操作进行安全检查。

请勿在通电情况下维护仪器。

带电状态下不要更换仪器的部件。

操作仪器时周围环境中不要有易燃 气体， 在有易燃气体的环境中操作任何电器设备都会导致安全危害。

不要安装替代部件或对仪器做任何未经授权的修改。

安全标志

表 13 在仪器上和手册中使用的安全标志

标志	说明
⚠	有此种标志的设备在被使用时，操作者应当阅读有关的*用户手册*以免对操作者和仪器造成损害。

化学和生物安全

警告

使用溶剂时请遵循相应的安全操作规程 （如佩带防护眼镜，安全手套和穿防护衣），请按照试剂商提供的安全数据表和处理溶剂的有关说明进行操作，当所用的试剂有毒有害时，这一点尤其重要。

警告

如果要在该仪器上测试致病的、有毒的或是有放射性的样品，操作者有责任确保全部必要的安全规程、指南、措施。其中也包括合理处置有基因修饰的样品。如果发生液体从容器中泄露的情况，务必根据实验室安全负责人的建议启动相应安全级别的防泄漏系统以及合适的净化和灭菌程序。

在使用试剂之前要仔细阅读印在包装盒上的警示信息。

泄漏，溢撒或遗失的样品可能悬浮于空气中，要根据适当的安全提示采取相应的措施。

Agilent 3100 OFFGEL 分馏器的盖子没有防止样品挥散和液体被污染的功能。

处理体液样品时务必小心，因为这些体液可能会传播疾病，目前还没有相应的检测确保体液不含微生物致病菌。一些毒性很强的致病微生物，如肝炎病毒 （B 型和 C 型），HIV 病毒 （I 型到 V 型），非典型分支杆菌，某些类型的真菌——更需要防止体液气化扩散。

对于使用过的有生物毒性的废物，如污染了的分割框架应根据当地或国家关于有毒生物样品处理规范条例加以处理。

对于有感染性的样品应根据实验室操作规范加以妥善处理，避免疾病的传播。

所有废弃液体和产物的处理应符合环境保护和健康安全的要求。

Sicherheit

Dieses Kapitel enthält wichtige Sicherheitshinweise zu dem Agilent 3100 OFFGEL Fractionator.

Sicherheitshinweise

Die folgenden allgemeinen Sicherheitsmaßnahmen müssen in allen Phasen des Betriebs, der Wartung und der Reparatur des Agilent 3100 OFFGEL Fractionator.

Die Bediener sollten vor der Installation, dem Betrieb und der Wartung des Geräts alle Sicherheitsanweisungen sorgfältig durchgelesen und verstanden haben. Die Nichtbeachtung dieser Vorsichtsmaßnahmen oder spezifischer Warnungen, die an anderen Stellen in diesem Handbuch enthalten sind, verstößt gegen die Sicherheitsstandards für Konstruktion, Produktion und geplante Nutzung des Geräts.

Agilent Technologies übernimmt keine Haftung im Falle der Missachtung dieser Voraussetzungen durch den Kunden.

WARNUNG

Ein Warnhinweis weist Sie auf Situationen hin, die Personenschäden oder eine Zerstörung der Ausrüstung verursachen können. Übergehen Sie nicht diesen Hinweis, bevor Sie die Warnung vollständig verstanden haben und die entsprechenden Bedingungen erfüllt sind.

ACHTUNG

Ein Achtungs-Hinweis weist auf eine Situation hin, die zu einer Beschädigung des Agilent 5100 ALP oder einem Datenverlust führen kann. Übergehen Sie nicht diesen Achtungs-Hinweis, bevor Sie ihn vollständig verstanden haben und die entsprechenden Bedingungen erfüllt sind.

HINWEIS

Gerätesicherheit
Dies ist ein Gerät der Sicherheitsklasse I (mit einem Anschluss für Schutzerde) und wurde gemäß internationalen Sicherheitsstandards hergestellt und getestet.

