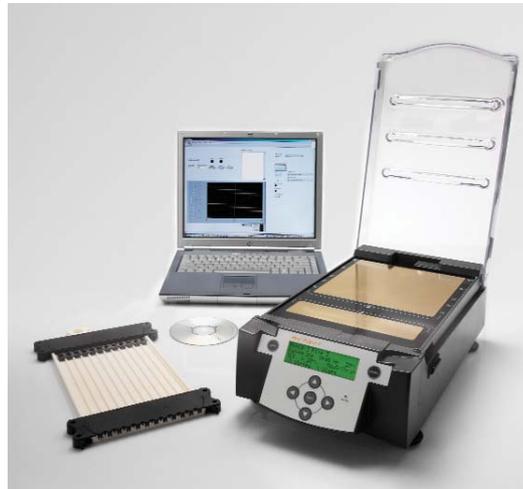


Ettan IPGphor II Control Software



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1. Introduction to Ettan IPGphor II Control Software

The Ettan™ IPGphor™ II Control Software is used to control the Ettan IPGphor II Isoelectric Focusing Unit from a PC. From the PC, protocols can be created, stored, and loaded to the Ettan IPGphor II. While isoelectric focusing of the Immobiline™ DryStrip gels progresses, the software records the isoelectric focusing (IEF) parameters over time. When the IEF run is completed, graph and log files are produced to document all steps of the IEF run. The graph and log files can be printed or saved. If desired, the log file can be exported to programs such as Microsoft® Excel®.

Multiple Ettan IPGphor II units may be controlled from one PC by using a RS232 expansion module. Expansion modules are available from National Instruments. Each Ettan IPGphor II unit's individual IEF parameters are recorded independently. (See hardware requirements and installation instructions).

2. Ettan IPGphor II Control Software: computer requirements and installation

The Ettan IPGphor II Control Software is designed to work with PC's that have Windows® 98 SE (Second Edition) or higher, Windows NT® or Windows 2000.

3. Connecting the Ettan IPGphor II to the PC

Use the serial cable that comes with the Ettan IPGphor II Isoelectric Focusing Unit to connect the serial port located in back of the Ettan IPGphor II instrument to the appropriate COM port of the PC or RS232 expansion module.

4. Ethernet connections and web browser for monitoring the Ettan IPGphor II from a remote location.

One Ettan IPGphor II instrument can be monitored through a web browser. This is only true within an internal network setting and cannot be viewed external to your computer network. Internet Explorer® 5.5 or later is required to monitor the Ettan IPGphor II instrument from another PC. The web browser must be set for LAN settings and the IP address of the Ettan IPGphor II instrument must be accessible. Contact your network administrator for assistance in establishing the connection.

5. Hardware requirements

If multiple Ettan IPGphor II Isoelectric Focusing Units are to be controlled by the Ettan IPGphor II Control Software the user must add a RS232 expansion box. Such expansion modules are available from National Instruments. Select the appropriate expansion module to fit with your computer connections (e.g. USB to RS232, PCMCIA to RS232, Ethernet to RS232). Up to 4 Ettan IPGphor II units can be controlled from one PC.

6. Software installation

Insert the Ettan IPGphor II Control Software CD. The installation procedure will start up automatically. Follow the onscreen instructions. If the installer does not start up automatically, select “Run...” from the Windows Start menu and enter “X:\Install.bat” (where “X” designates the CD drive that contains the Control Software CD).

Installation performs the following actions:

1

National Instruments Program NI-VISA 2.6 “Run time engine” is installed first [visa260runtime.exe].

2

The uninstall Ettan IPGphor II Control Software program will start first if a previous version of the Ettan IPGphor II Control Software is already installed [setup.exe]

3

Install IPGphor [setup.exe].

The default installation of the Ettan IPGphor II Control Software will be on the local C drive within the folder titled “Amersham Biosciences”.

An additional folder labeled IPGphor will be created on the C drive. This is the default folder in which saved protocols and saved log files will be stored. The program also loads a sample file of a protocol and a log file. The sample files will be loaded in the folders C:\IPGphor\Protocols and C:\IPGphor\Logfiles respectively.



Installing and uninstalling the Ettan IPGphor II Control Software to control multiple Ettan IPGphor II Isoelectric Focusing Units

Although up to 4 Ettan IPGphor II units can be controlled from a single PC, 4 separate copies of the Control Software must be installed on that single PC. To carry out the installation of a 2nd copy of the Control Software, select the CD drive containing the Control Software CD, then open the folder “InstallIPGphor2” and double-click on InstallIPGphor2.bat.

If a 3rd and/or 4th copy needs to be installed, repeat the procedure by opening the folder InstallIPGphor 3 (or InstallIPGphor 4) and double-clicking on InstallIPGphor3.bat (or InstallIPGphor4.bat).

The first time the program is started the default port will be COM 1. Select a different COM path (2 through 8) for each Ettan IPGphor II Isoelectric Focusing Unit, depending on the COM ports installed on the computer. Contact your network administrator for more information.

Installing and uninstalling NI-VISA 2.6

Before you install NI-VISA 2.6, we strongly recommend that you uninstall any versions of VISA prior to NI-VISA 2.0.1. NI-VISA 2.6 should upgrade properly over NI-VISA versions 2.0.1 through 2.5.2.

After uninstalling NI-VISA, some DLLs and executables may remain in the NI-VISA directories. To complete the uninstall process, you must restart your machine.

Installing and uninstalling Ettan IPGphor II monitor/embedded feature on the WEB client

In this feature the monitoring of one Ettan IPGphor II unit is possible.

1

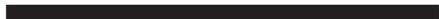
From the server computer go to the Option menu and select “Webserver enable”. Write down the TCP IP address from the pop-up message. You will need this for step 2 below. Exit the Ettan IPGphor II Control Software and restart for the change to take effect.

2

Go to the client computer. Open the web browser and enter the address <http://xxx/IPGphor#.htm>, where xxx is the TCP IP address of the server computer (obtained in step 1 above), and IPGphor# is the file name that is found on the window title of the Ettan IPGphor II Control Software that is webserver enabled (possible alternatives are IPGphor, IPGphor1, IPGphor2, IPGphor3, and IPGphor4).

