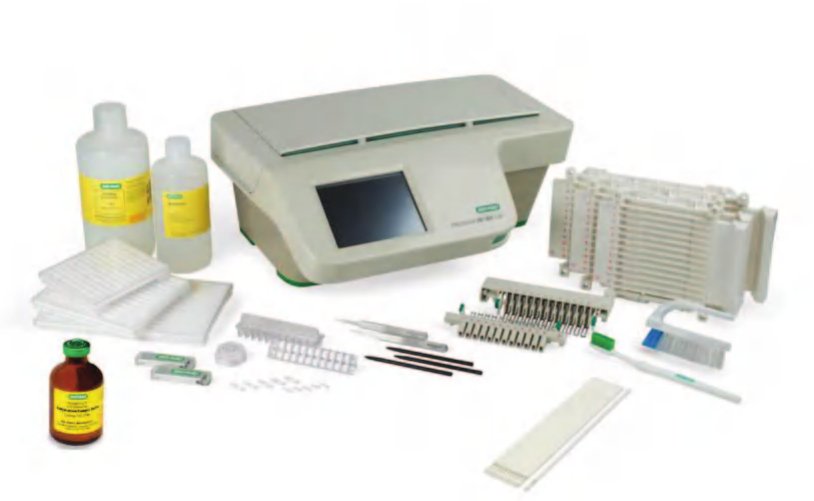
PROTEAN® i12™ IEF System

Instruction Manual

Catalog #164-6000, 164-6001



BIO-RAD

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Safety and Regulatory Compliance

This instrument has been certified to meet all applicable requirements of the EN61010-1 electrical equipment for measurement, control, and laboratory use standard and the class A standards for Electromagnetic Emissions, intended for laboratory equipment applications. It uses high output voltages that are electrically isolated from earth ground to minimize the risk of electrical shock to the user.

This product has also been tested to the requirements of CAN/CSA-C22.2 No. 61010-1, second edition, including Amendment 1, or a later version of the same standard incorporating the same level of testing requirements.

Certified products are safe to use when operated in accordance with the instruction manual.

Instrument Safety Warnings

This instrument should not be modified or altered in any way. Alteration of this instrument voids the warranty and safety certification and creates a potential safety hazard.

This instrument is intended for laboratory use only. Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent. Follow the safety specifications listed in this section and throughout this manual. Use only the power cord supplied with the instrument, making sure to choose the plug adaptor that corresponds to the electrical outlets in your region.

The following guidelines should be observed and followed:

- To ensure adequate cooling of the PROTEAN® i12™ IEF cell, be sure there is ≥6 cm clearance around the unit. Do not block the fan vents
- Do not use cloth or absorbent pads underneath the unit. These or other loose items may be pulled into the fan intake, causing damage to the unit due to overheating and voiding the warranty
- Connect the cell to a three-prong, grounded AC outlet using the three-prong AC power cord provided
- Do not operate the instrument in extreme humidity (>90%) or where condensation can short the internal electrical circuits of the cell. The PROTEAN i12 IEF cell has passed tests for operation at 10–31ºC, 0–90% relative humidity (noncondensing). Operating the cell outside these conditions voids the warranty
- Disconnect power to the PROTEAN i12 IEF cell before servicing. No user-serviceable parts are inside the instrument. Contact Bio-Rad service personnel for service
- Emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. Take appropriate measures to avoid interference

1 PROTEAN® i12™ IEF System

The PROTEAN i12 IEF system (Figure 1.1) is used for isoelectric focusing (IEF) on immobilized pH gradient (IPG) strips for the first dimension of two-dimensional (2-D) electrophoretic protein analysis. The PROTEAN i12 IEF cell can run 1–12 IPG strips in 7, 11, 13, 17, 18, and 24 cm focusing trays. Each channel in the i12™ focusing tray is powered by its own power supply, enabling precise control over each IPG strip. This makes it possible to run different sample types, different gradients, and multiple protocols all at the same time.

The i12 focusing trays and electrode assemblies accommodate all possible gel configurations (gel-side up or down, with or without electrode wicks). It also allows sample loading either by inclusion in the rehydration solution (in-gel loading) or with sample cups (sample cup loading).

The cell is fully programmable from the user interface; connection to an external computer is not required. Each protocol can contain up to ten steps in which voltage, manner of voltage ramping, current, and duration (hr or V-hr) are defined.

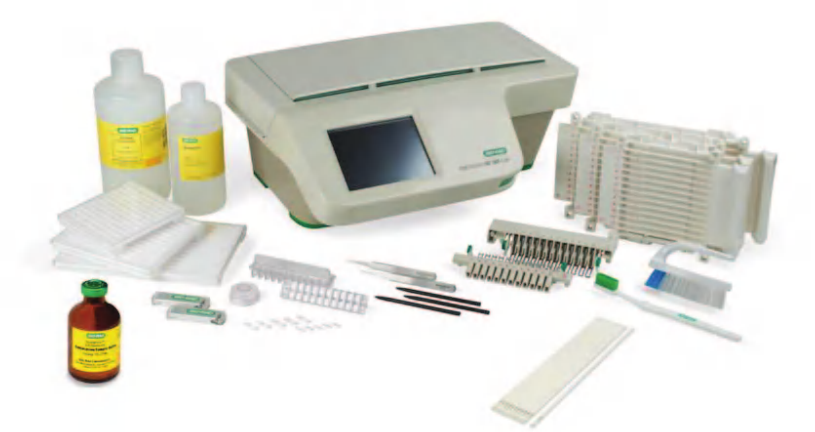


Fig. 1.1. PROTEAN i12 IEF system. The system includes the PROTEAN i12 IEF cell and numerous accessories.

Preprogrammed protocols stored in the internal memory serve as a convenient starting point for developing optimized, sample-specific IEF conditions. A USB flash drive can also be used as an alternative storage location or method of transfer for protocols and run data files.

PROTEAN i12 Reporter, a web-based application (www.i12reporter.com), is also available for uploading run data files, viewing electronic profiles for individual lanes, and comparing sample profiles from different runs. The application can be used to generate reports, print graphs, and create protocols. Protocols created with the application can be transferred to the PROTEAN i12 IEF cell using a USB flash drive.

1.1 System Components

The PROTEAN i12 IEF system comprises the PROTEAN i12 IEF cell, electrode assemblies, i12 focusing trays, and other accessories that make IEF and system maintenance possible.

1.1.1 PROTEAN i12 IEF Cell

The PROTEAN i12 IEF cell (Figure 1.2, Table 1.1) contains 12 individual power supplies, each dedicated to a single IPG strip. This individual power control for each lane allows use of IPG strips with different pH gradients and sample types, concentrations, and conductivities in a single run. It also allows programming of different protocols.

The touch-screen user interface is used to operate the cell, retrieve preprogrammed and user-defined protocols, create new or edit saved protocols, and access the internal memory for file management. New protocols, sample details, and run data are stored in the internal memory or on an external USB flash drive.

Table 1.1. PROTEAN i12 IEF cell components.

Component	Description
User interface	Controls the PROTEAN i12 cell; touch screen operated by hand, stylus, or mouse
Opaque lid	Protects light-sensitive labels from photobleaching
Safety lid	Safety interlock
Peltier platform	Holds one focusing tray and maintains temperature during the run
USB ports	4 USB ports (1 USB-A in front, 2 USB-A and 1 USB-B in back) for connection to a USB mouse and USB flash drive(s)
Power switch	For powering the cell on and off
Stylus storage	Slot for storage of stylus
Internal memory	System hardware; includes preprogrammed protocols and can be used to store user-defined protocols and data files
Leveling feet	Used to level the cell if needed

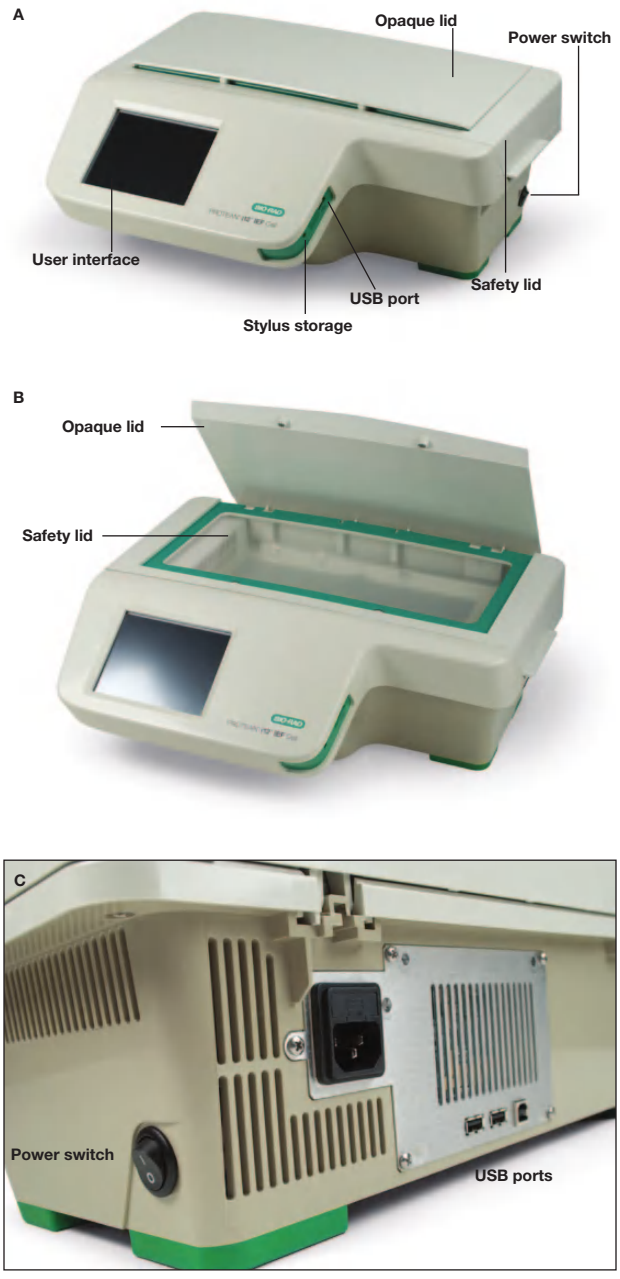


Fig. 1.2. PROTEAN i12 cell. A, Front view with all lids closed; B, Front view showing the safety lid closed and opaque lid open; C, Back view.

1.1.2 Accessories

The PROTEAN i12 IEF system includes the accessories listed in Table 1.2 and shown in Figure 1.3.

Table 1.2. Accessories for the PROTEAN i12 system. Accessories not included may be purchased separately (see Appendix G, Ordering Information).

Component	Quantity	Description
Included with the PROTEAN i12 IEF System		
i12 focusing trays with IPG strip retainers	1 each (7, 11, and 17 cm)	For IEF (and rehydration) of 1–12 IPG strips; each tray includes 2 IPG strip retainers to maintain contact between the IPG strip and electrodes for IEF with the gel-side down configuration
Electrode assemblies	1 set each	Positive (+) and negative (-) electrode assemblies; provide contact between each IPG strip and its power supply
i12 rehydration/equilibration trays	25 each (7, 11, and 17 cm)	Disposable trays for passive rehydration and equilibration of IPG strips
Electrode wicks	100 gel-side up 500 gel-side down	Collect salts and other charged impurities as well as proteins with isoelectric points (pI) outside the pH range of the IPG strip (use is recommended)
ReadyStrip™ IPG strips pH 3–10	12 each (7, 11, and 17 cm)	Medium for isoelectric separation of proteins
Mineral oil	500 ml	For overlay of IPG strips to prevent dehydration
Forceps	2 pair	For manipulation of IPG strips
Cleaning brushes	Set of 2	For cleaning the focusing tray and electrode assemblies
Cleaning concentrate	1 L	For cleaning the focusing tray and electrode assemblies
USB flash drive	2	Memory data storage device integrated with a USB interface; for storage and transfer of data from the PROTEAN i12 IEF cell
Stylus	3	Used to manipulate touch screen (user interface)
Leveling bubble	1	Indicates whether the IEF cell is level
Power cord	1	Connects the IEF cell to a power source
ReadyPrep™ rehydration buffer	10 ml	For rehydration and sample loading of IPG strips
Not Included with the PROTEAN i12 IEF System		
i12 focusing trays		(13, 18, and 24 cm) For IEF (and rehydration) of 1–12 IPG strips; each tray includes 2 IPG strip retainers
i12 rehydration/equilibration trays		(13, 18, and 24 cm) Disposable trays for passive rehydration and equilibration of IPG strips
Sample cup holder and sample cups		For cup loading of samples

Fig. 1.3. PROTEAN i12 accessories.

i12 Focusing Tray

Channels in the i12 focusing tray (Figure 1.4) hold IPG strips for IEF. Each focusing tray accommodates up to 12 IPG strips. Separate trays are available for 7, 11, 13, 17, 18, and 24 cm IPG strips. For flexibility, the PROTEAN i12 focusing tray and electrode assemblies accommodate all run configurations: gel-side down or up, with in-gel sample loading or sample cup loading. Each focusing tray holds one negative (-) and one positive (+) electrode assembly. Focusing trays can also be used for rehydration of IPG strips.

Electrode Assemblies

The electrode assemblies (Figure 1.4) each include 12 individual sets of negative and positive electrodes. The assemblies attach to the focusing tray to provide contact between each IPG strip and its power supply. They accommodate all focusing tray sizes.

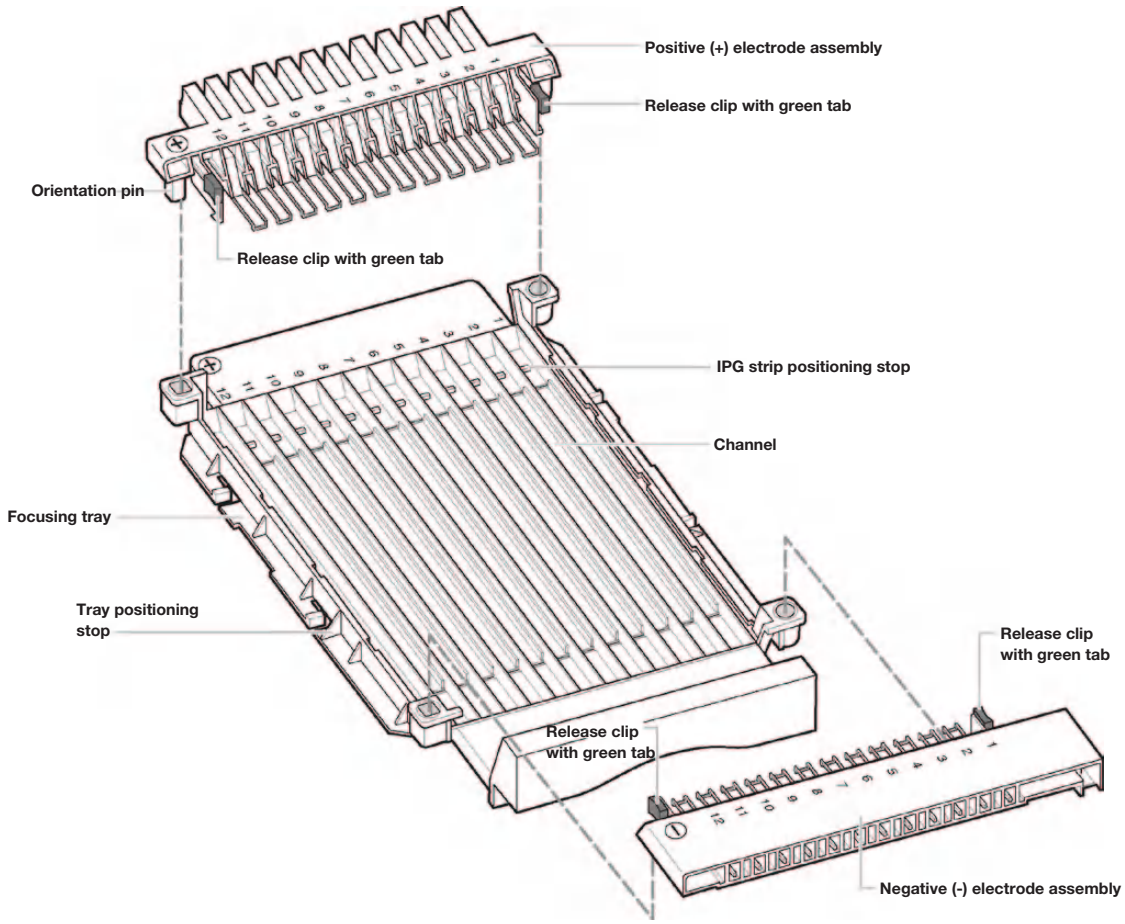


Fig. 1.4. PROTEAN i12 focusing tray and electrode assemblies.