Netzkabel

Für den Agilent 3100 OFFGEL Fractionator werden unterschiedliche Netzkabel als Optionen angeboten. Die Steckbuchse ist bei allen Kabeln identisch und wird in die Netzanschlussdose auf der Rückseite des Rechners gesteckt. Der Stecker ist bei jedem Kabel unterschiedlich und entspricht den Netzsteckdosen des betreffenden Landes.

WARNUNG **Betreiben Sie Ihr Gerät niemals an einem Netzanschluss ohne Erdung. Benutzen Sie niemals ein anderes Netzkabel als das von Agilent Technologies für Ihren Bereich deklarierte Netzkabel.**

WARNUNG **Benutzen Sie keine anderen als die von Agilent Technologies gelieferten Kabel, um eine einwandfreie Funktion und Übereinstimmung mit den gesetzlichen Vorschriften sicher zu stellen.**

Betrieb

Befolgen Sie die Installationsanweisungen, bevor Sie das Gerät an die Netzspannung anschließen. Zur Gewährleistung eines sicheren Betriebes.

Entfernen Sie während des Betriebs keine Geräteabdeckung. Vor dem Einschalten des Geräts müssen alle Erdungsanschlüsse, Verlängerungskabel, Autotransformatoren und angeschlossene Geräte an eine geerdete Steckdose angeschlossen werden. Jede Unterbrechung der Schutzerdung bedeutet ein potenzielles Stromschlagrisiko, das zu schweren Verletzungen führen kann. Wenn davon ausgegangen werden muss, dass der Schutz beeinträchtigt wurde, muss das Gerät ausgeschaltet und gegen unbeabsichtigten Betrieb gesichert werden.

Führen Sie keine Wartungsarbeiten am Gerät unter Spannung aus.

Ersetzen Sie keine Komponenten während das Gerät eingeschaltet ist.

Betreiben Sie das Gerät nicht in Gegenwart leicht entzündlicher Gase oder Dämpfe. Der Betrieb elektrischer Geräte in einer solchen Umgebung stellt ein hohes Sicherheitsrisiko dar.

Bauen Sie nur Originalteile ein und nehmen Sie keine nicht autorisierten Änderungen am Gerät vor.

Sicherheitssymbole

Tabelle 13 Sicherheitssymbole am Gerät und in diesem Handbuch

Symbol	Beschreibung
	Das Gerät ist mit diesem Symbol gekennzeichnet, wenn der Bediener das *Benutzerhandbuch* lesen sollte, um Verletzungen zu vermeiden und das Gerät vor Schäden zu schützen.

Chemische und biologische Sicherheit

WARNUNG

Beachten Sie beim Arbeiten mit Lösungsmitteln die entsprechenden Sicherheitsmaßnahmen (z. B. Tragen von Schutzbrille, Schutzhandschuhen und Schutzbekleidung) gemäß der Beschreibung im Datenblatt zur Materialhandhabung und Sicherheit des Lösungsmittelherstellers, insbesondere bei Verwendung toxischer oder gefährlicher Lösungsmittel.

WARNUNG

Wenn pathogene, toxische oder radioaktive Proben in diesem Gerät verwendet werden sollen, ist der Bediener verantwortlich für die Einhaltung aller notwendigen Sicherheitsrichtlinien, Vorsichtmaßnahmen und Praktiken. Dies betrifft auch die Handhabung genetisch modifizierter Organismen. Fragen Sie den Sicherheitsbeauftragten Ihres Labors nach den notwendigen Sicherheitsmaßnahmen für Ihre Anwendung und den korrekten Dekontaminierungs- und Sterilisierungsverfahren für den Fall, dass Flüssigkeit aus Behältern entweicht.

Lesen Sie vor der Verwendung alle Vorsichtshinweise auf den Originallösungsmittelbehältern.

Da durch Lecks, verschüttetes Lösungsmittel und Probenverluste Aerosole entstehen können, beachten Sie die entsprechenden Sicherheitsmaßnahmen.

Die Abdeckungen des Agilent 3100 OFFGEL Fractionator sind nicht als biologisch wirksame Dichtung für die Rückhaltung von Aerosolen und Flüssigkeiten ausgeführt.