Note: The uninstaller application will not notify you when rebooting is necessary. If you plan to re-install NI-VISA, you should always reboot the machine after running the uninstaller.

Note: To use the printing feature in Ettan IPGphor II Control Software, Internet Explorer 5.5 or later must be installed.



Ettan IPGphor II Control Software

7. Overview

The Ettan IPGphor II Control Software can be used to control up to 4 Ettan IPGphor II units at one time, each running a different set of run parameters. In addition to creating, saving and editing protocols the software monitors voltage, current and VHrs (volt-hours) of the run and graphs them as the run proceeds over time. The x and y scale values used to graph the Log Data change over time and are dependent on the maximum values reached. Contextual help will appear as the mouse is moved across active buttons, to guide the user in navigating and using the software. More information for several of these functions can be obtained by right clicking over the active button/hot spot.

8. Opening the Ettan IPGphor II Control Software

From the Windows Start menu select “Programs”. Select the IPGphor program from the menu. When controlling multiple Ettan IPGphor II units off one PC, each individual Ettan IPGphor II Control Software program (2, 3, or 4) must also be started from the Programs menu.

9. Communication between the Ettan IPGphor II and PC

When the Ettan IPGphor II Control Software is initiated the software confirms it is in communication with the Ettan IPGphor II Isoelectric Focusing Unit. If successful, a window will pop-up indicating communication has been established.



If the software does not recognize connection between it and the Ettan IPGphor II Isoelectric Focusing Units then an error dialog will appear.

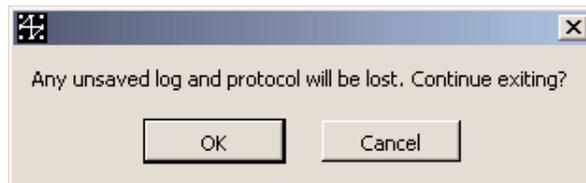


Note: Check to see that no other program is trying to access the communication port. Close all programs that may be accessing the COM port.

Note: If communication between the PC and the Ettan IPGphor II Isoelectric Focusing Unit is broken the software will not recognize a communication error but will no longer be collecting the data output from the instrument. Check the connections. Once connection is reestablished, the software will continue collecting data. Closing the program is not necessary.

Note: In the event of a power failure the software cannot collect data from the Instrument and the data will not be recoverable.

Check that the cable between the Ettan IPGphor II Isoelectric Focusing Unit and the PC is connected properly. Communication can be confirmed by selecting “Report” or “ID” from the Run drop-down menu. Communication can also be checked by exiting and then restarting the Ettan IPGphor II Control Software program. To exit the Ettan IPGphor II Control Software program, select “Exit” from the Logfile drop-down menu, use the control keyboard commands Ctrl + Q or press the X button located in the top right corner of the window. The software will notify the user that any unsaved protocols that may have been created will be lost.



Click OK to continue to exit as the software must be exited and connection to the instrument established before proceeding.

10. The Ettan IPGphor II Control Software

The Ettan IPGphor II Control Software has two main components; an IPGphor application window and a Protocol handling dialog. The IPGphor application window is used when controlling the Ettan IPGphor II Isoelectric Focusing Unit, viewing the data as the IEF run progresses and printing or exporting collected data. The second Protocol handling dialog is visible when Edit Protocol is selected. The Protocol handling dialog allows for creating, saving and loading protocols. The IPGphor application window and Protocol handling dialog can be resized, maximized or minimized.

11. IPGphor application window

Once the Ettan IPGphor II Software Control program is launched the application window will appear. In this main window the Ettan IPGphor II is controlled and readings taken at selected intervals to be logged and graphed.

This window also allows the user to:

- Open and view stored log files for previous runs
- Save, print and export the collected data
- Open the Protocol handling dialog to create new protocols
- Start, stop and pause the Ettan IPGphor II Isoelectric Focusing Unit being controlled
- Report the status of the Ettan IPGphor II Isoelectric Focusing Unit (instantaneous report of run conditions when requested)
- Capture the ID of the instrument (serial number and firmware version)
- Select the default log file directory
- Set the baud rate and the communication port
- Adjust the graph update interval
- Select the protocol to be run
- Enable the PC coupled to the Ettan IPGphor II Isoelectric Focusing Unit to be monitored remotely through a web browser

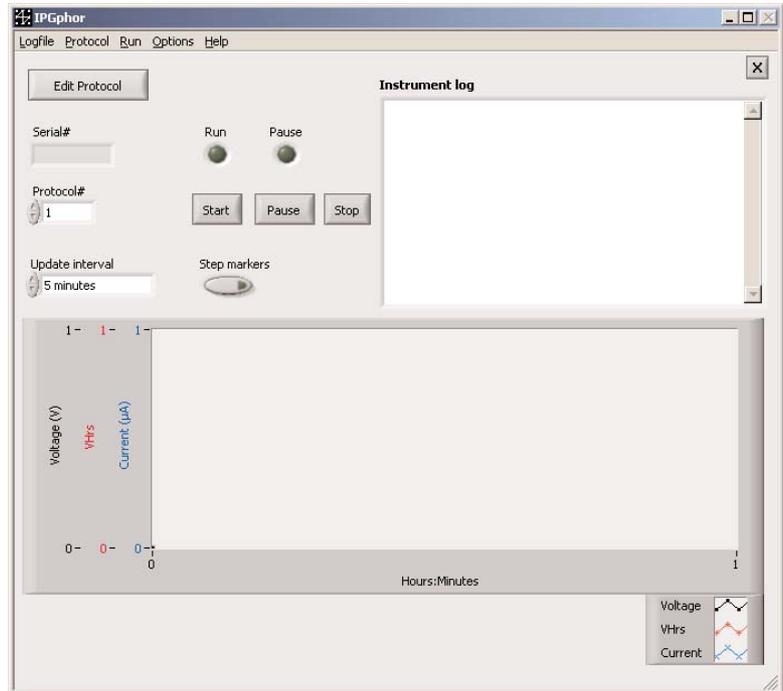


Fig 4. IPGphor application window.