1.2 Unpacking and Setup

1. Carefully inspect the shipping container for any damage that may have occurred during shipping. Severe damage to a container may indicate damage to its contents. If you suspect damage to the contents, immediately file a claim with the carrier in accordance with their instructions before contacting Bio-Rad Laboratories.
2. Open the shipping carton and lift the content out of its packing. Inspect the instrument for external damage. If any part is missing or damaged, contact Bio-Rad Laboratories immediately.
3. Place the PROTEAN i12 IEF cell on a firm, flat surface.
   a. Position the cell so that there is access to the USB ports (back panel) and power switch (right panel).
   b. To ensure adequate cooling, be sure that there is ≥6 cm clearance around the unit. DO NOT block the fan vents.
   c. Make sure the system is on a level surface. Place the leveling bubble in the center of the cooling platform and adjust the instrument leveling feet as needed.
4. Connect the cell to a three-prong, grounded AC outlet using the power cord provided with the cell.

Do not place cloth or absorbent pads underneath the instrument. These or other loose items may be pulled into the fan intake, causing damage to the unit due to overheating and voiding the warranty.

1.3 Workflow

Figure 1.5 summarizes the workflow described in this manual. For best results, use the reagents and protocols available in the ReadyPrep 2-D starter kit (catalog #163-2105) to familiarize yourself with the 2-D process and operation of the PROTEAN i12 IEF cell. For more details about performing 2-D electrophoresis, please refer to bulletin 2651, 2-D Electrophoresis for Proteomics: A Methods and Product Manual.

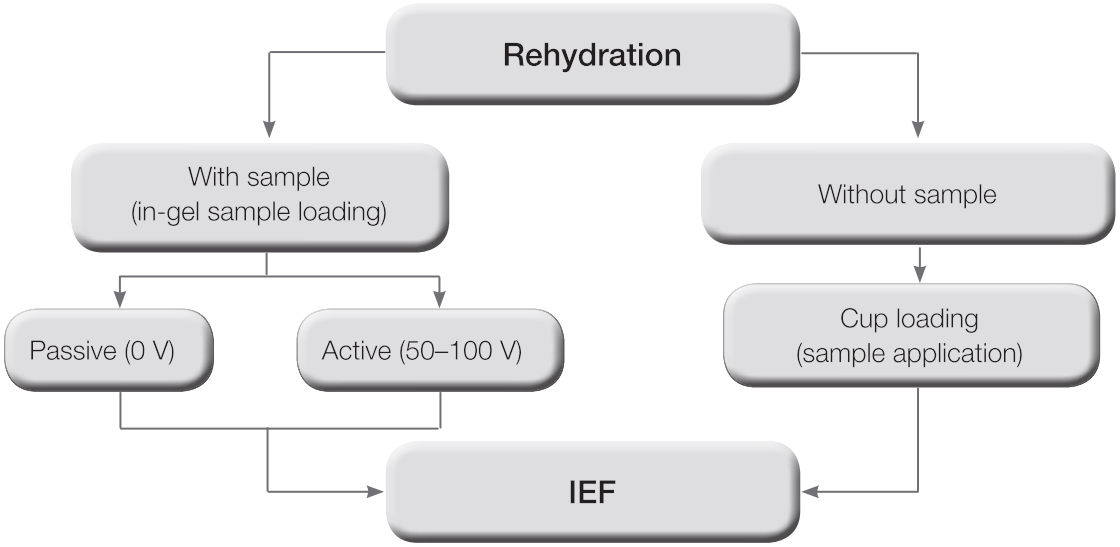


Fig. 1.5. IEF workflow overview.

2 Basic Operation

2.1 Setup

For best results, use the reagents and protocols available in the ReadyPrep™ 2-D starter kit (catalog #163-2105) to familiarize yourself with the 2-D process and operation of the PROTEAN® i12™ IEF cell.

2.1.1 i12™ Focusing Tray and Electrode Assemblies

The PROTEAN i12 electrode assemblies have 12 electrodes that fit the 12 channels in the i12 focusing tray. Each electrode accommodates the use of electrode wicks and both the gel side-up and gel side-down IEF configurations: a bridge on each electrode fits into the recessed area of the focusing tray to create the flat surface required for the gel-side down configuration, and the electrodes are spring-loaded, which allows them to exert a gentle downward pressure for the gel-side up configuration. Several features of the electrode assemblies ensure their correct and complete attachment to the focusing tray (Figure 2.1):

- Square and round orientation pins in the electrode assemblies ensure correct positioning of the cathode and anode in the focusing tray
- Release clips secure the electrode assemblies onto the focusing tray
- Green tabs on the release clips help push the electrode assemblies into place

To place the electrode assemblies in the i12 focusing tray:

1. Grasp the electrode assembly by the release clips and position the orientation pins as shown in Figure 2.1.
2. Push down on the green tabs until the locks click into place on the walls of the focusing tray. With the gel-side down configuration, make sure that each electrode is properly seated in the recessed area of the focusing tray.

   To remove electrode assemblies from the focusing tray, grasp the green tabs on the release clips and gently squeeze inward.

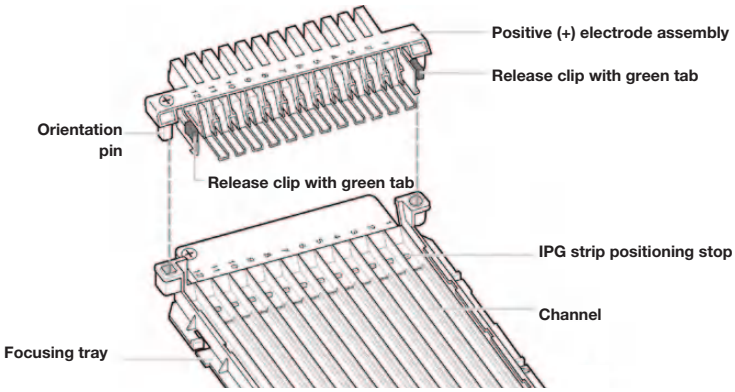


Fig. 2.1. Placement of an electrode assembly onto the i12 focusing tray.

2.1.2 Connecting the Electrodes

1. Position the assembly on the Peltier platform. Use the positioning guides and stops on the platform and focusing tray as guides (Figure 2.2, see inset).
2. Slide the assembly toward the positive (+) end until the positive (+) electrode assembly is completely inserted. When the the tray positioning stop reaches the positioning guide on the platform, the assembly is seated correctly (Figure 2.2).
3. Make sure the negative pin on the negative (-) electrode is in direct contact with the metal gounding strip on the instrument (Figure 2.2).

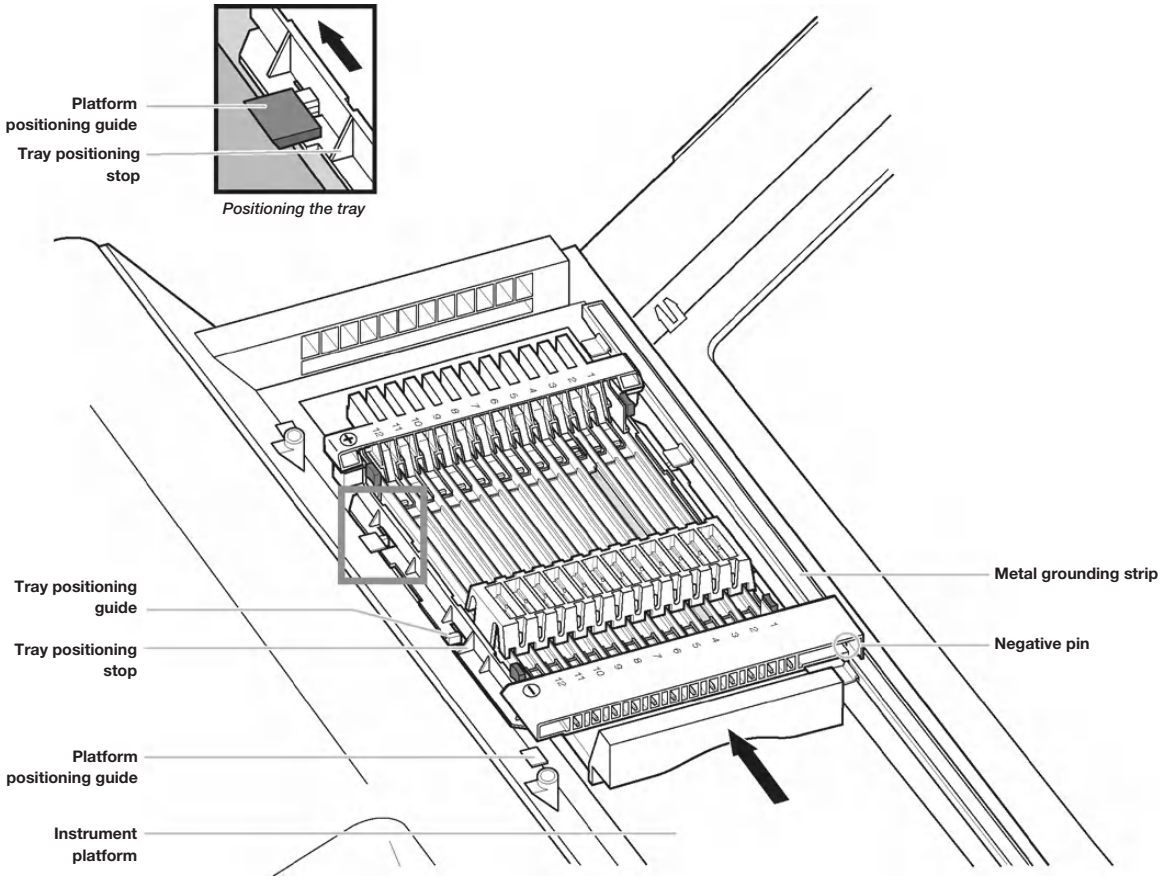


Fig. 2.2. Connecting the electrodes.

2.2 IPG Strip Rehydration and Sample Application

The choice of rehydration and sample loading method (in-gel sample loading or cup loading) and IPG strip configuration (gel-side down or gel-side up) dictates the workflow for the run. The first step is to rehydrate the IPG strips in rehydration solution, with or without sample (Table 2.1).

IPG strips can be rehydrated in either the i12 rehydration/equilibration trays or the i12 focusing tray:

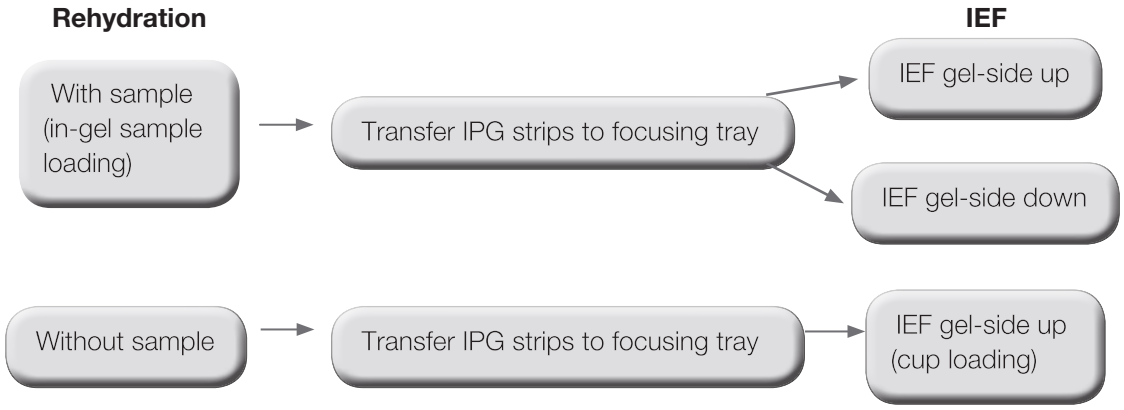
- For rehydration in rehydration/equilibration trays, see Section 2.2.1
- For rehydration in the focusing tray, see Section 2.2.2. Rehydration in the focusing tray with in-gel sample application can be programmed as part of the IEF run

> See Appendix B, Sample Loading Methods and Running Configurations, for guidelines for selecting the sample loading method and IPG strip configuration.

Table 2.1. Rehydration volumes, sample loads, and mineral oil volumes. The values listed are recommendations. Optimum sample load depends on sample type. See Appendix A, Reagent and Sample Preparation, for more details.

	IPG Strip Length				
	7 cm	**11 cm**	**17 cm**	**18 cm**	**24 cm**
Rehydration Solution	125 µl	200 µl	300 µl	315 µl	450 µl
Protein Load					
Coomassie (Brilliant) Blue	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg
Fluorescent stains	5–100 µg	20–200 µg	50–400 µg	50–400 µg	80–800 µg
Silver stains	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg
Mineral Oil	4 ml	5 ml	7 ml	7 ml	9 ml

2.2.1 IPG Strip Rehydration in the Rehydration/Equilibration Tray Followed by IEF



For the rehydration step:

1. Pipet the rehydration solution (with or without sample, see Table 2.1 for volumes and protein loads) along the center of the channel(s) of the i12 rehydration/equilibration tray. Take care to not introduce air bubbles when expelling the solution.
2. Using forceps, remove the cover sheet from the IPG strip, then gently place the IPG strip gel-side down onto the solution in the channel. Move the IPG strip back and forth slightly to ensure that the solution is distributed along the length of the IPG strips. Take care to not trap air bubbles beneath the IPG strip.

3. Overlay each IPG strip with mineral oil to prevent evaporation and precipitation of urea during rehydration (see Table 2.1 for recommended volumes). Apply the mineral oil to both ends of the channel and allow it to flow toward the middle of the channel. IPG strips can be left to rehydrate for up to 1 hr before adding the mineral oil.
4. Cover the tray and leave it on a level bench overnight (12–18 hr) for complete rehydration.
5. Transfer the rehydrated IPG strips to the focusing tray for IEF (see below).

IEF with Gel-Side Up

For IEF of IPG strips that were rehydrated in the presence of sample (in-gel loading):

1. Using forceps, remove the IPG strips from the rehydration tray, remove excess mineral oil, and place the rehydrated IPG strips gel-side up in the channels of the focusing tray (Figure 2.3, A). Position the positive (+) ends of the IPG strips against the positioning stops in each channel.
2. (Recommended) Wet the gel-side up wicks (notched) with distilled or deionized water and blot off excess water. Use two wicks per IPG strip: place a wick at each end of each IPG strip (Figure 2.3, B).
3. Position the electrode assemblies in the focusing tray and press down on the green tabs to snap the electrode assemblies into place (Figure 2.3, C). Place the focusing tray with the rehydrated IPG strips on the Peltier platform and connect the electrodes to the instrument (see Section 2.1, Setup).
4. Overlay each IPG strip with mineral oil (see Table 2.1 for recommended volumes).
5. Select or program the protocol(s) and start the run (see Section 2.3, Starting the Run).