Handhaben Sie Körperflüssigkeiten mit Vorsicht, da sie Krankheiten übertragen können. Kein bekannter Test bietet vollständige Sicherheit dafür, dass sie frei von Mikroorganismen sind. Einige der ansteckendsten – Hepatitis (B und C)- und HIV (I-V)-Viren, atypische Mykobakterien und bestimmte systemische Pilze – machen die Notwendigkeit für Schutz vor Aerosolen noch wichtiger.

Befolgen Sie immer die örtlichen Bundes- und Landesvorschriften für die Handhabung biologisch gefährlicher Stoffe bei der Entsorgung von gefährlichem biologischen Abfall, z. B. kontaminierte Well-Rahmen.

Handhaben Sie alle infektiösen Proben entsprechend guter Laborpraktiken und -methoden, um die Ausbreitung von Krankheiten zu vermeiden.

Entsorgen Sie alle Lösungsmittelabfälle und Abfallprodukte gemäß den entsprechenden Gesundheits- und Sicherheitsvorschriften.

Sécurité

Ce chapitre contient des informations de sécurité importantes concernant le Fractionneur Agilent 3100 OFFGEL.

Consignes de sécurité

Les consignes générales de sécurité suivantes doivent être observées lors des phases d'utilisation, d'entretien et de réparation du Fractionneur Agilent 3100 OFFGEL.

Toutes les consignes de sécurité doivent être lues et assimilées avant l'installation, l'utilisation et toute opération de maintenance de l'instrument. La non observation de ces consignes ou des avertissements spécifiques indiqués dans ce manuel constitue une violation des normes de sécurité relatives à la conception, à la fabrication et à l'utilisation normale de l'instrument.

Agilent Technologies ne saurait être tenu pour responsable en cas de non observation de ces consignes par le client.

DANGER **Cet avertissement vous signale une situation susceptible d'entraîner des blessures ou une détérioration de l'équipement. N'allez pas plus loin tant que vous n'avez pas pleinement compris et rempli les conditions indiquées.**

ATTENTION Cet avertissement vous signale des situations pouvant mettre en péril les fonctionnalités ou les performances de l'appareil Fractionneur Agilent 3100 OFFGEL ou conduire à des pertes de données. N'allez pas plus loin tant que vous n'avez pas pleinement rempli les conditions indiquées.

REMARQUE **Sécurité de l'instrument**
Il s'agit d'un instrument de classe I (équipé d'une borne de mise à la terre de protection), qui a été fabriqué et testé selon les normes internationales de sécurité.

Cordons secteur

Différents cordons secteur sont proposés en option avec le Fractionneur Agilent 3100 OFFGEL. L'extrémité femelle est identique pour tous les cordons secteur. Elle se branche dans la prise secteur prévue à cet effet à l'arrière de l'instrument. Le côté mâle de chaque cordon secteur est spécifique au pays ou à la région de destination.

DANGER **Ne jamais faire fonctionner un instrument si la borne de terre de l'alimentation secteur n'est pas effectivement reliée à la terre. Ne jamais utiliser un cordon secteur différent de celui spécifiquement fourni par** Agilent Technologies **pour la région d'utilisation.**

DANGER **Afin d'assurer un fonctionnement correct et conforme à la réglementation sur la compatibilité électromagnétique, ne jamais utiliser un cordon secteur différent de celui spécifiquement fourni par** Agilent Technologies**.**

Utilisation

Avant la mise sous tension, suivez les instructions d'installation pour utiliser l'instrument en toute sécurité.

Ne pas retirer les capots de l'instrument pendant son fonctionnement. Avant de mettre l'instrument sous tension, toutes les bornes de mise à la terre, les rallonges, les auto-transformateurs et les périphériques qui y sont connectés doivent être branchés sur une prise munie d'une fiche de terre de protection. Toute interruption de la ligne de terre constitue un risque d'électrocution pouvant engendrer des blessures corporelles graves. S'il s'avère que la protection n'est plus assurée, l'utilisation de l'instrument devra être rendue impossible.

Eviter tout entretien de l'instrument sous tension.