Description of IPGphor application window (Fig 4)

Instrument Log

The instrument log records data retrieved from the Ettan IPGphor II Isoelectric Focusing Unit once a run has started.

Edit protocol

The edit protocol button takes the user to the Protocol handling dialog. Here the user can create, save, load and store new protocols as well as access previously saved protocols.

Serial

Indicates the serial number of the Ettan IPGphor II Isoelectric Focusing Unit that is controlled by the software.

Protocol

Indicates the Protocol # selected to start from the Control Software.

Step markers

When activated, marks the locations of a step change by placing the vertical gridlines on the graph at the time of a step change. When inactive uniformly spaced vertical gridlines are displayed on the graph.

Update interval

Changes the time interval at which the software captures data from the Ettan IPGphor II Isoelectric Focusing Unit.

Start, Pause and Stop

Controls the Ettan IPGphor II Isoelectric Focusing Unit.

Run and Pause indicators

Indicates the status of the Ettan IPGphor II Isoelectric Focusing Unit as it is monitored by the Control Software.

Graph

The Ettan IPGphor II Control Software graphs the data (Voltage, Volt-hours and Current) as it is retrieved from the Ettan IPGphor II Isoelectric Focusing Unit. The x and y scale values change to the maximum value as the run progresses. The default colors for the data markers and trendlines are black for the Voltage, red for the accumulated VHrs and light blue for the Current in μA .

Ettan IPGphor II Control Software application window menus and keyboard commands

Drop-down menus at the top left of the Control Software application window can be accessed by clicking on the selected header to view the drop-down menu options or can be accessed through the following keyboard commands.

menu bar		description
Logfile		
<u>O</u> pen	Ctrl+O	Opens a log file (from a previously saved text file).
<u>S</u> ave	Ctrl+S	Saves captured run data as a text file.
<u>P</u> rint	Ctrl+P	Prints the current log data shown in the Instrument Log window.
Print <u>G</u> raph	Ctrl+G	Prints the graph currently displayed.
Print <u>A</u> ll	Ctrl+A	Prints the Instrument Log data and the graph currently displayed.
<u>E</u> xport	Ctrl+E	Exports log data to spreadsheet.
<u>E</u> xit	Ctrl+Q	Stops and exits the program.
Protocol		
Open Protocol Window	Ctrl+W	Opens the Protocol handling dialog.
Run IPGphor control functions		
<u>S</u> tart	Ctrl+1	Starts a protocol (indicate no. of IPG strips to be run).
<u>S</u> top	Ctrl+3	Stops a protocol.
<u>P</u> ause	Ctrl+2	Pauses a protocol.
<u>R</u> eport		Retrieves current data from the Ettan IPGphor II instrument and displays it in the Instrument Log window.
<u>I</u> D		Retrieves the Ettan IPGphor II instrument serial number & firmware revision.
Options		
<u>F</u> olders	Ctrl+F	Allows the user to set the default folders for log files.
<u>P</u> ort		Set serial port options and Baud rate. (Com port number; 1200 or 9600 Baud)
<u>G</u> raph Update Interval		Selectable update rates at 1, 2, 3, 4, 5, 10, 15, 20, 25 or 30 minute intervals.
Webserver <u>E</u> nable		Starts a Webserver. Monitoring the Ettan IPGphor II Control Software application window from a Webserver is possible. To activate the Webserver the Ettan IPGphor II Control Software program must be exited and restarted.
Webserver <u>D</u> isable		Stops the Webserver. To deactivate the Webserver, the Ettan IPGphor II Control Software program must be exited and restarted.
Help		
<u>H</u> elp	Ctrl+F1	Help on use of the software.
<u>A</u> bout		Program ID information.

Using the Tab key on the PC can scroll active or editable fields. Shift+Tab will move the cursor back.

Setting Serial Interface Parameters

The baud rate and the communication port used to communicate with the Ettan IPGphor II Isoelectric Focusing Unit can be set or checked by selecting “Port” under the Options drop-down menu. A baud rate of 1200 or 9600 can be used but must match the baud rate set on the unit. A baud rate of 1200 is the default setting on the Ettan IPGphor II Isoelectric Focusing Unit.

Setting the default folder

The default location to which log files will be saved is set to C:\IPGphor\Logfiles. This option can be changed by selecting “Folder” under the Options drop-down menu. Navigate to the folder or location you wish for saved data to be stored and click on the **Select Cur Dir** button. Whenever files are saved from the IPGphor application window, the new default folder will be displayed as the new location for files to be saved.

Webserver enable

The Ettan IPGphor II Control Software can be used to monitor an Ettan IPGphor II Isoelectric Focusing Unit from another PC. In order to monitor the Ettan IPGphor II Isoelectric Focusing Unit the Webserver must be enabled and the Ettan IPGphor Control Software program closed and restarted. Internet Explorer 5.5 or later is required.

On the server computer select “Webserver Enable” from the Options drop-down menu. Record the http:// (TCP IP) address as indicated on the pop-up window. Close the Ettan IPGphor II Control Software. The Ettan IPGphor II Control Software program should now be restarted.

In the remote PC, open the web browser. For Internet Explorer select “Internet Options” under the Tools menu bar and select the Connections tab. Select LAN settings. Mark bypass proxy server for local addresses. Enter the web address http://xxx/IPGphor#.htm where xxx is the TCP IP address of the server computer and IPGphor # is the Ettan IPGphor II software installed file (IPGphor, IPGphor 1, IPGphor 2, IPGphor 3 or IPGphor 4).

If established properly, the Ettan IPGphor II Control Software application window should be displayed and the Ettan IPGphor II Isoelectric Focusing Unit run can be monitored from a remote location.

Webserver disable

Selecting “Webserver Disable” from the Options drop-down menu disables Webserver. The program needs to be closed and restarted for the change to take effect.

ID

Selecting “ID” from the Run drop-down menu can capture the serial number of the Ettan IPGphor II Isoelectric Focusing Unit currently controlled by the software. The ID of the instrument controlled will be displayed in the Instrument Log window. This is helpful when multiple Ettan IPGphor II units are controlled from one PC.