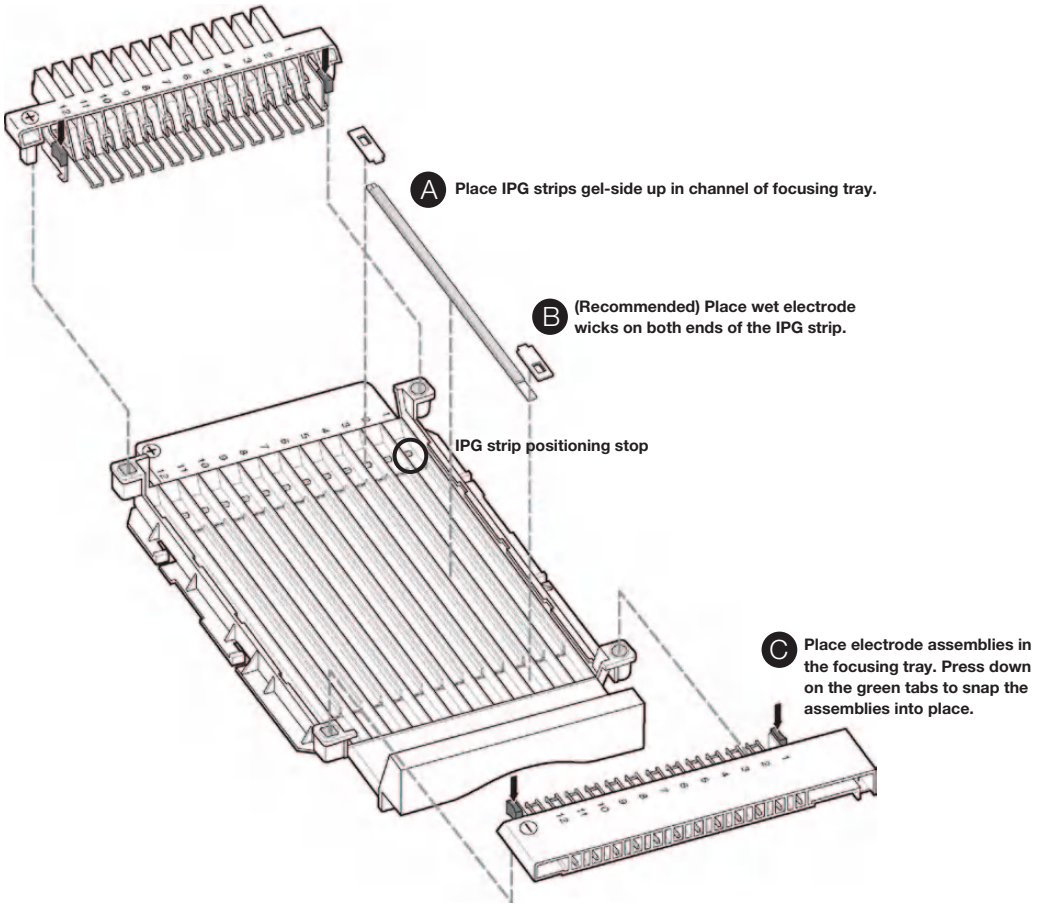


Fig. 2.3. Placement of an IPG strip gel-side up into the i12 focusing tray.

IEF with Gel-Side Down

For IEF of IPG strips that were rehydrated in the presence of sample (in-gel sample loading):

1. Position the electrode assemblies in the focusing tray and press down on the green tabs to snap the electrode assemblies into place (Figure 2.4, A; see Section 2.1.1, i12 Focusing Tray and Electrode Assemblies).
2. (Recommended) Wet the rectangular (gel-side down) wicks with distilled or deionized water and blot off excess water. Use two wicks per IPG strip: place a wick on top of each electrode (Figure 2.4, B).
3. Using forceps, place the rehydrated IPG strips gel-side down in the channels of the focusing tray (Figure 2.4, C). Position the positive (+) ends of the IPG strips against the positioning stops in each channel.
4. Place the focusing tray on the Peltier platform and connect the electrodes to the instrument (see Section 2.1.2, Connecting the Electrodes).
5. Overlay each IPG strip with mineral oil (see Table 2.1 for recommended volumes).
6. Place the IPG strip retainers on top of the IPG strips at both the positive and the negative ends (Figure 2.4, D). Without IPG strip retainers in place, gases formed during electrolysis may lift IPG strips off the electrodes, interrupting electrical contact.
7. Select or program the protocol(s) and start the run (see Section 2.3, Starting the Run).

> To avoid movement of the IPG strip retainers, which can damage the IPG strips, place them into the focusing tray after the focusing tray has been secured on the Peltier platform.

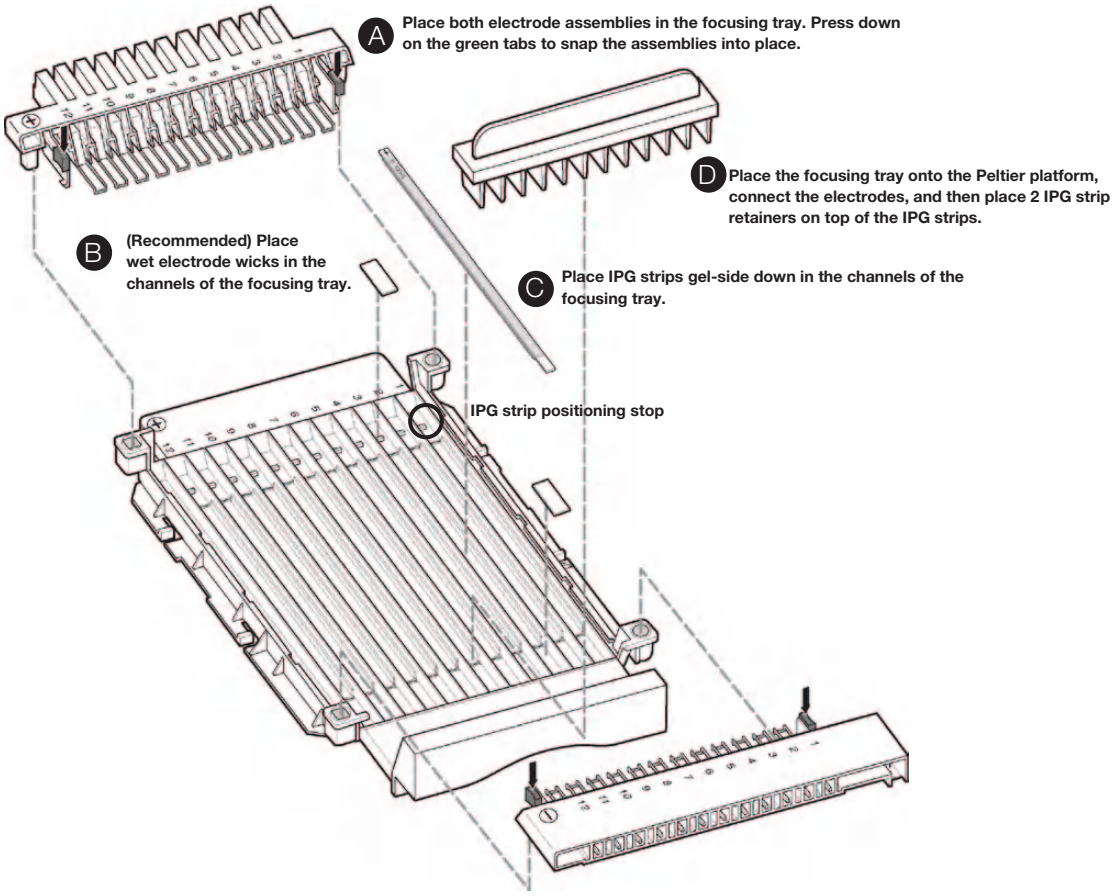


Fig. 2.4. Placement of an IPG strip gel-side down into the i12 focusing tray.

Cup Loading (IEF with Gel-Side Up)

Sample cups (catalog #164-6020) offer an alternative method of sample loading. Their use can improve resolution, especially at extreme pH ranges (see Appendix B, Sample Loading Methods and Running Configurations, for guidelines on when to use cup loading). The PROTEAN i12 sample cup assembly consists of a sample cup holder that holds 1–12 disposable sample cups.

1. Using forceps, place the rehydrated IPG strips gel-side up in the channels of the focusing tray (Figure 2.3, A). Position the positive (+) end of the IPG strips against the positioning stops in each channel.
2. (Recommended) Wet the gel-side up electrode wicks (notched) with deionized water and blot off excess water. Use two wicks per IPG strip: place a wick at each end of each IPG strip (Figure 2.3, B).
3. Position the electrode assemblies in the focusing tray and press down on the green tabs to snap the electrode assemblies into place (Figure 2.3, C). Place the focusing tray on the Peltier platform and connect the electrodes to the instrument (see Section 2.1.2, Connecting the Electrodes).
4. Prepare the sample cup assembly by placing the sample cups into the slots of the sample cup holder corresponding to the channel with the rehydrated IPG strip (Figure 2.5).
5. Clamp the sample cup assembly onto the edges of the focusing tray, on top of the IPG strips and next to either electrode (Figure 2.5). (Placement depends on the pH gradient and the sample. See Appendix B, Sample Loading Methods and Running Configurations.)
6. Load 25–250 µl sample into the sample cups (larger volumes of dilute samples may be loaded, up to 400 µl). Overlay both the sample in the sample cup and the IPG strip with mineral oil.
7. Select or program the protocol(s) and start the run (see Section 2.3, Starting the Run).

> Two flexible arms on the sample cup holder apply gentle pressure onto the sample cup to ensure a complete seal between the IPG strip and the sample cup.
>
> Once the sample cup holder is positioned, any horizontal movement will damage the IPG strips.

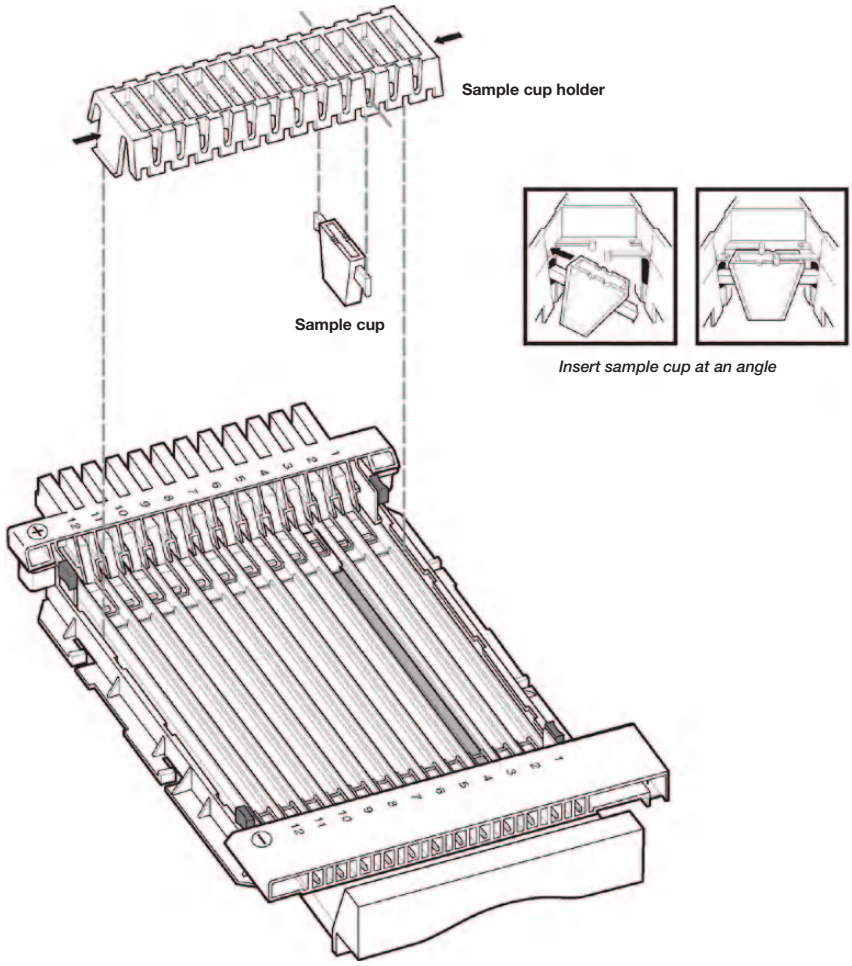
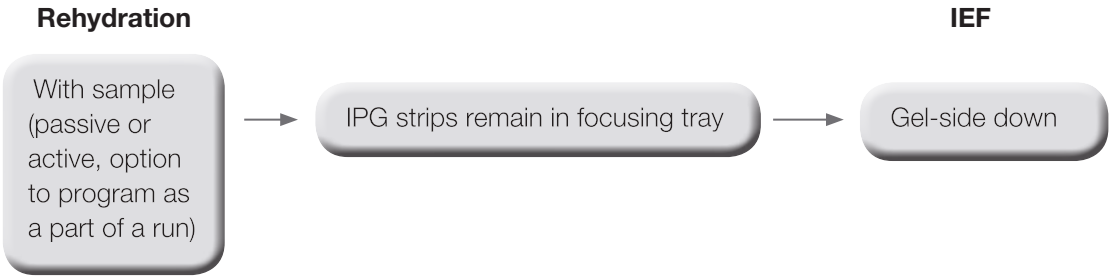


Fig. 2.5. Placement of the sample cup assembly onto the focusing tray.

2.2.2 IPG Strip Rehydration in the Focusing Tray Followed by IEF

Rehydration can be programmed as a part of the run, followed by the IEF protocols. Alternatively, the IPG strips can be rehydrated independently and the protocol(s) started when most convenient.



1. Position the electrode assemblies in the focusing tray as described in Section 2.1.1.
2. Pipet the rehydration solution containing the protein sample along the center of the channel(s) of the focusing tray (see Table 2.1 for recommended volumes and protein loads). Do not introduce air bubbles when expelling the solution.
3. Using forceps, remove the cover sheet from the IPG strip, then gently place the IPG strip gel-side down onto the sample in the channel of the tray. To ensure even rehydration, move the IPG strips back and forth slightly to distribute the solution along the lengths of the IPG strips. Check that no bubbles are trapped beneath the IPG strip and that some rehydration solution extends beyond the electrode contacts.
4. Place the focusing tray with the IPG strips on the Peltier platform and connect the electrodes to the instrument (see Section 2.1.2).
5. Immediately overlay each IPG strip with mineral oil to prevent evaporation and precipitation of urea during rehydration. Apply the mineral oil to both ends of the channel and allow it to flow toward the middle of the channel. See Table 2.1 for recommended volumes of mineral oil.
6. Position the IPG strip retainers on top of the IPG strips at both the anode and the cathode to maintain electrical contact with the IPG strips during IEF. Without the IPG strip retainers, electrolysis gasses may lift IPG strips off of the electrodes, interrupting electrical contact.

> Place IPG strip retainers after the focusing tray has been placed into the instrument to avoid movement of the strip retainers.