Toujours débrancher le cordon secteur pour remplacer un composant de l'instrument.

Ne pas faire fonctionner l'appareil en présence de gaz inflammables ni de fumée. Faire fonctionner un appareil électrique dans un tel environnement constitue un risque certain.

Utiliser exclusivement des pièces d'origine Agilent et ne pas effectuer de modification non autorisée de l'instrument.

Symboles de sécurité

Table 13 Symboles de sécurité utilisés sur les instruments et dans les manuels

Symbole	Description
	L'appareil est marqué de ce symbole lorsque l'utilisateur doit consulter le *Manuel de l'utilisateur* afin de se prémunir contre les risques de blessure corporelle et de protéger l'appareil contre tout dommage.

Sécurité chimique et biologique

DANGER

Lors de travaux avec des solvants, observez les procédures de sécurité appropriées (port de lunettes, gants et vêtements de protection, par exemple) indiquées dans la fiche technique de manipulation et de sécurité du produit fournie par son fabricant, tout spécialement lorsqu'il s'agit de solvants toxiques et dangereux.

DANGER

Si des échantillons pathogènes, toxiques ou radioactifs doivent être utilisés dans cet instrument, il est de la responsabilité de l'utilisateur de s'assurer que toutes les règles de sécurité, directives, précautions et pratiques sont respectées en conséquence. Ceci concerne également la manipulation des organismes génétiquement modifiés. Demandez au responsable de la sécurité de votre laboratoire de vous conseiller en ce qui concerne le niveau de confinement requis par votre application et les procédures de décontamination ou de stérilisation à suivre en cas de fuites de fluides.

Avant toute utilisation de solutions, respectez toutes les précautions indiquées sur leur emballage d'origine.

Les fuites, écoulements ou pertes d'échantillon pouvant générer des aérosols, observez les règles de sécurité adéquates.

Les capots du Fractionneur Agilent 3100 OFFGEL n'ont pas été conçus pour assurer l'étanchéité aux aérosols et aux liquides.

Manipulez les fluides corporels avec précaution, ceux-ci étant susceptibles de transmettre des maladies. Aucun test connu ne peut garantir qu'ils sont exempts de micro-organismes. Certains des plus virulents, tels que les virus de l'hépatite (B et C) et le HIV (I-V), les mycobactéries atypiques et certains champignons systémiques, exigent une protection renforcée contre les aérosols.

Suivez toujours les règles nationales et locales de manipulation lors de la mise au rebut de matériel usagé à risque biologique, par exemple, des cadres à compartiments contaminés.

Manipulez tous les échantillons infectieux selon les procédures et les méthodes de tout bon laboratoire afin d'éviter la propagation de maladies.

L'élimination des solutions et produits usagés doit être effectuée en conformité avec les directives de santé et de sécurité environnementale en vigueur.

Sicurezza

Questo capitolo contiene informazioni importanti per la sicurezza relative al sistema Frazionatore OFFGEL Agilent 3100.

Informazioni per la sicurezza

Le seguenti norme di sicurezza generale devono essere osservate durante tutte le fasi di funzionamento, manutenzione e riparazione del sistema Frazionatore OFFGEL Agilent 3100.

Le istruzioni riguardanti la sicurezza devono essere lette e comprese prima dell'installazione, dell'utilizzo e della manutenzione dello strumento. Il mancato rispetto di tali norme o di avvertenze specifiche riportate nel presente manuale viola gli standard di sicurezza per progettazione, produzione ed uso previsto dello strumento.

Agilent Technologies non può in nessun modo essere ritenuta responsabile del mancato rispetto delle normative in vigore da parte dell'utilizzatore.

ATTENZIONE **Un segnale di allarme richiama l'attenzione su una situazione che potrebbe causare danni alle persone o alla strumentazione. Non procedere oltre se non si è certi di aver compreso le istruzioni e soddisfatto le condizioni indicate.**

AVVERTENZA Un segnale di avvertimento richiama l'attenzione su una situazione che protrebbe compromettere la funzionalitá o le prestazioni del sistema Frazionatore OFFGEL Agilent 3100 e causare perdite di dati. Non procedere oltre se le situazioni indicate non sono state risolte.