12. Protocol handling dialog

To create a protocol click the **Edit Protocol** button in the top lefthand corner of the Ettan IPGphor II Control Software application window or select “Open Protocol Window” from the Protocol drop-down menu.

The Protocol handling dialog will open to allow the user to create an IEF protocol (Fig 5).

The window will appear with default values of 0 for the rehydration time, gradient steps, 20 °C for the rehydration and IEF set temperature. 100 Volts, 50 μ A and 5 minutes run time are the default settings for step 1. The values for these parameters can be changed by either using the up and down buttons  to the left of each field or by inserting a new value for the field.

To change the time of the step it frequently is quicker to highlight and enter the desired value by using your keyboard than it is to use the up and down arrows to change the value.

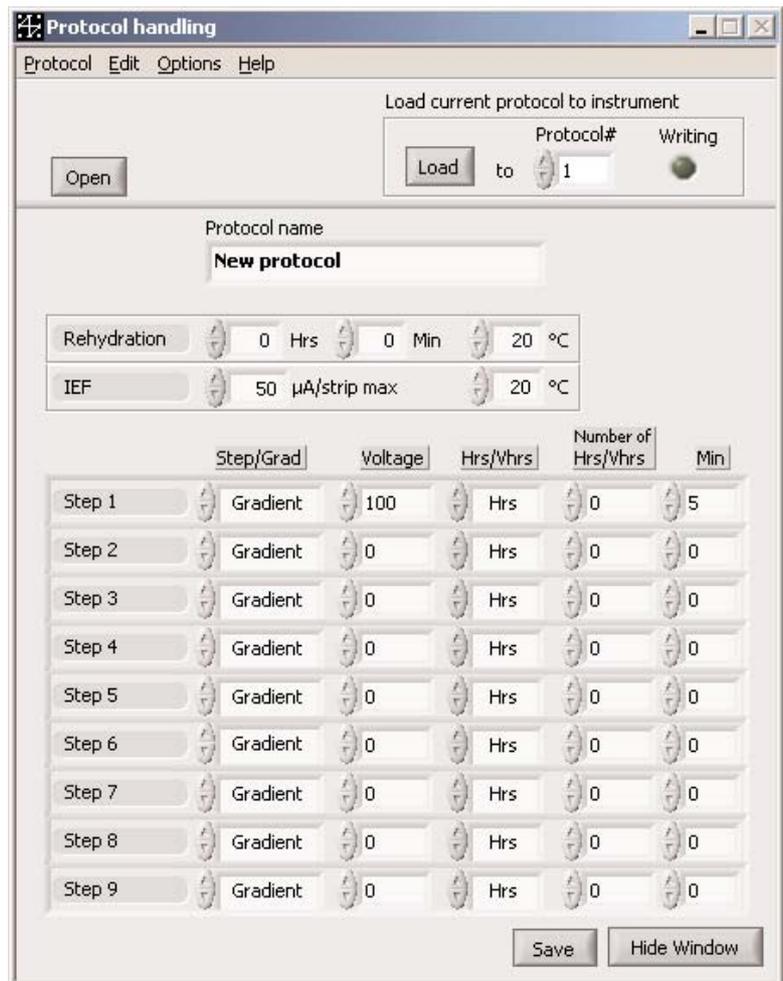


Fig 5. The Protocol handling dialog.

Protocol handling dialog: menus and keyboard commands

Drop-down menus at the top left of the Protocol handling dialog can be accessed by clicking on the selected header to view the drop-down menu options or can be accessed through the following keyboard commands.

menu bar		description
Protocol		
<u>N</u> ew	Ctrl+N	Creates an "empty" protocol.
<u>O</u> pen	Ctrl+O	Opens a protocol from file.
<u>S</u> ave	Ctrl+S	Saves captured run data.
<u>P</u> rint	Ctrl+P	Prints the current protocol.
<u>L</u> oad	Ctrl+L	Loads the current protocol displayed to the Ettan IPGphor II Isoelectric Focusing Unit.
<u>L</u> oad All		Loads retrieved protocols (files 1–10) to the Ettan IPGphor II Isoelectric Focusing Unit (batch).
<u>R</u> etrieve All		Retrieves all stored protocols (files 1–10) from Ettan IPGphor II Isoelectric Focusing Unit (batch).
<u>H</u> ide <u>W</u> indow	Ctrl+W	Stops and exits the dialog.
Edit		
<u>C</u> u <u>t</u>	Ctrl+X	Cuts selected text.
<u>C</u> o <u>p</u> y	Ctrl+C	Copies selected text.
<u>P</u> aste	Ctrl+V	Pastes text.
Options		
<u>F</u> olders	Ctrl+F	Allows the user to set the default folder for saved protocol files.
Help		
<u>H</u> elp	Ctrl+F1	Help on use of the software.
<u>A</u> bout		Program ID information.

Using the Tab key on the PC allows the cursor to scroll through active or editable fields. In the Protocol handling dialog, Ctrl+↓ will move the cursor down into the editable fields then Tab will allow scrolling through those fields. Ctrl+↑ will move the cursor out of the individual step lines. Shift+Tab allows the cursor to scroll backwards.

13. Creating a new protocol

Protocol name

To name a protocol, highlight and delete the words “New protocol” and then enter the name you wish to identify the file by.

Protocol name
New protocol

The filename can contain an indefinite number of characters but only the first 16 characters will be displayed on the Ettan IPGphor II Isoelectric Focusing Unit display.

For example, the protocol could be identified with the length of the IPG strip and the pH range that is being run.

Protocol name
24 cm 5.5-6.7

Range of parameters allowed for each step

Rehydration time:

Rehydration time can be set from 1 minute to 99 hrs:59 minutes.

Temperature during rehydration:

Temperature during rehydration can be set between 15 °C and 30 °C. This value can be controlled in 1 °C increments.

µA:

The µA limit of the IEF run can be set from 1 to 200 µA/strip. 50 µA/strip is the recommended value for IEF for all IPG strips. This value can be changed from 1–200 µA in 1 µA increments.