7. Rehydration in the focusing tray with in-gel sample application can be programmed as a part of the IEF run or be performed separately. To program rehydration as part of the run:
   a. Select or program the protocol(s) for the lanes containing IPG strips (see Section 3.1).
   b. Program the global rehydration conditions in the Run Settings screen (see Section 3.2). If electrode wicks are used, include a pause to insert electrode wicks when the rehydration step is completed.
   c. Start the run (see Section 2.3, Starting the Run).

> For rehydration not programmed as part of the run, leave the tray on the Peltier platform or on a level bench overnight (12–18 hr) for complete rehydration.
>
> Global rehydration conditions are applied to all IPG strips in a run.

2.3 Starting the Run

Turn on the PROTEAN i12 IEF cell by pressing the power switch on the right side of the instrument. A self-diagnostic program runs for approximately 10 sec. On the user interface, a message reads *Self Test in Progress*. If a component fails, the diagnostic program stops, and an error message appears (see Chapter 7, Troubleshooting).

Once the cell is powered on, the Main screen appears (Figure 2.6). Start the run by selecting one of the options described in Figure 2.6.

Fig. 2.6. PROTEAN i12 IEF cell Main screen.

3 Running a Protocol

Select **Run** in the Main screen to select, assign, and run an existing (preprogrammed or user-defined) protocol. Multiple protocols can be assigned simultaneously to different IPG strips in a single run. Power is not applied to lanes without assigned protocols (these are designated **Not Assigned**).

This chapter describes how to select a protocol, enter run and sample details, and start a run (Figure 3.1). For details about creating or editing protocols, refer to Chapters 4 and 5.

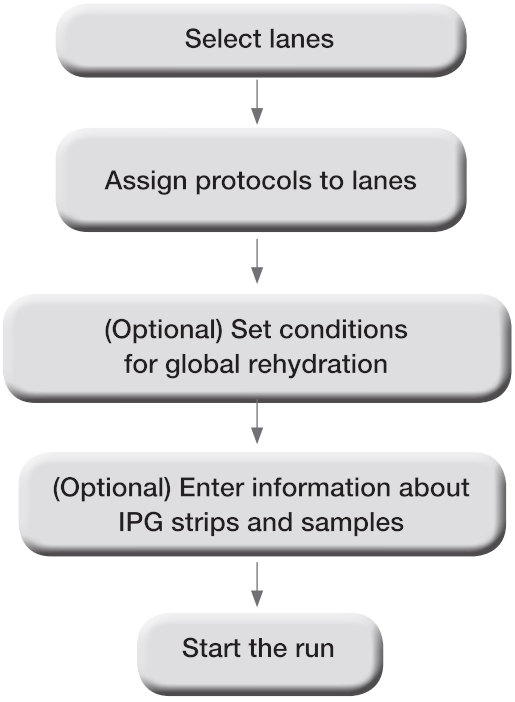
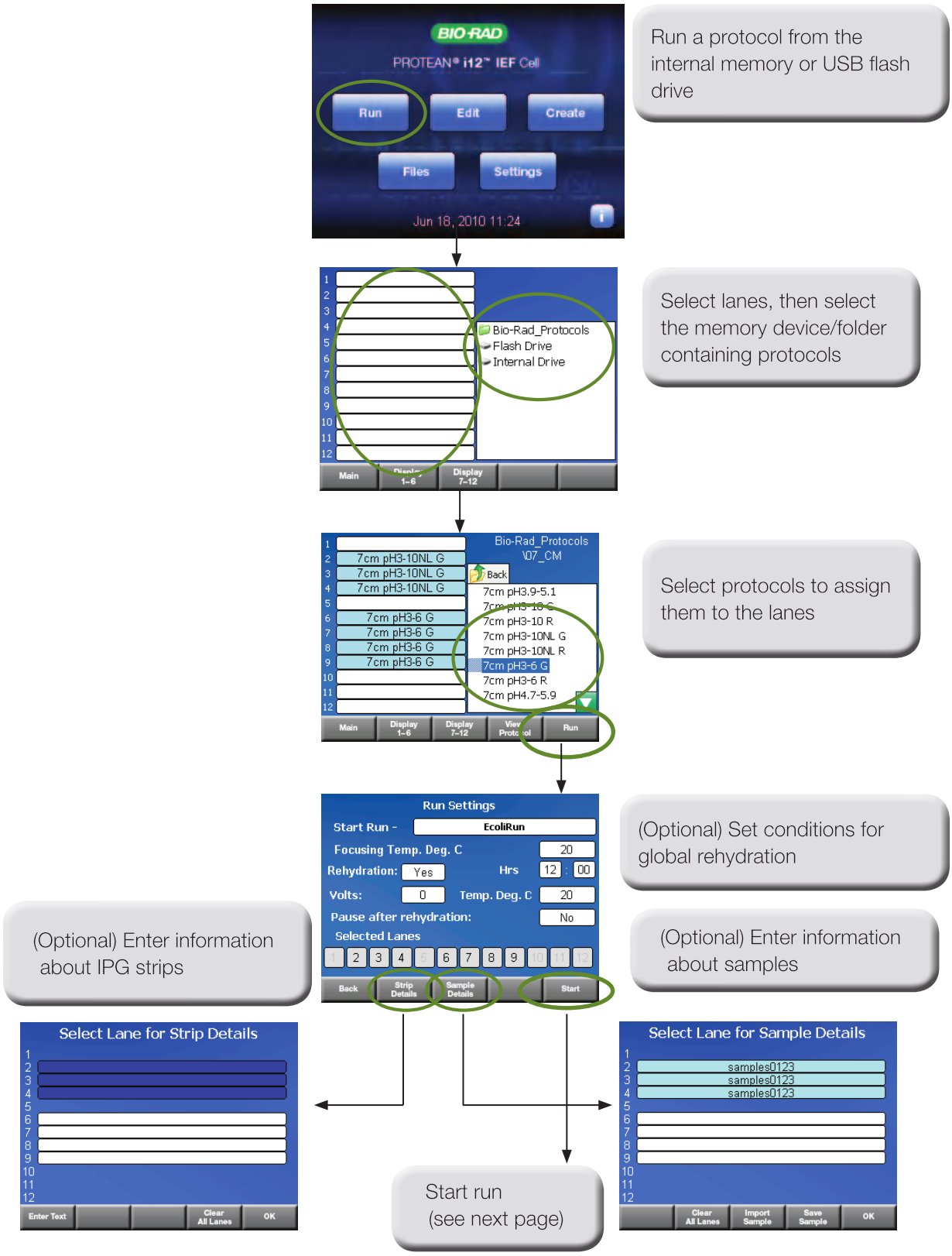
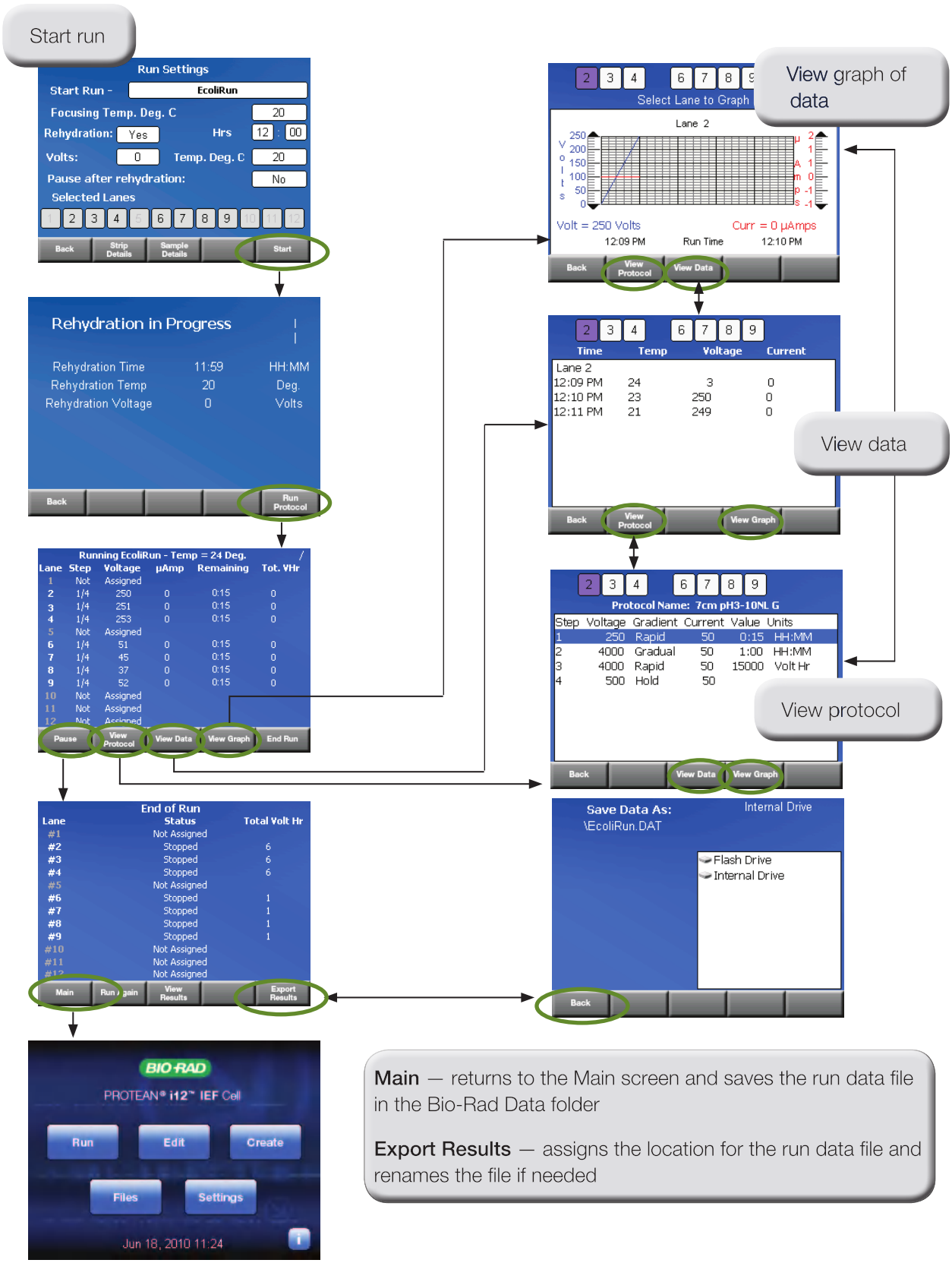


Fig. 3.1. General workflow for running an existing protocol.

3.1 Workflow

See Section 3.2, Screen Details, for details about the options and functions for each screen.

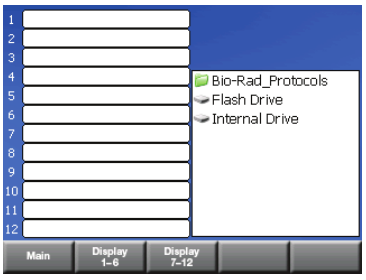
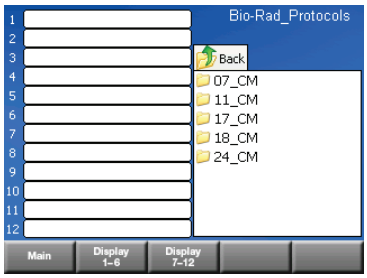
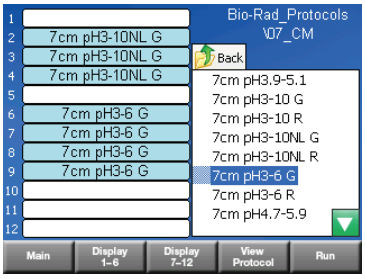


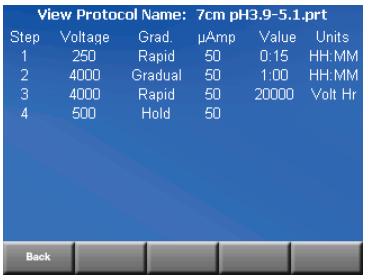
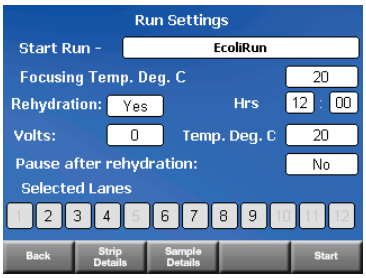


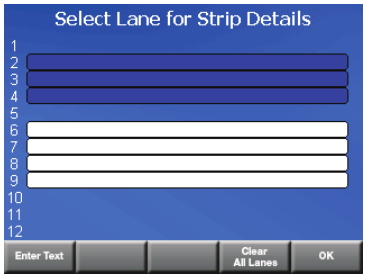
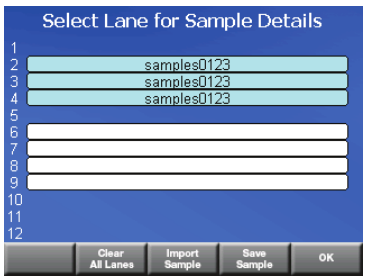
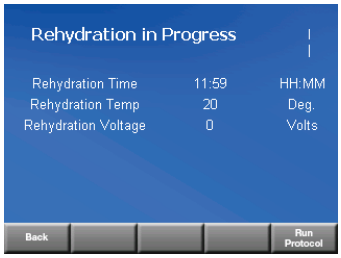
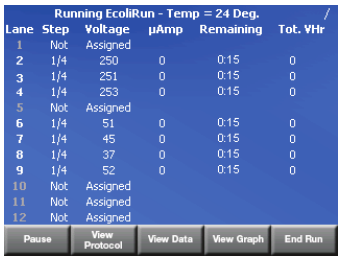
Main — returns to the Main screen and saves the run data file in the Bio-Rad Data folder

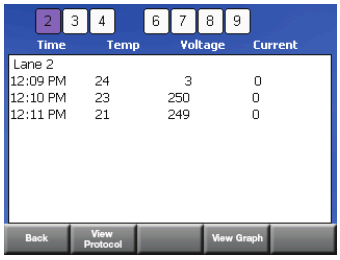
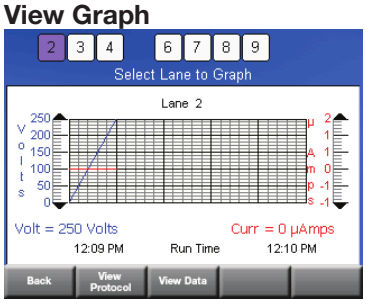
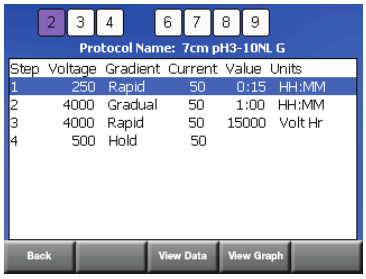
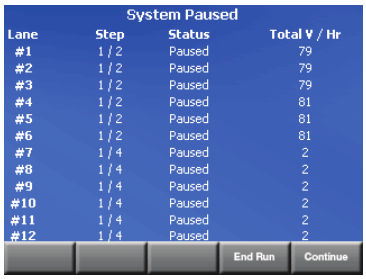
Export Results — assigns the location for the run data file and renames the file if needed