NOTA

Sicurezza dello strumento
Lo strumento appartiene alla Classe di sicurezza I (dotato di terminale di protezione con messa a terra) ed è stato prodotto e collaudato secondo standard di sicurezza internazionali.

Cavo di alimentazione

Il sistema Frazionatore OFFGEL Agilent 3100 può essere utilizzato con vari tipi di cavi di alimentazione, acquistabili a parte. Il lato con il connettore femmina è identico su tutti i tipi di cavi e deve essere collegato alla presa di alimentazione sul retro dello strumento. Il lato con il connettore maschio varia a seconda dei cavi e dei tipi di prese utilizzati nei vari paesi.

ATTENZIONE **Non collegare lo strumento ad una presa di corrente sprovvista di messa a terra. Non utilizzare un cavo di alimentazione diverso da quello fornito da** Agilent Technologies **per l'uso nel proprio paese.**

ATTENZIONE **Per un funzionamento ottimale e per rispettare le normative di sicurezza EMC, è importante utilizzare solo i cavi di alimentazione forniti da** Agilent Technologies**.**

Funzionamento

Prima di alimentare il sistema, seguire attentamente le istruzioni per l'installazione affinché lo strumento possa funzionare in sicurezza.

Non togliere mai i coperchi della strumentazione durante il funzionamento. Prima dell'accensione, tutti i terminali protettivi, le prolunghe, gli autotrasformatori e qualsiasi dispositivo collegato, devono essere collegati ad una presa dotata di messa a terra. Qualsiasi interruzione della messa a terra protettiva è una potenziale causa di scosse elettriche in grado di provocare lesioni gravi alle persone. Quando è possibile che la protezione sia danneggiata, lo strumento deve essere scollegato e reso inutilizzabile.

Non effettuare operazioni di manutenzione senza aver staccato la corrente.

Non sostituire i componenti dello strumento.

Non utilizzare lo strumento in presenza di gas o vapori infiammabili. L'uso di qualsiasi tipo di apparecchiatura elettrica in ambienti di questo tipo può comprometterne la sicurezza.

Non installare parti di ricambio o effettuare modifiche non autorizzate allo strumento.

Simboli di sicurezza

Tabella 13 Simboli di sicurezza utilizzati per gli strumenti e per la documentazione tecnica

Simbolo	Descrizione
	L'apparecchiatura è contrassegnata da questo simbolo quando l'utente deve consultare il *Manuale per l'utente* per evitare danni all'operatore e proteggere gli strumenti da eventuali danni.

Sicurezza chimica e biologica

ATTENZIONE **Lavorando con i solventi osservare sempre le norme di sicurezza in vigore (indossare gli occhiali e gli abiti antinforturnistici) come descritto nella scheda tecnica del materiale fornita dal rivenditore, specialmente se si utilizzano solventi tossici o pericolosi.**

ATTENZIONE

Se si intende utilizzare campioni tossici o radioattivi sullo strumento, è responsabilità dell'utente garantire che tutte le norme di sicurezza in vigore, linee guida, precauzioni e pratiche vengano rispettate adeguatamente. Questo anche in caso di manipolazione di organismi geneticamente modificati. Informarsi presso il responsabile della sicurezza del laboratorio sul livello di protezione richiesta per l'applicazione in uso e sui procedimenti di decontaminazione e sterilizzazione da seguire, in caso i fluidi fuoriescano dai contenitori.

Osservare attentamente tutte le avvertenze riportate sui contenitori delle soluzioni originali prima dell'uso.

Poiché perdite, fuoriuscite e versamenti di campione possono produrre aerosol, seguire procedure di sicurezza adeguate.

I coperchi del sistema Frazionatore OFFGEL Agilent 3100 non sono stati progettati per fungere da tenute biologiche per il contenimento di liquidi o aerosol.