Temperature of the run:

Temperature during IEF can be set between 15 °C and 30 °C. This value can be controlled in 1 °C increments. Since strips are run in high concentrations of urea, colder temperatures can lead to formation of urea crystals that block proper focusing. Ideally, IPG strips should be run at temperatures between 20 °C and 25 °C.

Step/Grad

The two options for the voltage change pattern are “Step”, which sets the voltage at the selected value for the new step and then holds the voltage constant for the step duration; and “Gradient,” which increases the voltage linearly with respect to time from the value set in the previous step to the value set for the current step. If the first step is set in gradient mode, the voltage will increase starting from 0 Volts.

Note: Depending on the μA limit of the IEF run, the conductivity of the IPG strip, rehydration solution and the sample Ettan IPGphor II Isoelectric Focusing Unit may not operate at the set maximum voltage.

Voltage:

The voltage of any step may be set from 1–8000 Volts. A value of 0 in this field will cause the program to end regardless of what values are set for any subsequent steps.

Hrs/VHrs:

Each step of the protocol can be programmed to run for a specified number of hours (in hours and minutes) or for a specific number of VHrs. Volt-hours is the product of the voltage multiplied by the number of hours for each step.

Each VHrs step may be set from 1–99,999 VHrs. If more than 100,000 VHrs is desired it will have to be split between two steps.

The maximum value for Hours and minutes is 99 hours and 59 minutes. At least 1 minute or 1 Vhr must be programmed for a step or the program will end at that step regardless of what values are set for any subsequent steps.

Editing fields

Editing modes for Hrs/VHrs and Step/Gradient

The Hrs/VHrs and Step/Gradient fields each have two options which can be selected by using the up/down arrows located to the left of each field. Alternatively, clicking on one of these fields produces a pop-up menu, allowing you to select one of the options.

For example: To change the voltage transition mode from gradient to step-n-hold mode, click in the Step/Grad field you wish to change. A pop-up menu will appear with the two modes allowed. Select a mode by holding down the mouse and highlighting the mode desired. Unclick the mouse and the selected mode will appear in the window.



In the VHrs mode, the dialog will change slightly as the two fields (hrs and minutes) are not required to program this step. Only the total VHrs for that step will be entered.

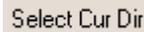


Editing numbers in the Voltage, Hrs, VHrs and minutes fields.

For fields that require a time or number to be entered, use the up and down  buttons to the left of the field to change the value 1 digit at a time. Alternatively, you can highlight and use the keyboard to enter the desired value.

Saving protocols

Once a protocol is created it can be saved for future reference. To save a file either click on the  button, use the keyboard shortcut Ctrl + S or select “Save” from the Protocol drop-down menu. The default location for the saved files is in the local C directory under C:\MPGphor\Protocols. The file will be specified by the protocol name and saved as a custom pattern Excel file (.xls).

If desired, the user can change the default directory by selecting “Folder” under the Options drop-down menu. The default protocols folder will be shown. If desired, navigate to the location where files are to be stored and click on the  button.

Printing Protocols

A protocol may be printed from the Protocol handling dialog. To print a protocol, either use the keyboard command Ctrl + P or select “Print” from the Protocol drop-down menu.

A dialog box will appear. Select the printer, the print orientation (Portrait or Landscape), and the number of copies.

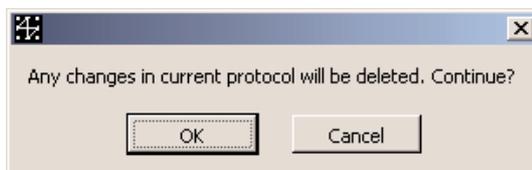


Printer and print orientation selections can be made either by clicking on the up/down buttons located to the left of the respective fields to scroll through the available choices, or by clicking in the field to produce a pop-up menu. Select the number of copies by using the up/down buttons located to the left of that field.

Opening previously saved protocols

Multiple numbers of protocols can be saved. Once saved protocols can be recalled either by clicking the **Open** button, by using the keyboard command Ctrl + O or by selecting “Open” from the Protocol drop-down menu.

Any steps programmed in the current protocol file will be deleted and the software prompt notifies the user of this action.



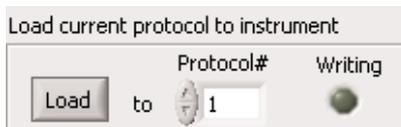
If changes to the current protocol do not need to be saved click **OK** and continue. To save any changes to the current protocol, click **Cancel** to abort opening the stored protocol. Save the current protocol before attempting to reopen the stored protocol.

To continue opening a stored protocol select the saved file from the Protocols folder and click the **Open** button. The stored protocol will now be displayed in the Protocol handling dialog.

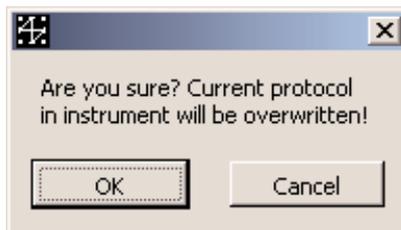
If necessary, make any adjustments to the values in the fields before proceeding to load the protocol.

Loading protocols

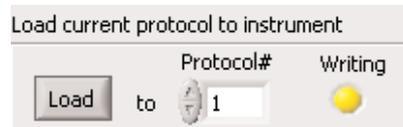
To load the protocol displayed in the Protocol handling dialog first select a Protocol #. The Protocol # (1–10) chosen here designates the protocol number on the Ettan IPGphor II Isoelectric Focusing Unit which will be overwritten by your loaded protocol. Select the Protocol # by using the up/down buttons located to the left of the field.



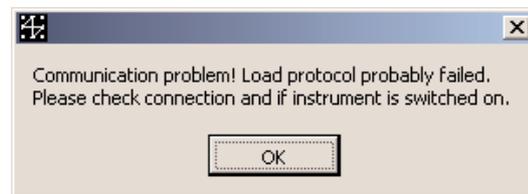
To load a protocol, either click the **Load** button select “Load” from the Protocol drop-down menu or use the keyboard command Ctrl + L. The software will prompt the user that the protocol occupying that protocol number on the Ettan IPGphor II Isoelectric Focusing Unit itself will be overwritten. If this is acceptable, click **OK** to continue.