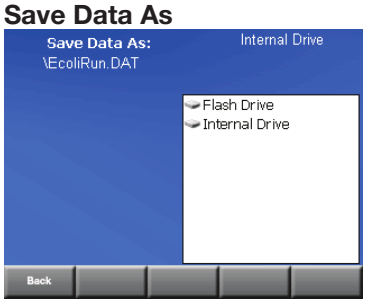
3.2 Screen Details

Screen	Details/Procedure
Main 	**Run** — opens the protocol assignment screen for selection of protocol(s) stored in the internal memory or on a USB flash drive (only preprogrammed Bio-Rad protocols or saved .prt protocol files appear).
Protocol Assignment Screens	
Protocol Assignment  	Display the available lanes (in a 12- or 6-lane format), available memory devices, and Bio-Rad folder (with preprogrammed protocols). To assign a protocol to a lane(s): 1. Touch any lane to select it. Selected lanes are highlighted in blue. 2. Select the memory device/folder for display of stored protocols. 3. Select the protocol, which is highlighted and immediately assigned to the highlighted lane(s). To select a preprogrammed protocol, select the Bio-Rad folder and then select the protocols for the IPG strip length. The protocols appear in the content box. 4. Repeat this procedure to assign different protocols to different lanes.
Lanes Assigned 	Displays the name of the assigned protocol in each selected (highlighted) lane. **View Protocol** — displays the details of the protocol highlighted in the content box **Run** — opens the Run Settings screen

Screen	Details/Procedure
View Protocol 	Displays the protocol parameters for the selected protocol. **Back** — returns to the Lanes Assigned screen To create and edit protocols, use the **Create** or **Edit** option in the **Main** screen (see Chapter 4).
Run Settings 	Displays the name of the run data file and offers options for setting the focusing temperature and conditions for a global rehydration step. It also offers links to options for entering sample and IPG strip details. The 12 lane numbers appear, with active lanes listed in boldface and unassigned lanes grayed out. To enter or change the name of the run data file, select the text box next to **Start Run** to access the keyboard and enter the name. **Focusing Temp. Deg. C** — sets the temperature for IEF (10–25ºC, default 20ºC) **Rehydration** — select **Yes** to program a rehydration step Rehydration parameters: ▪ **Hrs** — rehydration time (0–99:59 hr, default 12 hr) ▪ **Volts** — rehydration voltage (0 or 50–100 V, default 0 V) ▪ **Temp. Deg. C** — rehydration temperature (10–25ºC, default 20ºC) ▪ **Pause after rehydration** — select **Yes** if a pause is required for insertion of electrode wicks **Start** — starts the run **Strip Details** — opens the Strip Details screen **Sample Details** — opens the Sample Details screen Protocol details, data points, and sample and IPG strip details for each assigned lane are included in the run data file. Run data files with the same name are overwritten if saved in the same folder. When the run is complete, the option to rename the run data file and select a location will be available. The default storage location for a run data file is Internal Drive/Bio-Rad Data.

Screen	Details/Procedure
Strip Details  **Sample Details** 	Used to enter information about the IPG strips (pH range, length, lot, etc.) and samples in the assigned lanes. Press an assigned lane to select it and then press **Enter Text**. **Enter Text** — opens the keyboard used to enter the details **Clear All Lanes** — removes all entries **OK** — accepts the entered details and returns to the Run Settings screen. The entered strip detail is included with the saved run data file **Save Sample** — saves the entered sample details in a specified location and folder. Sample details are saved to the lane that was specified To import an existing sample detail file: 1. Press **Import Sample** to access previously saved sample detail files. 2. Select the location and the sample detail file, then press **Load Sample**. The sample details populate the assigned lanes.
Run Screens	
Rehydration in Progress 	Displays the rehydration conditions and the time remaining during rehydration. When the rehydration time has elapsed, the IEF run starts unless a pause is selected. If a rehydration pause is selected, the message, *Rehydration Completed* appears. **Run Protocol** — terminates rehydration and starts step 1 of the protocol(s) in the assigned lane(s) **Back** — terminates rehydration and returns to the Run Settings screen
Running 	Displays active parameter values for each assigned lane, the time remaining for the active step, and the total accumulated V-hr for the run. **View Protocol**, **View Data**, and **View Graph** open the respective screens. **Pause** — pauses the run and opens the **System Paused** screen, where the run may be continued or terminated **End Run** — terminates the run and opens the **End Of Run** screen

Screen	Details/Procedure
View Data  **View Graph**  **View Protocol** 	Displays data recorded for each assigned lane while the run is in progress. The information for the first assigned lane appears; press the remaining assigned lane numbers to display the related details. Toggle between the screens to review all the information for the highlighted lane. **View Data** — displays all the data points for the selected lane. Data points are collected at 5 min intervals unless otherwise specified in the Settings screen. The collection frequency range is 1–15 min per data point **View Graph** — displays a graph of voltage and current as a function of time for the selected lane **View Protocol** — displays the protocol details for the selected lane **Back** — returns to the Running screen
System Paused 	Appears if the run is paused (for example, to safely remove completed or faulty IPG strips, remove/add electrode wicks, etc.). **End Run** — terminates the run **Continue** — continues the run

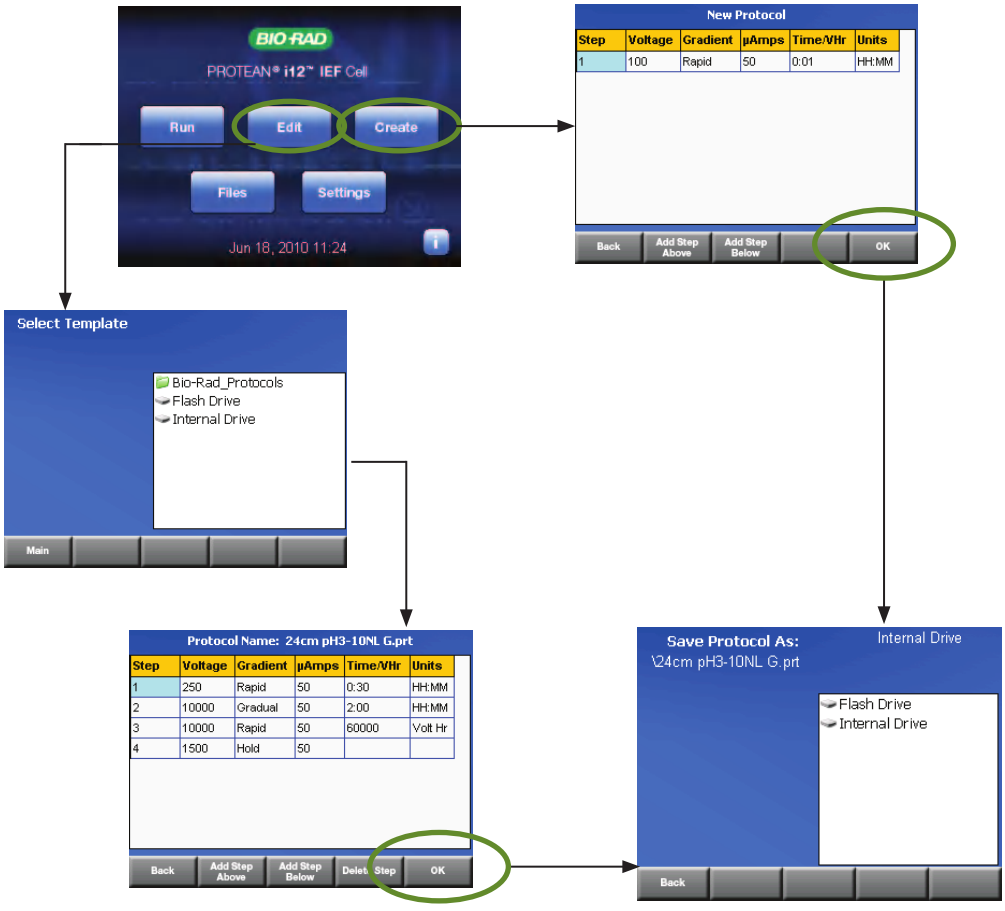
Screen	Details/Procedure
End of Run 	Appears at the end of a run and displays the total V-hr for each assigned lane. **View Results** — opens the View Data screen with options to View Protocol and View Graph for each of the assigned lanes **Run Again** — returns to the Run Settings screen **Export Results** — opens the Save Data As screen **Main** — opens the Main screen and automatically saves the run data file in the Bio-Rad data folder. Run data files with the same name are overwritten; to rename the file, select Export Results
Save Data As 	Used to rename and export the run data (.dat) file to a specific location in the internal memory or to a USB flash drive. 1. Select a storage location and select or create a folder. 2. Press **Save** to open the alphanumeric keyboard. The run data file name appears. Select **Save** to accept the name, or enter a new name and select **Save**. 3. The run data file is saved in the selected location, and the Main screen appears.

The run data file (.dat) is a text file that can be opened and imported into a spreadsheet. For details on the file and how to display the data with Excel software, see Chapter 6, Data Export and Analysis.

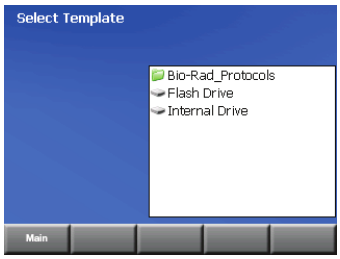
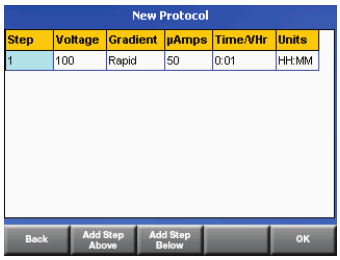
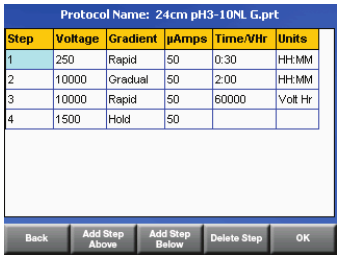
4 Creating and Editing Protocols

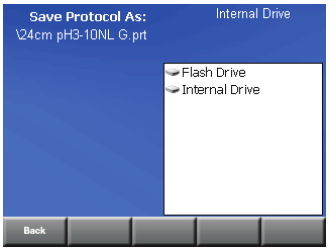
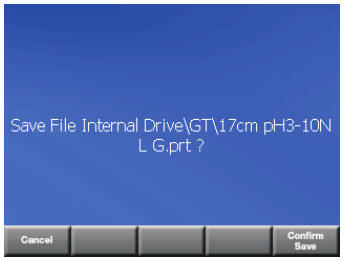
Use the **Edit** and **Create** options in the Main screen to edit an existing protocol or create a new protocol. Up to ten steps can be programmed and stored in the internal memory or a USB flash drive.

4.1 Workflow



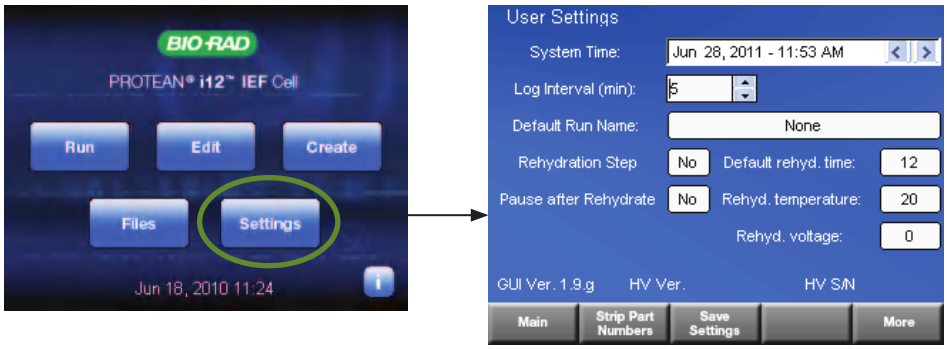
4.2 Screen Details

Screen	Details/Procedure
Main 	**Edit** — opens the **Select Template** screen with options for editing an existing protocol **Create** — opens the **New Protocol** screen with options for creating a protocol Bio-Rad protocols cannot be overwritten. Save any changes as a new protocol.
Select Template 	Used to select the protocol that will serve as the template (the protocol you wish to edit). Select the memory device, the folder, then the protocol. The **Edit Protocol** screen opens, displaying the name and parameters of the selected protocol.
New Protocol  **Edit Protocol** 	Displays the steps and settings for a new or template protocol: the New Protocol screen displays a single step, and the Edit Protocol screen displays steps in a saved protocol. Press **Add Step Above** or **Add Step Below** to add steps as needed (a protocol can contain up to ten steps). Touch a cell in the table to edit the settings for the parameters listed (see Appendix C, IEF Protocols for more details): **Voltage** — 0 V or 50–10,000 V **Gradient**: ▪ **Rapid** — voltage limited by the set current value ▪ **Linear** — voltage increases in a linear fashion from the starting voltage to the maximum set voltage. Linearity is not achieved if the current limit is reached before the required voltage is reached ▪ **Gradual** — bases the voltage change on a delayed voltage ramping algorithm that gradually increases over the time specified ▪ **Hold** — maintains the voltage, recommended at a field strength of ~50 V/cm of strip length, until the run is stopped manually. Can be used as the final step to prevent protein diffusion when IEF is complete. Steps cannot be added beyond the hold step **µAmps** — current (0–100 µA) **Time/VHr** — 00:01–99:59 hr or 1–999,999 V-hr **Units** — toggle between HH:MM and Volt Hr; previously entered values reset to 00:01 and 1, respectively **OK** — opens the Save Protocol As screen

Screen	Details/Procedure
Save Protocol As 	To save the new or edited protocol: 1. Select the location and select or create a folder. 2. Press **Save** to access the alphanumeric keyboard, where the name is displayed at the top of the screen. 3. Enter or edit the name of the protocol, then press **Save**. 4. The Confirm Save screen appears.
Confirm Save 	Displays location to which file will be saved. **Cancel** — returns to the Save Protocol screen **Confirm Save** — saves and then opens the Main screen. *Overwrite* appears if the same file name is being saved to the same location

5 Setting Defaults and Managing Folders

5.1 Setting Default Parameters (Settings)



Use the Settings options to set default parameters and to customize screens:

- **System Time** — sets the month, day, year, or time. Use the right and left arrow buttons to make adjustments
- **Log Interval (min)** — sets the data collection frequency (1–15 min; default 5 min). Select the field and use the up and down arrows to change the value
- **Default Run Name** — name automatically associated with a run, and the run data are stored in the Bio-Rad Data folder in the internal memory when the run is complete. Stored run data files with names identical to new files being stored in the same location are overwritten
- **Rehydration Step** — sets global rehydration step defaults. If the rehydration step is selected, all rehydration step options appear in the Run Settings screen. If not selected, the details can be assigned in the Run Settings screen
- **Pause after Rehydrate** — select to include a pause after rehydration
- **Default rehyd. time** — sets the rehydration time (0–99 hr; default 12 hr)
- **Rehyd. temperature** — sets the rehydration temperature (10–25ºC; default 20ºC)
- **Rehyd. voltage** — sets the voltage for rehydration (0 V, 50–100 V; default 0 V)

Save Settings — saves the settings as displayed

More Settings — touch screen calibration and firmware updates

Strip Part Numbers — lists the part numbers of ReadyStrip™ IPG strips (based on length and pH range)

> If changes are made and Save Settings is not pressed, the new values are implemented (except System Time) in the current run, but they revert to the previous settings when the system is shut down.