Maneggiare i fluidi corporei con estrema cura, poiché possono trasmettere malattie. Non esistono test in grado di garantire che siano completamente privi di microrganismi. La presenza di virus mortali, come epatite (B e C) ed HIV (I-V), microbatteri atipici ed alcuni funghi sistemici, aumenta ulteriormente la necessità di protezione dagli aerosol.

Attenersi sempre alle normative in vigore localmente quando si devono smaltire rifiuti contenenti materiali biologicamente pericolosi ad esempio, telai di comparti contaminati.

Maneggiare i campioni infetti secondo la buona prassi di laboratorio ed utilizzare metodi adatti per prevenire la diffusione della malattia.

Smaltire tutte le soluzioni ed i prodotti di scarico in base alle linee guida per la sicurezza e la salute ambientale.

Seguridad

En este capítulo encontrará información de seguridad importante relativa al Fraccionador 3100 OFFGEL de Agilent.

Información de seguridad

Las siguientes precauciones generales de seguridad deben observarse durante todas las fases de funcionamiento, mantenimiento o reparación del Fraccionador 3100 OFFGEL de Agilent.

Deben leerse y comprenderse perfectamente todas las instrucciones de seguridad antes de la instalación, manejo y mantenimiento del instrumento. Si no se cumplen estas normas o los avisos específicos que aparecen en diversas partes de este manual, se invalidan los estándares de seguridad de diseño, fabricación y utilización de este instrumento.

Agilent Technologies no se responsabiliza del incumplimiento por parte del usuario de estas normas.

AVISO **Un aviso advierte sobre una situación que podría derivar en lesiones personales o daños al equipo. No continuar tras un aviso hasta haber entendido y cumplido totalmente las condiciones indicadas.**

PRECAUCIÓN Una precaución advierte de situaciones que podrían limitar la funcionalidad o el rendimiento del Fraccionador 3100 OFFGEL de Agilent y dar lugar a una pérdida de datos. No continuar tras un mensaje de precaución hasta haber tomado en consideración plenamente las condiciones indicadas.

NOTA

Seguridad del instrumento
Este es un instrumento de seguridad de Primera Clase (dotado de un terminal de toma de tierra) y ha sido fabricado y comprobado de acuerdo a las normas internacionales de seguridad.

Cables de alimentación

Con el Fraccionador 3100 OFFGEL de Agilent se ofrecen diferentes cables de alimentación opcionales. El extremo hembra de todos ellos es idéntico. Éste encaja en la entrada de alimentación de la parte trasera del instrumento. El extremo macho de todos los cables de alimentación es diferente y está diseñado para adaptarse a los enchufes de pared de cada país o región.

AVISO **No utilice nunca el instrumento en una toma de alimentación que no esté conectada a tierra. No utilice nunca un cable de alimentación que no haya sido suministrado por** Agilent Technologies **y diseñado para su zona geográfica.**

AVISO **No utilice nunca cables que no hayan sido suministrados por** Agilent Technologies **para asegurar un correcto funcionamiento así como el cumplimiento de las normativas de seguridad o de compatibilidad electromagnética.**

Operación

Antes de conectar el instrumento a la alimentación eléctrica, debe seguir las instrucciones de instalación para hacer funcionar el instrumento con seguridad:

No retirar las cubiertas del instrumento mientras esté funcionando.
Antes de encender el instrumento, todos los cables de tierra, alargadores, auto-transformadores y aparatos conectados al mismo deben conectarse a tierra mediante un enchufe adecuado. Si se interrumpe la conexión a tierra, podrían producirse lesiones graves. Siempre que se sospeche que la conexión a tierra se ha interrumpido, debe bloquearse el aparato para evitar cualquier manipulación.

Evitar todo mantenimiento del instrumento mientras esté bajo tensión.

No se deben sustituir los componentes del instrumento mientras esté encendido.

No manejar el instrumento en presencia de gases o vapores inflamables. El funcionamiento de cualquier instrumento eléctrico en estas circunstancias constituye un riesgo para la seguridad.

No instalar componentes de repuesto que no correspondan al instrumento ni realizar modificaciones no autorizadas.