A yellow highlighting of the Writing icon indicates the protocol is being loaded. The IPGphor application window is updated with the loaded protocol.



If the PC and Ettan IPGphor II Isoelectric Focusing Unit are in communication the protocol will be loaded into the designated protocol number on the instrument. The display on the Ettan IPGphor II will indicate the new file name and save the protocol into the protocol number that was designated when loading.



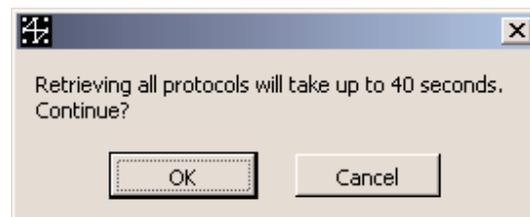
If the PC and Ettan IPGphor II Isoelectric Focusing Unit are not in proper communication an error message will appear. Check that the cable between the Ettan IPGphor II Isoelectric Focusing Unit and the PC is connected properly. Communication can be confirmed by selecting “Report” or “ID” from the Run drop-down menu.

Retrieving all preprogrammed IPGphor protocols

Files that exist within the Ettan IPGphor II Isoelectric Focusing Units memory can be captured to the PC. This allows the archiving of protocols that are no longer frequently used and frees up needed file space on the Ettan IPGphor II Isoelectric Focusing Unit.

Under the Protocol drop-down menu select “Retrieve all”. A communication window will appear to confirm the action.

Click  to continue to retrieve the files.



The software will indicate it is reading protocols (1–10) by displaying a message in the top portion of the dialog.



Files are saved under the default protocol folder with the default name: Instrument protocols, date and time.

A message displayed in the dialog box indicates protocols (1–10) on the Ettan IPGphor II Isoelectric Focusing Unit are being retrieved.

Once retrieved the user is prompted to save the protocols to disk with a “Save As...” dialog box. Files are saved within the default protocol folder with a default name: Instrument protocols *date time*. The files are stored as a custom pattern (Excel) text file.

Null protocols retrieved from the Ettan IPGphor II Isoelectric Focusing Unit (i.e., those lacking any steps) are not saved. In order to be saved to disk a protocol must contain programmed steps.

If the Ettan IPGphor II Isoelectric Focusing Unit is not in proper communication with the computer, an error message will appear.

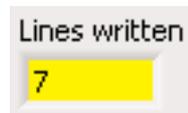


Check that the cable between the Ettan IPGphor II Isoelectric Focusing Unit and the PC is connected properly. Communication can be confirmed by selecting “Report” or “ID” from the Run drop-down menu.

Loading retrieved Ettan IPGphor II protocols

“Load all” allows the user to reload stored protocols (1–10) to the Ettan IPGphor II Isoelectric Focusing Unit that have been retrieved as indicated in the section above. When “Load all” is selected from the Protocol drop-down menu (Protocol handling dialog), the software opens the Protocols folder on the C drive. Select the instrument protocol set you wish to load.

A message in the top portion of the window indicates the protocols (1–10) are being loaded and displays the number of lines written to the Ettan IPGphor II Isoelectric Focusing Unit.



Closing the Protocol handling window

Once a protocol has been loaded, click on the  button to close the Protocol handling dialog.

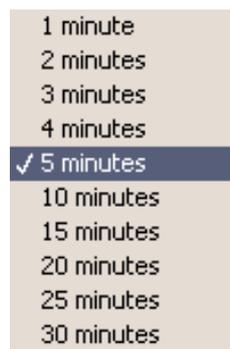
14. Running a protocol

Once a file has been created and loaded to the Ettan IPGphor II Isoelectric Focusing Unit the instrument can be controlled by either using the software or by manually starting the instrument using the IPGphor keypad.

Prior to starting the software confirm the protocol to be run. The software automatically prepares to run the last protocol loaded (written) to the instrument. Select the correct protocol # to run by confirming the protocol on the Ettan IPGphor II Isoelectric Focusing Unit display. If the correct protocol is not on the display, use the Ettan IPGphor II Isoelectric Focusing Unit keypad or use the up/down button located to the left of the # field to select the desired protocol.

Adjusting the log file data capture time

The time interval that data is captured from the Ettan IPGphor II Isoelectric Focusing Unit can be adjusted by changing the Update interval on the IPGphor application window. Use the up/down button to adjust the interval or click on the time field and select one of the time options with the pop-up menu.



You can also adjust the interval by selecting “Graph update interval” from the Options drop-down menu. Allowed intervals are 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes. For long runs it is recommended to set the interval for at least 10 minutes to avoid Instrument log data printouts that are many pages long.

Once everything has been properly set, the software can be used to control the Ettan IPGphor II Isoelectric Focusing Unit.

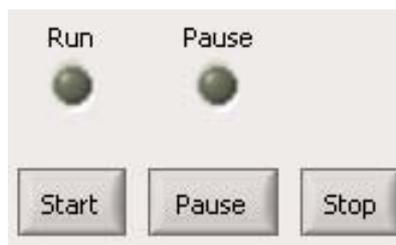
Note: Pressing **Start** on the Ettan IPGphor II Isoelectric Focusing Unit causes the software to automatically save the data displayed in the application window. The file is saved in the default directory with the name of the protocol, the date and the time the file was saved. For example:

18 cm 6–11 05.15.2003 03.54.59 PM.txt).

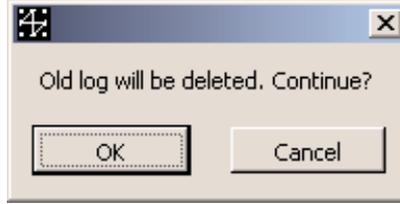
To avoid autosaving the data, start the Ettan IPGphor II Isoelectric Focusing Unit using the software. The software prompts the user to select whether the displayed data should be deleted.