5.2 Managing Files and Folders (Files)

The PROTEAN® i12™ IEF cell stores saved and preprogrammed protocols and run data and sample details files. Use the options under Files to:

- Delete files and folders
- Copy files to a different location
- Create new folders

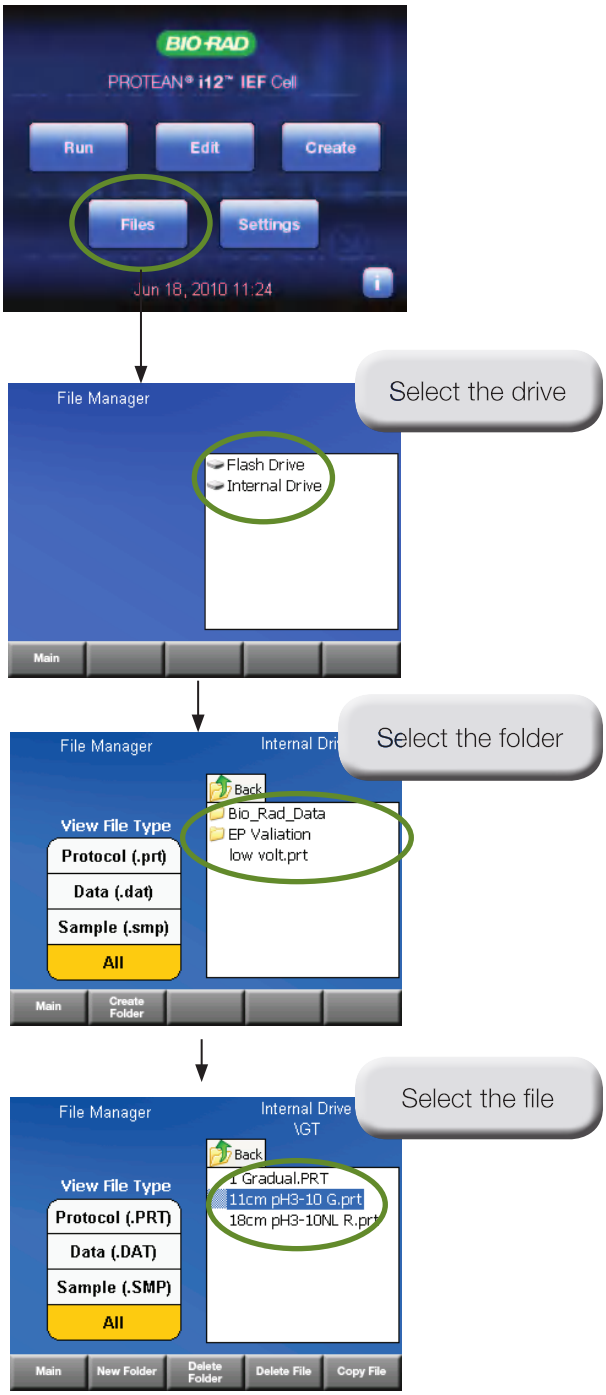
5.2.1 Navigating the Files Structure

1. Press **Files** on the Main screen.
2. Select the drive.
3. Select a folder to display its contents.

- **View File Type** — displays files of a certain type
- **Create Folder** — creates a new folder
- **Delete Folder** — opens the Delete Confirmation screen and options to Cancel or Delete
- **Main** — returns to the Main screen

4. Select a file. The file name is highlighted and options to **Delete File** and **Copy File** appear.

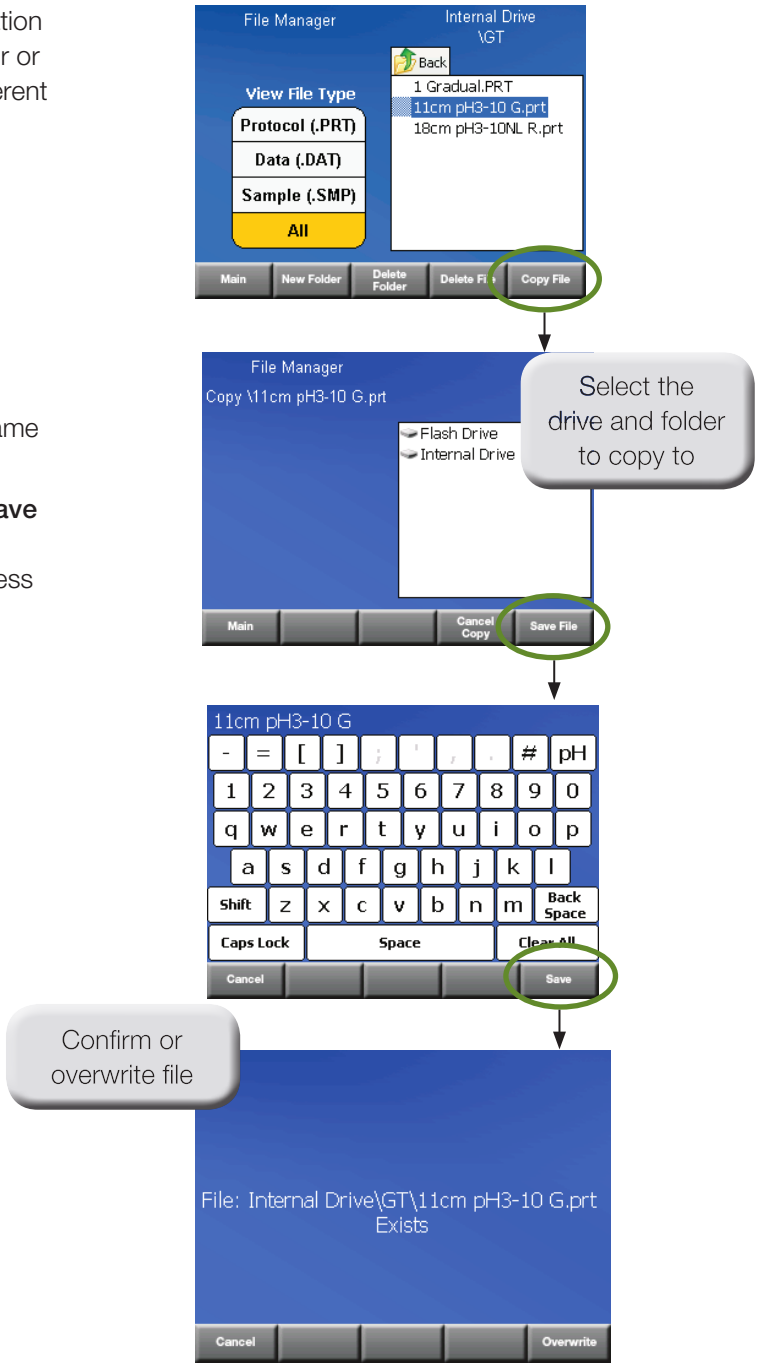
- **Delete File** — opens the Delete Confirmation screen and options to Cancel or Delete
- **Copy File** — opens the Copy File To window (see Section 5.2.2)



5.2.2 Copying Files

A file can be copied to the same folder and location (if saved with a new name) or to a different folder or different location using the same name or a different name.

1. Select a file following the workflow in Section 5.2.1.
2. Press **Copy File**.
3. Select the drive and folder as the location to which to copy folder (the destination).
4. Press **Save File** to enable an alphanumeric keypad with the file name. Confirm the file name or enter a new name, then press **Save**.
5. In the confirmation screen, press **Confirm Save** or **Overwrite** to copy the file to the selected location and return to the Main screen, or press **Cancel** to return to the File Copied screen.



6 Data Export and Analysis

6.1 File Types

PROTEAN® i12™ software stores three types of files:

- .dat — run data files, which contain a record of the protocols run, IPG-strip– or sample-specific information entered by the user, and a continuous record for each IPG strip of current, voltage, temperature, and V-hr accumulated, as well as metadata such as the date, time, instrument serial number, firmware, and software version
- .prt — protocol files
- .smp — sample files

Run data files (.dat) can be viewed and manipulated using either Microsoft Excel software or the PROTEAN i12 Reporter web-based application.

6.2 Export to Microsoft Excel Software

To export data to Excel software:

1. Follow the directions in Section 5.2.2, Copying Files, to copy run data files to the USB flash drive.
2. Transfer the USB flash drive to a computer (PC) and launch Microsoft Excel.
3. Select **File > Open**. In the Open dialog, select **All Files** as the file type. Find and click the .dat file to select it, then click **Open**.
4. In the **Text Import Wizard**, choose **Delimited** as the file type option, then click **Next** and select **comma** as the delimiter. The .dat file information appears in an Excel worksheet.

6.3 Export to PROTEAN i12 Reporter (www.i12Reporter.com)

The PROTEAN i12 Reporter is a web-based application that enables the creation of protocols (.prt files) and the display of run data files (.dat files) on a remote computer. It can be used to display the electronic run profiles for each lane, compare data from different runs, and generate and print reports.

Uploading Data

To upload data (.dat) files to the PROTEAN i12 reporter, first load them onto the flash drive from the PROTEAN i12 cell. Then upload them into the application.

1. Follow the directions in Section 5.2.2, Copying Files, to copy run data files to a USB flash drive.
2. Transfer the USB flash drive to a computer (PC), then launch the browser and the PROTEAN i12 Reporter application (www.i12Reporter.com).
3. In the PROTEAN i12 Reporter Main page (Figure 6.1), under **Upload Your Run Data**, click **Browse** and navigate to the run data files (.dat). You can upload up to six files at a time.

Fig. 6.1. PROTEAN i12 Reporter Main screen.

4. Select the files and click **Upload**. The Run Details screen opens. The data appear under tabs across the top of the page (Figure 6.2). If multiple data files are uploaded, the **Compare Lanes** tab also appears.

Viewing Data

To view the data, select the run and the viewing option in the Run Details screen (Figure 6.2):

- **Voltage vs. Time**, **Current vs. Time** or **Voltage/Current vs. Time** options generate graphs of the data
- **Protocols** displays the IEF protocols and any sample or strip information entered for the run
- **Run Data** displays the raw data in table format
- **Events** shows if the run was paused and when it ended
- **Create Report** allows you to customize a report with the data and graphs

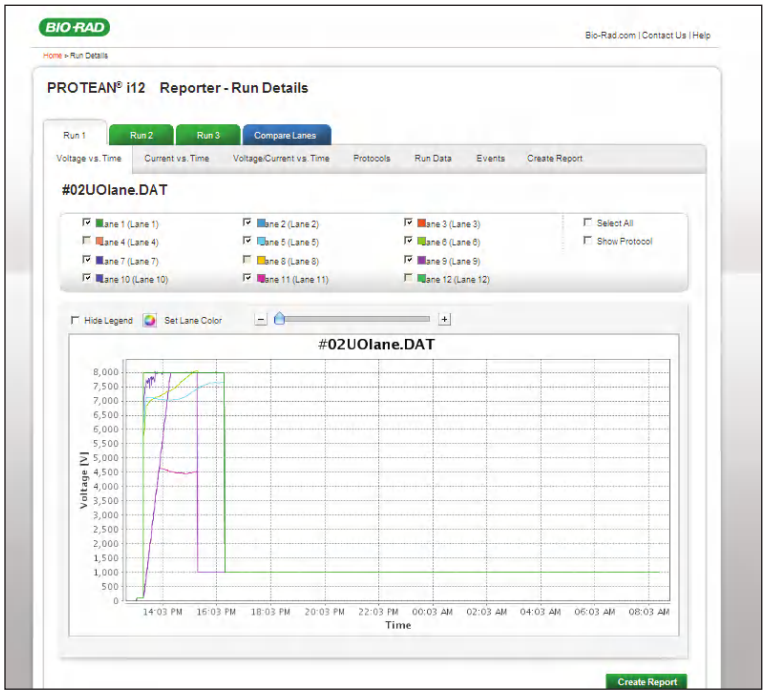


Fig. 6.2. PROTEAN i12 Reporter Run Details screen.

Creating Reports

Click **Create Report** to generate a report of the run data. Use the options in the Create Report screen to customize your report:

- Choose how to display a logo, username, affiliation, or copyright
- Choose which elements to include in the report: run data with events log, protocols, sample and strip details, or graphs

Click **Print Report** to print the report from a designated printer.

Click **Save Report** to save the report as a .pdf file.

Viewing and Editing Protocols

On the **Main** page, under **Protocols**:

- Click **View Bio-Rad Protocols** to open the Bio-Rad Protocols screen, which displays all Bio-Rad preprogrammed protocols. Click on a protocol to view its details (Figure 6.3). Click **Edit Protocol** to edit the settings and save as a new protocol. You can then print the new protocol or upload it into the PROTEAN i12 IEF cell
- Click **Create New Protocol** to open the Create and Edit an IEF protocol screen (Figure 6.4). Use the options to set the voltage, gradient, and time parameters and create a protocol of up to ten steps

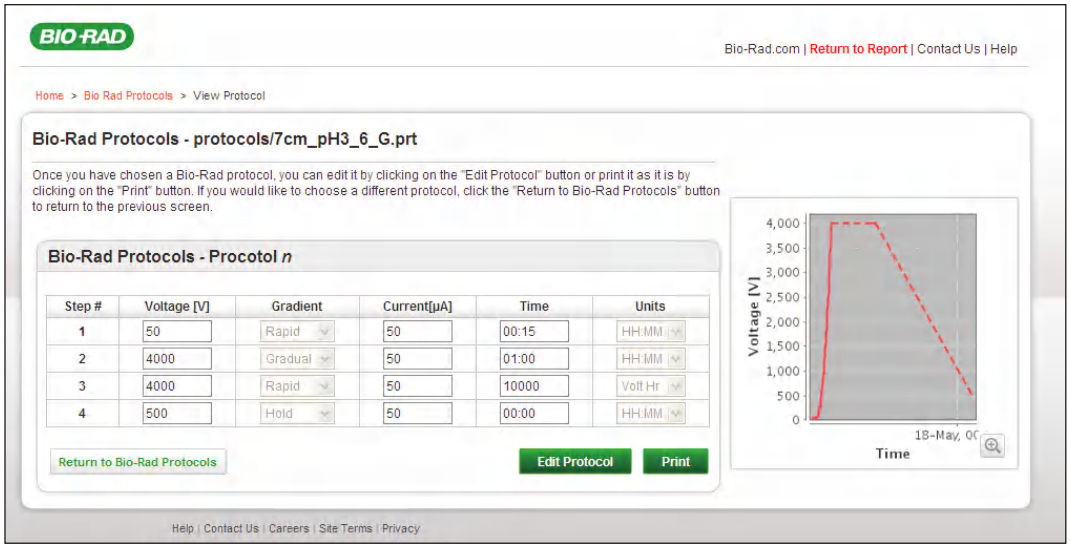


Fig. 6.3. PROTEAN i12 Reporter Bio-Rad Protocols screen.

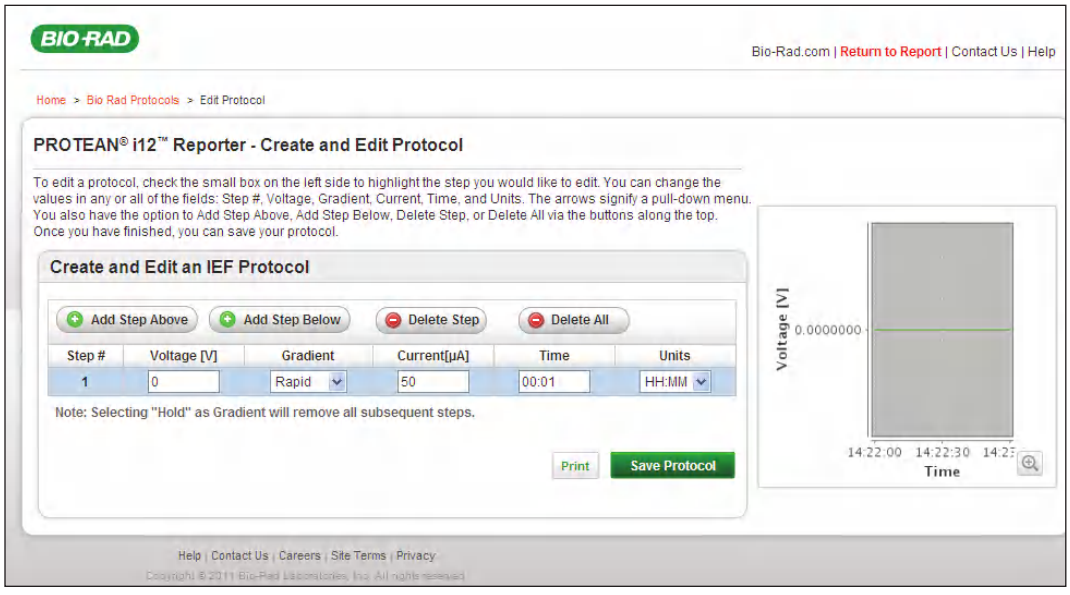


Fig. 6.4. PROTEAN i12 Reporter Create and Edit Protocol screen.