Símbolos de seguridad

Tabla 13 Símbolos de seguridad empleados en los instrumentos y en los manuales

Símbolo	Descripción
	El instrumento aparece marcado con este símbolo para indicar que el usuario debe consultar el *Manual de usuario* con el fin de evitar posibles lesiones al operador y para proteger el equipo contra posibles daños.

Seguridad química y biológica

AVISO

Cuando se trabaje con disolventes, debe seguir los procedimientos de seguridad apropiados (por ejemplo gafas, guantes y ropa adecuada) descritos en las especificaciones sobre el tratamiento de material y datos de seguridad que suministra el proveedor, especialmente cuando se utilicen productos tóxicos o peligrosos.

AVISO

Si se tiene previsto utilizar muestras patógenas, tóxicas o radioactivas en este instrumento, es responsabilidad del usuario asegurar que se observen adecuadamente todos los reglamentos, directrices, precauciones y prácticas de seguridad. Esto también incluye la manipulación de organismos modificados genéticamente. Solicitar asesoramiento al responsable de seguridad del laboratorio acerca del nivel de contención necesario para cada aplicación y sobre los procedimientos adecuados de descontaminación o esterilización que deben seguirse si se producen escapes de líquidos de los recipientes.

Deben tenerse en cuenta toda la información de aviso impresa en los recipientes originales de las soluciones con anterioridad a su uso.

Puesto que las fugas, vertidos o pérdidas de muestras pueden generar aerosoles, deben tenerse en cuenta las precauciones de seguridad adecuadas.

Las cubiertas del Fraccionador 3100 OFFGEL de Agilent no se han diseñado como cierres biológicos herméticos para la contención de aerosoles o líquidos.

Los fluidos corporales deben manipularse con cuidado, ya que pueden transmitir enfermedades. Ninguna prueba conocida ofrece una certeza total de que estén exentos de microorganismos. Algunos de los más virulentos, como los virus de la hepatitis (B y C) y el HIV (I-V), microbacterias atípicas y determinados hongos sistémicos, ponen de relieve la necesidad de una protección contra los aerosoles.

Siempre se deben observar los reglamentos oportunos en materia de manipulación de sustancias biológicas peligrosas al desechar residuos biológicos de alto riesgo, ej. estructuras de compartimento contaminadas.

**Todas las muestras infecciosas se deben manipular de acuerdo con los procedimientos y métodos de laboratorio adecuados, con el fin de evitar la propagación
de enfermedades.**

Todos los residuos de soluciones y productos deben desecharse de acuerdo con directrices adecuadas de seguridad e higiene medioambientales.

9 Appendix

This chapter contains details on non-condensing conditions as well as ordering information for consumables and spare parts.

Non-condensing Conditions

The Agilent 3100 OFFGEL Fractionator is specified to be operated only under non-condensing conditions and with a cooling platform temperature of maximum 10 °C below ambient temperature.

For example, assuming a typically experimental setup of 20 °C on the cooling platform and an ambient temperature of 25 °C, the humidity must be below 73 %. A plot of humidity versus ambient temperature for the cooling platform at 20 °C, 25 °C and 30 °C is depicted in Figure 1. For non-condensing conditions the humidity has to be below the plotted curves.