Starting a run

To start the Ettan IPGphor II Isoelectric Focusing Unit either click the  button in the application window, select “Start” from the Run drop-down menu, use the keyboard command Ctrl + 1 or press **Start** on the Ettan IPGphor II Isoelectric Focusing Unit.



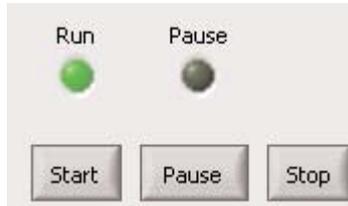
Old file logs from the last run are deleted prior to the instrument starting the run. The software notifies the user of this action.



The instrument prompts the user to indicate how many IPG strips will be run.



The indicator icon for Run changes to green when the run has been started.



Note: If communication between the PC and the Ettan IPGphor II Isoelectric Focusing Unit is broken the software will not recognize communication error but will no longer be collecting the data output from the instrument. Check the connections. Once connection is reestablished, the software will continue collecting data. Closing the program is not necessary.

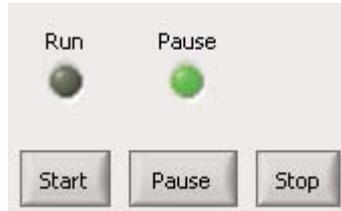
Note: In the event of a power failure the software cannot collect data from the Instrument and the data will not be recoverable.

The Ettan IPGphor II Isoelectric Focusing Unit display shows the run has started and the red HV light turns on.

While the run is starting the Instrument Log displays the instrument ID, the firmware version, the name of the protocol, and the programmed steps of the protocol. Data is collected at time 0, at the occurrence of a step change, and at the desired time interval that has been set prior to the run. The update interval can be adjusted during the run but previous data points cannot be deleted or added.

The graph will be updated as data points are collected and the x and y axis scale will change as the run progresses.

To pause the protocol either click the  button on the application window, select “Pause” from the Run drop-down menu, use the keyboard command Ctrl + 2 or press the **Stop** button on the Ettan IPGphor II Isoelectric Focusing Unit control panel. The indicator icon for Pause changes to green while the Ettan IPGphor II Isoelectric Focusing Unit is in pause mode and the red HV light on the Ettan IPGphor II Isoelectric Focusing Unit turns off.



To stop the protocol either click the  button on the application window, select “Stop” from the Run drop-down menu, use the keyboard command Ctrl + 3 or press the **Stop** button twice on the Ettan IPGphor II Isoelectric Focusing Unit control panel. Both the Run and Pause indicator icons darken and the red HV light on the Ettan IPGphor II Isoelectric Focusing Unit turns off.

Monitoring the run

As the run progresses, the Ettan IPGphor II Isoelectric Focusing Unit conditions are recorded in the Instrument log window and the data points graphed in real time. The user may scroll through the log file values by using the scroll bar to the right of the log window.

Step markers

The Step markers button functions to indicate where a step transition has occurred (e.g. changing from step 1 to step 2, etc.). To mark where the steps have occurred, click the button so that the green indicator is on.



To return to uniform vertical gridline marks along the x axis click the button so that the indicator is darkened .



Note: Additional data points can be collected while the unit is running by selecting the “Report” from the drop-down menu. However collecting multiple data points over a 1 minute interval may result in multiple y values on the same plot.

15. Capturing run data

Storing log files

The log of a file can be stored and recalled for future reference. To store a log file, select “Save” from the Logfile drop-down menu. The default file-name is the date and time (e.g. 05.02.2003 01.59.43 PM) but this can be edited. The default location for the saved file is C:\IPGphor\Logfiles. These files can be reloaded into the software at any time to view data and graphs.

Printing log files and graphs

To print the log file only, select “Print” from the Logfile drop-down menu or use the keyboard command Ctrl + P.

To print the graph only, select “Print Graph” from the Logfile drop-down menu or use the keyboard command Ctrl + G*.

To print the log file and the graph, select “Print All” from the Logfile drop-down menu or use the keyboard command Ctrl + A*.

A dialog box will appear. Select the printer, the print orientation (Portrait or Landscape), and the number of copies.



Printer and print orientation selections can be made either by clicking on the up/down buttons  located to the left of the respective fields to scroll through the available choices, or by clicking in the field to produce a pop-up menu. Select the number of copies by using the up/down buttons located to the left of that field.

Exporting the log files to Excel

To export the log files to Excel either select “Export” from the Logfile drop-down menu or use the keyboard command Ctrl + E.

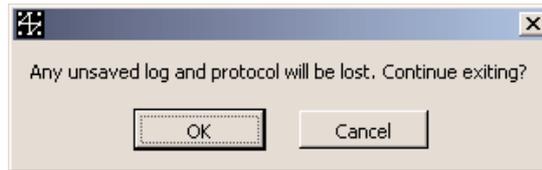
When opening in Excel, keep the default values (Delimited, Delimiters = Tab, Text Qualifier = “ and Column data = General).

*The size of the graph printed is dependent on the size the graph is viewed onscreen. To enlarge the graph either expand or maximize the Logfile window.

Note: Before exiting the Ettan IPGphor II Control Software make sure that all data collected has been saved, printed or exported. Closing the software will result in the loss of unsaved data.

16. Exiting the software

To close the program, either select “Exit” from the Logfile drop-down menu, use the keyboard command Ctrl + Q or press the X button in the top right corner of the window. When the software is closed a dialog box appears to confirm whether the program should be exited.



If the instrument is in run mode a different dialog box will appear.



When OK is clicked, a second dialog box requesting the user to stop the Ettan IPGphor Isoelectric Focusing Unit appears. Click to stop the unit and continue exiting.



Note: If communication between the PC and the Ettan IPGphor II Isoelectric Focusing Unit is broken the software will not recognize a communication error but will no longer be collecting the data output from the instrument. Check the connections. Once connection is reestablished, the software will continue collecting data. Closing the program is not necessary.

Note: In the event of a power failure the software cannot collect data from the Instrument and the data will not be recoverable.

17. Troubleshooting

Printing seems to hang when using the control software:

- Network printer was selected and the computer is not logged into the network. Log in, or if printing is no longer required press Cancel until the login dialog box disappears.