7 Troubleshooting

This chapter offers troubleshooting advice for the PROTEAN® i12™ IEF system. For further help or advice, please contact the Bio-Rad Technical Support department.

In the United States, the Technical Support department is open Monday–Friday, 5:00 AM–5:00 PM, Pacific time.

Phone: 1-800-424-6723
Fax: 1-510-741-5802
Email: LSG_TechServ_US@bio-rad.com (for U.S. and international customers)

Online technical support and worldwide contact information are available at **www.consult.bio-rad.com**.

Table 7.1. Troubleshooting guide.

Problem	Cause	Solution
No current in a lane	Poor contact between IPG strip and electrode	Make sure the gel side of the IPG strip is in direct contact with the electrode. For the gel-side down configuration, make sure to use the IPG strip retainers. See Chapter 2 for proper placement of IPG strips
	No IPG strip in lane	Make sure that the lanes selected for the run contain IPG strips before starting a run
	Incomplete wetting of electrode wick	Wet the electrode wicks completely as instructed in Chapter 2
	Incomplete rehydration of IPG strip	Check the rehydration volumes and times for the IPG strips used
No current in any lane	No contact between electrode assembly and IPG strips	Make sure: ▪ Electrode assembly is properly seated in focusing tray ▪ IPG strips are positioned correctly, (for example, that gel is in direct contact with the electrode)
	No contact between electrode assembly and instrument	Make sure: ▪ Gold contact pin of negative (-) assembly is in direct contact with cathode bar on instrument ▪ Positive (+) assembly is completely inserted into anode of instrument
	No IPG strips in lanes	Make sure IPG strips are positioned in lanes before starting a run
	No conductivity in IPG strips	Make sure IPG strips are rehydrated with correct reagents

Problem	Cause	Solution
Voltage does not increase beyond a low value	High levels of ionic contaminants in rehydration and sample solutions	Several hours may be needed for ionic contaminants to leave IPG strips. Keep salt concentration below 40 mM
Voltage does not reach the programmed value or reaches it very slowly	Programmed voltage may not be reached due to the sample composition Ampholyte concentration is too high. Up to 1% Bio-Lyte® ampholytes may be used, but ampholytes increase conductivity; therefore, voltage will be lower with increasing concentrations	No action needed Lower the ampholyte concentration
Error message appears	Situation-dependent	Shut down the instrument using the power switch and then restart it. If this fails to resolve the issue, contact Bio-Rad Technical Support

8 Cleaning and Maintenance

PROTEAN® i12™ IEF Cell

The external case is composed of cycoloy (PC/ABS). Keep it clean with occasional dusting or wiping down with a wet paper towel.

Electrode Assemblies

The electrode assembly holder is made of polycarbonate, and the electrodes are platinum-plated titanium. To clean the assemblies, remove them from the focusing tray and rinse with water. Dry them thoroughly before reusing.

i12™ Focusing Trays

To clean the polycarbonate i12 focusing trays, remove the excess mineral oil and clean with a nonabrasive detergent (for example, Bio-Rad cleaning concentrate) using the cleaning brushes provided. Rinse the trays thoroughly with deionized water to remove all detergents. Dry them thoroughly before reusing.

i12 Rehydration/Equilibration Trays

These polystyrene trays are disposable.

Sample Cup Holder and Sample Cups

The sample cup holder and sample cups are made of polycarbonate. The sample cups are disposable to prevent cross-contamination, but the sample cup holder can be cleaned and reused. Soak the sample cup holder in Bio-Rad cleaning concentrate or other mild detergent, rinse it with deionized water, and dry it thoroughly before reusing.

IPG Strip Retainers

Clean the polycarbonate IPG strip retainers by soaking them in Bio-Rad cleaning concentrate or other mild detergent. Rinse them with deionized water and dry them thoroughly before reusing.

Appendix A
Reagent and Sample Preparation

Rehydration Solution

Prepare or dilute samples into a rehydration or loading solution that contains urea, a nonionic or zwitterionic detergent, carrier ampholytes, a reducing agent such as dithiothreitol (DTT), and bromophenol blue tracking dye (Table A.1). Optimum composition depends on the sample, and the guidelines in Table A.1 should serve as a starting point for any optimization; additional or alternative components may be useful as well. For more comprehensive guidelines, see 2-D Electrophoresis for Proteomics: a Methods and Product Manual (bulletin 2651).

To prevent protein contamination, for example from skin keratin, wear laboratory gloves when handling IPG strips and the apparatus and solutions used in IPG strip preparation.

Protein Sample Loads for IEF

The total amount of protein to load per IPG strip depends on the sample, the pH range and length of the IPG strip, and the detection system used (Table A.2). Below are guidelines for protein loads that produce acceptable 2-D patterns. In general:

- Use less protein for silver staining and more for Coomassie Blue staining. Fluorescent stains such as Flamingo™, Oriole™, and SYPRO® Ruby have a wider dynamic range and a correspondingly wider tolerance for protein load
- Samples of greater complexity have protein mass distributed among a larger number of protein species, and narrow pH ranges have less sample protein focusing within the pH range of the IPG strip. Increased protein loads may, therefore, be required for samples of higher protein complexity and for narrow-range separations
- The maximum that can be loaded onto each IPG strip is 500 µg for 7 cm, 1 mg for 11 cm, 3 mg for 17 cm/18 cm, and 4 mg for 24 cm IPG strips
- In some cases, overloading of protein is acceptable and can help to reveal low-abundance proteins of interest

Table A.1. IPG strip rehydration solution composition. Vary the concentrations of the individual components as needed within the range given.

Component	Concentration	
	Standard	**Range**
Urea	8 M	7–9.5 M
Thiourea	—	0–2 M*
CHAPS	2%	1–4%
DTT	50 mM	15–100 mM
Ampholytes (w/v)**	0.2%	0.1–0.4%
Bromophenol blue	0.001%	0.001%

* Thiourea may be used with urea for more effective solubilization and focusing of hydrophobic proteins (Rabilloud et al. 1997).
**For example, Bio-Lyte ampholytes. Use the pH range corresponding to the IPG strip selected.

Table A.2. Rehydration volumes and sample loads. Protein concentration in samples prepared for IEF can be difficult to determine accurately due to interference from detergents, reductants, and other sample components. For best results in protein quantitation, use the *RC DC*™ protein assay kit (catalog #500-0121 and #500-0122).

	IPG Strip Length				
	7 cm	**11 cm**	**17 cm**	**18 cm**	**24 cm**
Rehydration Solution	125 µl	200 µl	300 µl	315 µl	450 µl
Protein Load					
Coomassie (Brilliant) Blue	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg
Fluorescent stains	5–100 µg	20–200 µg	50–400 µg	50–400 µg	80–800 µg
Silver stains	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg

Appendix B
Sample Loading Methods and Running Configurations

When planning an IEF experiment, one must choose between a number of methods for sample application, two options for IPG strip configuration (gel-side up or gel-side down), and whether to use electrode wicks. The PROTEAN® i12™ IEF cell accommodates all of these options using a single set of electrodes and focusing trays specific for each commercially available IPG strip length.

Sample Loading Methods

In the original procedure for IEF on IPG strips described by Görg et al. (1988), IPG strips were rehydrated without sample, placed gel-side up for IEF. The sample was applied in sample cups that were open at the bottom and pressed against the gel (cup loading).

A procedure was later described (Rabilloud et al. 1994, Sanchez et al. 1997) in which the sample was included in the rehydration solution and introduced uniformly along the IPG strip during rehydration (in-gel loading). In this approach, sample was diluted into a volume of rehydration solution appropriate for the IPG strip length, and the IPG strip was placed over the sample with its gel-side down. This method simplified sample application and, in some cases, improved results, particularly with dilute samples or larger quantities of sample protein. In-gel sample loading also allows rehydration and IEF to be conducted as one continuous unattended operation: if in-gel sample loading is conducted in the focusing tray with the electrodes in place and in contact with the gel, the IEF instrument may be programmed to start IEF without user intervention following suitable time for rehydration.

In-gel sample loading may also be conducted under low voltage (active rehydration). In this technique, in-gel sample loading is conducted in the focusing tray under a relatively low voltage (50–100 V). This can improve entry of high molecular weight proteins.

Despite greater complexity in setup, cup loading is beneficial in certain circumstances. It generally gives better results when IEF is conducted on basic pH gradients (for example, pH 7–10) or when optimum resolution of basic proteins is desired on wide pH gradients (Görg et al. 2000, Barry et al. 2003). Cup loading may also be beneficial for samples containing high molecular weight or hydrophobic proteins (Görg et al. 2004). Sample cups should be placed close to an electrode: positive (+) electrode placement is recommended for basic gradients, and negative (–) electrode placement is recommended for acidic gradients. On wide gradients, the best resolution is generally observed at the end of the IPG strip opposite the site of cup placement. Use anodic placement to improve resolution of basic proteins and cathodic placement for acidic proteins. Factors influencing the choice of sample loading method are summarized in Table B.1.

IPG Strip Configuration

The orientation (gel-side up or gel-side down) of the IPG strip during IEF is largely determined by the sample loading method employed:

- Cup loading requires gel-side up strip placement so that the sample cup may be placed in contact with the gel surface
- In-gel sample loading is conducted gel-side down. If the IEF cell is programmed for an unattended start following rehydration, IEF must be conducted gel-side down as well
- If in-gel sample loading is performed in the rehydration/equilibration tray, IEF may be performed either gel-side up or gel-side down. This is largely a matter of user preference, though improved resolution may be observed with the gel-side up, particularly with higher protein loads

Electrode Wicks

Electrode wicks serve as a sink for ionic sample contaminants and proteins with pIs outside the pH range of the IPG strip used. They also prevent drying of the ends of the IPG strips during IEF. Electrode wicks may be placed between an electrode and the IPG strip in either running configuration (with the PROTEAN i12 IEF cell, specific electrode wicks are provided for each configuration). Rehydration in the focusing tray cannot be performed with electrode wicks in place. In-gel sample loading with an unattended IEF start, therefore, precludes the use of electrode wicks. However, a pause may be programmed following rehydration during which electrode wicks may be inserted. In many cases, the use of electrode wicks has little effect on separation quality, and they may be omitted for convenience in either running configuration if satisfactory results are obtained in their absence.

Table B.1. Advantages and disadvantages of different sample loading methods.

Method	Advantages	Disdvantages
In-Gel Loading	Simple sample application	Poor resolution of basic proteins
	No precipitation at point of sample application	
	Accommodates dilute samples and larger protein loads	
Passive	Focusing can follow rehydration without manual intervention if performed within the IEF instrument	Not all proteins, particularly large or hydrophobic proteins, will be taken up
Active	More effective with certain proteins, particularly those of high molecular weight	Rehydration must occur within the IEF instrument
Cup Loading	More effective for basic proteins	Setup more complicated; the cup must form a seal with the IPG strip
	Can improve resolution at extremes of the pH gradient (the end opposite the point of application)	High protein loads are difficult to accommodate; concentrated samples are required
		Sample precipitation may occur at the point of application

Appendix C
IEF Protocols

Optimum IEF conditions depend on the composition and complexity of the sample and on the pH range and length of the IPG strip. The PROTEAN® i12™ IEF cell has preprogrammed protocols for each length and pH range that can suit most circumstances and that also serve as convenient starting points for optimization.

IEF Protocols

IEF should begin under a gradual increase in voltage followed by a prolonged focusing phase at the maximum voltage advisable for the IPG strip length used. Focusing occurs until a set number of Volt-hours (V-hr) have accumulated.

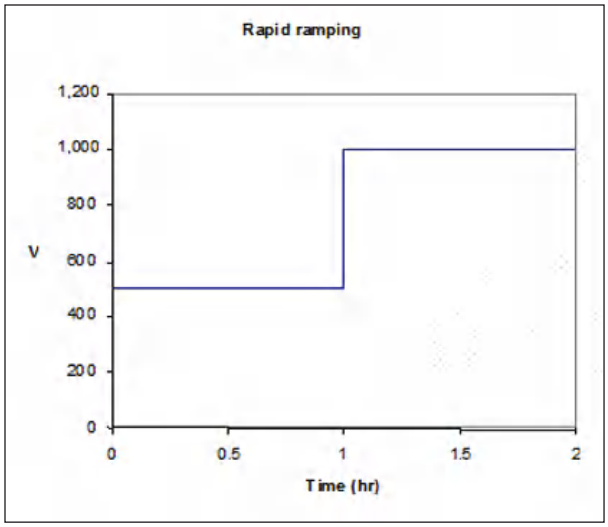
Optimal duration (in V-hr) depends on the length of the IPG strip and the pH gradient. Current is limited at a recommended 50 µA per IPG strip (though up to 100 µA is possible), and the resistance of the IPG strip increases over the course of IEF as ions are depleted from the IPG strip. Ohm's law[1] dictates that when current is held constant, voltage increases as resistance increases. Voltage, therefore, increases of its own accord from its initial low value over the course of the run.

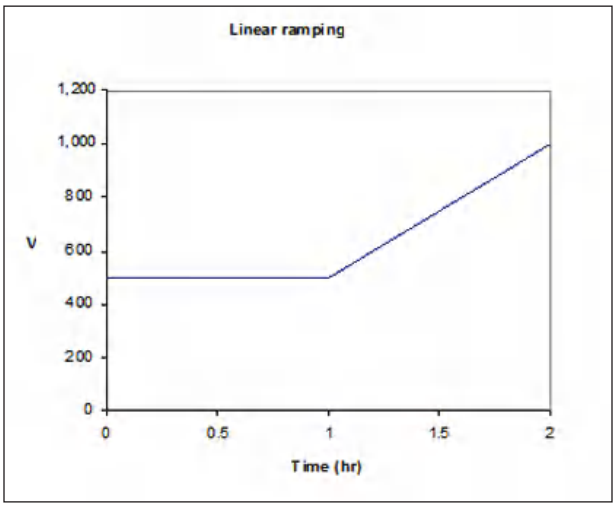
The individual lane control provided by the PROTEAN i12 IEF cell ensures that the current limit is not exceeded in any IPG strip, even in situations where conductivity differs significantly among samples run at the same time. A one-step protocol is adequate in most circumstances, as the voltage will rise gradually without need for a phased protocol with programmed voltage ramping. A gradual protocol, however, may be used with heavy protein loads or when high levels of charged contaminants are present. Both rapid (single-step, "R") and gradual (ramped, multistep, "G") protocols are provided for most IPG strip types. Gradual focusing is recommended for micro-range IPG strips, so only gradual preprogrammed protocols are provided for narrow-range IPG strips.