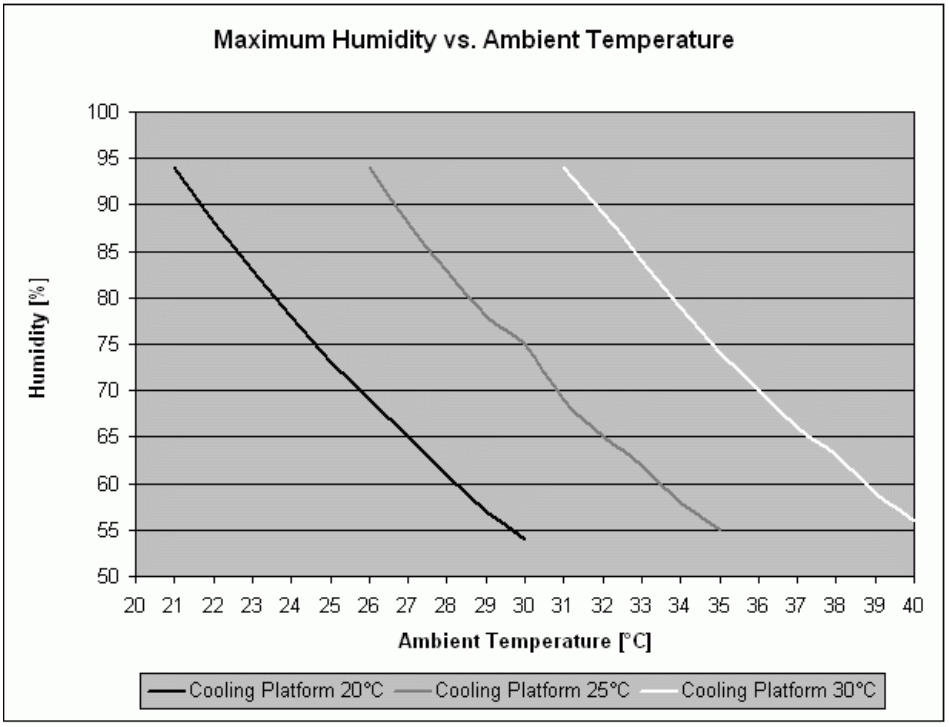


Figure 1 Plot of humidity versus ambient temperature assuming the cooling platform to be set at 20 °C, 25 °C or 30 °C. For non-condensing condition the humidity has to be below the plotted curves.

Ordering Information

List of Consumables

Table 14 Agilent 3100 OFFGEL Fractionator consumable kits

Product	Order Number	Product Description
3100 OFFGEL, 3-10, Low Res Kit	5188-6425	includes frames (12 well), IPG-strips pH 3-10 (13 cm), cover seals and fractionation reagents for 12 samples.
3100 OFFGEL, 3-10, High Res Kit	5188-6424	includes frames (24 well), IPG-strips pH 3-10 (24 cm), cover seals and fractionation reagents for 12 samples.
3100 OFFGEL, 4-7, Low Res Kit	5188-6427	includes frames (12 well), IPG-strips pH 4-7 (13 cm), cover seals and fractionation reagents for 12 samples.
3100 OFFGEL, 4-7, High Res Kit	5188-6426	includes frames (24 well), IPG-strips pH 4-7 (24 cm), cover seals and fractionation reagents for 12 samples.
3100 OFFGEL, Starter Kit	5188-6444	includes frames (6x 12 well, 6x 24 well), IPG-strips pH 3-10 (3x 13 cm, 3x 24cm), IPG-strips pH 4-7 (3x 13 cm, 3x 24cm), cover seals and fractionation reagents for 12 samples
OFFGEL, Protein test sample	5188-6428	includes beta-lactoglobulin as a protein test sample, enough for more than 100 tests.

List of Spare Parts

Table 15 Agilent 3100 OFFGEL Fractionator spare parts

Product	Order Number	Product Description
Tray Set	G3100-60007	includes 4 replacement trays
Electrode Assy	G3100-60002	includes 1 tray, 1 fixed electrode and 1 movable electrode
USB-Memory Stick Kit	G4208-68700	maximum capacity is 2GB
12 Well Frame Set	5188-8012	Set includes 12 well frames (12 wells), cover seals, electrode. pads, glycerol solution, cover fluid and manual. To be used with 13 cm IPG gel strips and respective ampholytes; for use with 5188-6444, 5188-6425, 5188-6427.
24 Well Frame Set	5188-8013	Set includes 12 well frames (24 wells), cover seals, electrode pads, glycerol solution, cover fluid and manual. To be used with 24 cm IPG gel strips and respective ampholytes; for use with 5188-6444, 5188-6424, 5188-6426.

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In This Book

Technical information about the Agilent 3100 OFFGEL Fractionator is given:

- Site and equipment requirements
- Fractionation principles
- Installation and configuration
- Using the browser interface
- Maintenance and Troubleshooting
- Spare parts and consumables
- Safety and related information

Printed in Germany
04/2010

G3100-90001

Agilent Technologies