Cannot monitor through a web browser

- Only one Ettan IPGphor II Control Software copy can be monitored from a remote web browser. From the server computer go to the Option menu and Enable or Disable the Webserver as necessary. Restart the correct Ettan II IPGphor Software copy that correlates to the Ettan IPGphor II Isoelectric Focusing Unit to be monitored. Software copies are denoted by IPGphor(x) where (x) is the number (1–4) assigned to the Ettan IPGphor II Isoelectric Focusing Units being monitored.
- If a proxy server is used right click on Internet Explorer and select “Internet Options” under the Tools drop-down menu. Select the Connection tab. Select LAN settings. Mark bypass proxy server for local addresses or contact your network administrator. The viewer will only work within a firewall.

Communication error:

- Check that the Ettan IPGphor II Isoelectric Focusing Unit is switched on.
- Check that the serial cable is properly connected to the PC and the Ettan IPGphor II Isoelectric Focusing Unit.
- Check to see that no other program is trying to access the communication port. Close all programs that may be accessing the COM port.

Ordering information

product	quantity	code number
Ettan IPGphor II Isoelectric Focusing Unit		
Basic unit. Order strip holders separately	1	80-6505-03
Fuses, 1.6 A, 250V, 5 × 20 mm	5	80-6108-69
Cable Serial 9 Pin F/F with Null Modem Adapter	1	80-6506-36
Ettan IPGphor II Control software	1	80-6506-55
Strip Holder Covers		
7 cm	2	80-6455-44
11 cm	2	80-6455-63
13 cm	2	80-6455-82
18 cm	2	80-6456-01
24 cm	2	80-6479-76
Strip Holders for use with Immobiline DryStrip gels		
7 cm, complete	6	80-6416-11
11 cm, complete	6	80-6416-30
13 cm, complete	6	80-6416-49
18 cm, complete	6	80-6416-68
24 cm, complete	6	80-6469-88
7 cm, complete	1	80-6416-87
11 cm, complete	1	80-6417-06
13 cm, complete	1	80-6417-25
18 cm, complete	1	80-6417-44
24 cm, complete	1	80-6470-07
Manifold Kit	1	80-6498-38
Immobiline DryStrip Reswelling Trays		
7–18 cm	1	80-6371-84
7–24 cm	1	80-6465-32
IPG Buffer		
pH 4–7	1 ml	17-6000-86
pH 3–10 L	1 ml	17-6000-87
pH 3–10 NL	1 ml	17-6000-88
pH 3.5–5.0	1 ml	17-6002-02
pH 4.5–5.5	1 ml	17-6002-04
pH 5.0–6.7	1 ml	17-6002-05
pH 5.5–6.7	1 ml	17-6002-06
pH 6–11	1 ml	17-6001-78
Rehydration Solutions		
DeStreak Rehydration Solution	1 ml	17-6003-19
DeStreak Reagent	5 × 3 ml	17-6003-18

product	quantity	code number
Ettan preparation kits and reagents		
2-D Fractionation Kit	10 preps	80-6501-04
Albumin and IgG Removal Kit	10 preps	RPN6300
Protease Inhibitor	1 ml	80-6501-23
Nuclease Mix	0.5 ml	80-6501-42
Thiourea	100 g	RPN6301
Iodoacetamide	25 g	RPN6302
Sample Grinding Kit	50 samples	80-6483-37
2-D Clean-Up Kit	50 samples	80-6484-51
SDS-PAGE Clean-Up Kit	50 samples	80-6484-70
2-D Quant Kit	500 assays	80-6483-56
Mini Dialysis Kit, 1 kDa cut-off, 250 µl	1	80-6483-75
Mini Dialysis Kit, 1 kDa cut-off, 2 ml	1	80-6483-94
Mini Dialysis Kit, 8 kDa cut-off, 250 µl	1	80-6484-13
Mini Dialysis Kit, 8 kDa cut-off, 2 ml	1	80-6484-32
PlusOne™ reagents		
Urea	500 g	17-1319-01
CHAPS	1 g	17-1314-01
Triton™ X-100	500 ml	17-1315-01
Dithiothreitol (DTT)	1 g	17-1318-01
Bromophenol Blue	10 g	17-1329-01
Immobiline DryStrip Cover Fluid	1000 ml	17-1335-01
Immobiline DryStrip gels		
7 cm		
pH 4–7	12	17-6001-10
pH 3–10	12	17-6001-11
pH 3–10NL	12	17-6001-12
pH 6–11	12	17-6001-94
11 cm		
pH 4–7	12	18-1016-60
pH 3–10	12	18-1016-61
pH 6–11	12	17-6001-95
13 cm		
pH 4–7	12	17-6001-13
pH 3–10	12	17-6001-14
pH 3–10NL	12	17-6001-15
pH 6–11	12	17-6001-96
18 cm		
pH 4–7	12	17-1233-01
pH 3–10	12	17-1234-01
pH 3–10NL	12	17-1235-01
pH 3.5–4.5	12	17-6001-83
pH 4.0–5.0	12	17-6001-84
pH 4.5–5.5	12	17-6001-85
pH 5.0–6.0	12	17-6001-86
pH 5.5–6.7	12	17-6001-87
pH 6–9	12	17-6001-88
pH 6–11	12	17-6001-97

product	quantity	code number
24 cm		
pH 3.5–4.5	12	17-6002-38
pH 4.0–5.0	12	17-6002-39
pH 4.5–5.5	12	17-6002-40
pH 5.0–6.0	12	17-6002-41
pH 5.5–6.7	12	17-6002-42
pH 3–7 NL	12	17-6002-43
pH 3–10	12	17-6002-44
pH 3–10 NL	12	17-6002-45
pH 4–7	12	17-6002-46
pH 6–9	12	17-6002-47
Cleaning solution, IPGphor Strip Holder	950 ml	80-6452-78
2-D Electrophoresis Principles and Methods	1	80-6429-60
Equilibration tube set for 24 cm IPG Strips	12	80-6467-79
IEF electrode strips	100	18-1004-40

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