The PROTEAN i12 IEF cell has three ramping modes for each step in a focusing protocol (Figure C.1):

- **Rapid ramping mode** — the voltage limit is kept constant throughout the protocol step. The voltage limit changes abruptly as the protocol transitions from one step to the next
- **Linear ramping mode** — the voltage limit increases linearly within the programmed time frame, starting with the final voltage of the previous step and ending with the programmed voltage for the current step
- **Gradual ramping mode** — the voltage limit is increased quadratically according to: $V = B + (N^2 \times (E - B)/T^2)$, where B = starting voltage, E = ending voltage, N = elapsed time, and T = total time

[1] V = IR, where V=voltage, I=current and R=resistance.





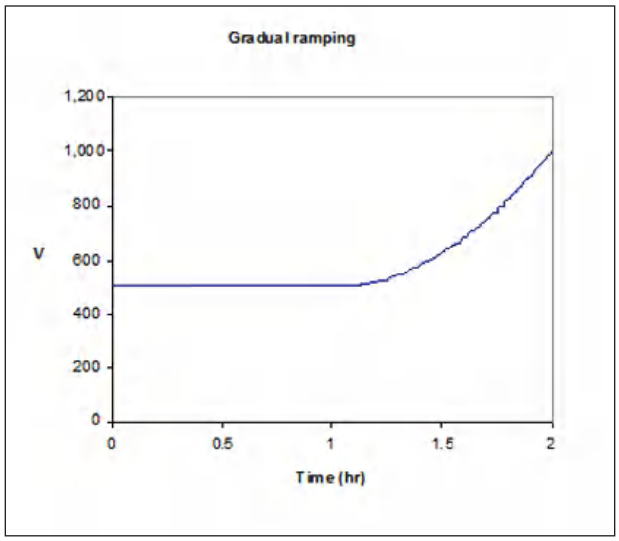


Fig. C.1. Voltage profiles in a two-step protocol. The ramping mode for step 2 is either rapid, linear, or gradual. Step 1 is 500 V for 1 hr, step 2 is 1,000 V for 1 hr, and current is non-limiting.

Note, however, that IEF is performed under current-limited conditions, so the maximum programmed voltage may never be reached, depending on the programmed voltage, the nature of the sample, and the intrinsic resistance of the IPG strip used.

Since the duration of the prolonged focusing phase is specified in V-hr, the actual duration depends on the average voltage during focusing. Focusing may conclude at different times for IPG strips run at the same time with the same protocol. It is, therefore, important to include a hold step during which the IPG strip is held at a relatively low voltage to maintain focusing until the IPG strip can be removed from the instrument.

Table C.1. Recommended focusing and hold voltages.

	Voltage (V)	
IPG Strip (cm)	Focusing	Hold
7	4,000	500
11	8,000	750
17	10,000	1,000
18	10,000	1,000
24	10,000	1,500

Bio-Rad Protocols (All protocols have a 50 µA current limit, though 100 µA is possible.)

7cm pH3–10 R
7cm pH3–10 NL R
7cm pH4–7 R
7cm pH5–8 R

Step	Voltage (V)	Ramp	Time	Units
1	4,000	Rapid	15,000	Volt Hr
2	500	Hold		

7cm pH3–10 G
7cm pH3–10 NL G
7cm pH4–7 G
7cm pH5–8 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:15	HH:MM
2	4,000	Gradual	1:00	HH:MM
3	4,000	Rapid	15,000	Volt Hr
4	500	Hold		

7cm pH3–6 R

Step	Voltage (V)	Ramp	Time	Units
1	4,000	Rapid	10,000	Volt Hr
2	500	Hold		

7cm pH3–6 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:15	HH:MM
2	4,000	Gradual	1:00	HH:MM
3	4,000	Rapid	20,000	Volt Hr
4	500	Hold		

7cm pH3.9–5.1
7cm pH4.7–5.9

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:15	HH:MM
2	4,000	Gradual	1:00	HH:MM
3	4,000	Rapid	20,000	Volt Hr
4	500	Hold		

7cm pH5.5–6.7
7cm pH6.3–8.3

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:15	HH:MM
2	4,000	Gradual	1:00	HH:MM
3	4,000	Rapid	25,000	Volt Hr
4	500	Hold		

7cm pH7–10 R

Step	Voltage (V)	Ramp	Time	Units
1	4,000	Rapid	16,000	Volt Hr
2	500	Hold		

7cm pH7–10 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:15	HH:MM
2	4,000	Gradual	1:00	HH:MM
3	4,000	Rapid	16,000	Volt Hr
4	500	Hold		

11cm pH3–10 R
11cm pH3–10 NL R
11cm pH4–7 R
11cm pH5–8 R

Step	Voltage (V)	Ramp	Time	Units
1	8,000	Rapid	26,000	Volt Hr
2	750	Hold		

11cm pH3–10 G
11cm pH3–10 NL G
11cm pH4–7 G
11cm pH5–8 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:20	HH:MM
2	8,000	Gradual	1:00	HH:MM
3	8,000	Rapid	26,000	Volt Hr
4	1,500	Hold		

11cm pH3–6 R

Step	Voltage (V)	Ramp	Time	Units
1	8,000	Rapid	20,000	Volt Hr
2	750	Hold		

11cm pH3–6 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:20	HH:MM
2	8,000	Gradual	1:00	HH:MM
3	8,000	Rapid	20,000	Volt Hr
4	750	Hold		

11cm pH3.9–5.1
11cm pH4.7–5.9

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:20	HH:MM
2	8,000	Gradual	1:00	HH:MM
3	8,000	Rapid	32,000	Volt Hr
4	750	Hold		

11cm pH5.5–6.7
11cm pH6.3–8.3

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:20	HH:MM
2	8,000	Gradual	1:00	HH:MM
3	8,000	Rapid	40,000	Volt Hr
4	750	Hold		

11cm pH7–10 R

Step	Voltage (V)	Ramp	Time	Units
1	8,000	Rapid	29,000	Volt Hr
2	750	Hold		

11cm pH7–10 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:20	HH:MM
2	8,000	Gradual	1:00	HH:MM
3	8,000	Rapid	29,000	Volt Hr
4	750	Hold		

17cm pH3–10 R
18cm pH3–10 R
17cm pH3–10 NL R
18cm pH3–10 NL R
17cm pH4–7 R
18cm pH4–7 R
17cm pH5–8 R
18cm pH5–8 R

Step	Voltage (V)	Ramp	Time	Units
1	10,000	Rapid	43,000	Volt Hr
2	1,000	Hold		

17cm pH3–10 G
18cm pH3–10 G
17cm pH3–10 NL G
18cm pH3–10 NL G
17cm pH4–7 G
18cm pH4–7 G
17cm pH5–8 G
18cm pH5–8 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	43,000	Volt Hr
4	1,000	Hold		

17cm pH3–6 R
18cm pH3–6 R

Step	Voltage (V)	Ramp	Time	Units
1	10,000	Rapid	32,000	Volt Hr
2	1,000	Hold		

17cm pH3–6 G
18cm pH3–6 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	32,000	Volt Hr
4	1,000	Hold		

17cm pH3.9–5.1
18cm pH3.9–5.1
17cm pH4.7–5.9
18cm pH4.7–5.9

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	50,000	Volt Hr
4	1,000	Hold		

17cm pH5.5–6.7
18cm pH5.5–6.7
17cm pH6.3–8.3
18cm pH6.3–8.3

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	63,000	Volt Hr
4	1,000	Hold		

17cm pH7–10 R
18cm pH7–10 R

Step	Voltage (V)	Ramp	Time	Units
1	10,000	Rapid	46,000	Volt Hr
2	1,000	Hold		

17cm pH7–10 G
18cm pH7–10 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	46,000	Volt Hr
4	1,000	Hold		

24cm pH3–10 R
24cm pH3–10 NL R
24cm pH4–7 R
24cm pH5–8 R

Step	Voltage (V)	Ramp	Time	Units
1	10,000	Rapid	60,000	Volt Hr
2	1,500	Hold		

24cm pH3–10 G
24cm pH3–10 NL G
24cm pH4–7 G
24cm pH5–8 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	60,000	Volt Hr
4	1,500	Hold		

24cm pH3–6 R

Step	Voltage (V)	Ramp	Time	Units
1	10,000	Rapid	44,000	Volt Hr
2	1,500	Hold		

24cm pH3–6 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	44,000	Volt Hr
4	1,500	Hold		

24cm pH3.9–5.1
24cm pH4.7–5.9

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	70,000	Volt Hr
4	1,500	Hold		

24cm pH5.5–6.7
24cm pH6.3–8.3

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	88,000	Volt Hr
4	1,500	Hold		

24cm pH7–10 R

Step	Voltage (V)	Ramp	Time	Units
1	10,000	Rapid	63,000	Volt Hr
2	1,500	Hold		

24cm pH7–10 G

Step	Voltage (V)	Ramp	Time	Units
1	250	Rapid	0:30	HH:MM
2	10,000	Gradual	2:00	HH:MM
3	10,000	Rapid	63,000	Volt Hr
4	1,500	Hold		

Appendix D
IEF for Peptide Fractionation Prior to LC-MS

IEF fractionation of proteolytically digested samples may be performed prior to analysis by reversed-phase liquid chromatography coupled to mass spectrometry with electrospray ionization (LC-MS) in “bottom-up” or “shotgun” proteomics experiments. IEF and reversed-phase LC are fully orthogonal separation techniques, and combining them in a two-dimensional peptide separation workflow greatly increases the depth of proteome coverage compared to reversed-phase LC alone (Cargile et al. 2005). The IEF dimension can be conveniently run on IPG strips using the PROTEAN® i12™ IEF cell. Details on sample preparation, run conditions, peptide elution, and preparation for LC-MS may be found in bulletin 6140.

Appendix E
References

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Appendix F
Specifications

Input power	100–240 VAC, 50/60 Hz
Fuse	2 ea. 2 Amp time delay 5 x 20 mm
Power input	IEC 60320 standard cord set with ground
Power output	
Voltage	0, 50–10,000 V, 1 V increments
Current	0–100 µA, 1 µA intervals
Power	0–1 W per lane
Peltier platform	
Tray capacity	1 tray
Temperature	10–25ºC ±1.0ºC @ max ambient 23ºC 18–25ºC ±1.0ºC @ max ambient 31ºC
Focusing trays	
Material	Polycarbonate
IPG strip length	7, 11, 13, 17, 18, and 24 cm
Capacity	1–12 IPG strips per focusing tray
Channel volume	7 cm—7 ml, 11 cm—10 ml, 13 cm—11.2 ml, 17 cm—14.2 ml, 18 cm—15.2 ml, 24 cm—20.2 ml
Rehydration/equilibration trays	
Material	Polystyrene
Capacity	1–12 IPG strips per tray
IPG strip length	7, 11, 13, 17, 18, and 24 cm
Channel volume	7 cm—6.8 ml, 11 cm—9.6 ml, 13 cm—10.5 ml, 17 cm—14.2 ml, 18 cm—16 ml, 24 cm—19 ml

Environmental requirements

For indoor use only, at altitudes up to 6,000 ft

Operate at 10–35ºC ambient, with maximum 90% relative humidity

Regulatory	
Safety	EN 61010-1:2001, IEC 61010-1:2001 Use NRTL to test for compliance to UL61010-1:2004 and CAN/CSA C22.2 No. 61010-1-04
EMC	EN61326 (1997 w/A1:98) Class A FCC Code of Federal Regulations, Title 47, Part 15, Subpart B, Class A
Other approvals	RoHS/WEEE Research Materials to determine level of EFUP
Dimensions	7.3 in x 13.6 in x 15.1 in
Weight	19 lbs
User Interface	
Display	QVGA resolution (320 x 240) touch screen or mouse control
Programmable	Yes
Ramping	Step, linear, gradual, and hold voltage ramping for each focusing step. Hold mode as a final step to prevent diffusion when IEF is complete
Protocol capacity	2 GB
Data collection	.dat format

Appendix G
Ordering Information

Catalog #	Description
164-6000	PROTEAN® i12™ IEF System, 90–240 VAC, includes basic unit, positive and negative electrode assemblies, 7 cm, 11 cm, and 17 cm focusing trays with IPG strip retainers, 1 pack each of 7 cm, 11 cm, and 17 cm rehydration/equilibration trays, 2 pairs of forceps, 2 packs electrode wicks for gel-side down and gel-side up applications, mineral oil, 2 cleaning brushes, cleaning concentrate, 2 USB flash drives, 3 styluses, pH 3–10 ReadyStrip™ IPG strips in 7 cm, 11 cm, and 17 cm lengths, rehydration sample buffer, and instruction manual. 13 cm, 18 cm, and 24 cm trays and cup loading accessories can be purchased separately
164-6001	PROTEAN i12 IEF Cell, 90–240 VAC basic unit includes cell, positive and negative electrode assemblies
164-6107	i12™ 7 cm Focusing Tray, includes 2 IPG strip retainers
164-6111	i12 11 cm Focusing Tray, includes 2 IPG strip retainers
164-6113	i12 13 cm Focusing Tray, includes 2 IPG strip retainers
164-6117	i12 17 cm Focusing Tray, includes 2 IPG strip retainers
164-6118	i12 18 cm Focusing Tray, includes 2 IPG strip retainers
164-6124	i12 24 cm Focusing Tray, includes 2 IPG strip retainers
165-4035	i12 7 cm Rehydration/Equilibration Tray, with lids, 25 pack
165-4025	i12 11 cm Rehydration/Equilibration Tray, with lids, 25 pack
164-6313	i12 13 cm Rehydration/Equilibration Tray, with lids, 25 pack
165-4015	i12 17 cm Rehydration/Equilibration Tray, with lids, 25 pack
165-4041	i12 18 cm Rehydration/Equilibration Tray, with lids, 25 pack
165-4043	i12 24 cm Rehydration/Equilibration Tray, with lids, 25 pack
164-6040	IPG Strip Retainers, 2 pack
164-6020	i12 Sample Cup Holder, with 25 pack of sample cups
164-6021	i12 Sample Cups, 25 pack
164-6030	Gel-Side Up Electrode Wicks, 100 pack
164-6031	Gel-Side Down Electrode Wicks, 500 pack
164-6012	Negative Electrode Assembly
164-6011	Positive Electrode Assembly
164-6010	Electrode Assembly Pair, one positive and one negative electrode assembly

165-4072	Cleaning Brushes, 2 pack
161-0722	Cleaning Concentrate
165-6060	USB Flash Drive, 2 pack
164-6050	Stylus, 3 pack
165-4070	Forceps, 1 pair
163-2129	Mineral Oil
163-2105	ReadyPrep™ 2-D Starter Kit
163-2106	ReadyPrep Rehydration/Sample Buffer, 1 bottle, 20 ml
163-2107	ReadyPrep Equilibration Buffer I, 1 bottle, 20 ml
163-2108	ReadyPrep Equilibration Buffer II, 1 bottle, 20 ml
163-2091	ReadyPrep Proteomics Grade Water, 500 ml
161-0610	Dithiothreitol (DTT), 1 g
161-0611	Dithiothreitol (DTT), 5 g
163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml
163-2109	Iodoacetamide, 30 g
161-0731	Urea, 1 kg
161-0719	Tris, 1 kg
163-2111	ReadyPrep Overlay Agarose, 1 bottle, 50 ml
163-2094	Bio-Lyte 3/10 Ampholyte, 100x, 1 ml
163-2093	ReadyStrip 100x pH 7–10 Buffer, includes only ampholytes, 1 ml
163-2098	ReadyStrip 100x pH 3.9–5.1 Buffer, includes only ampholytes, 1 ml
163-2097	ReadyStrip 100x pH 4.7–5.9 Buffer, includes only ampholytes, 1 ml
163-2096	ReadyStrip 100x pH 5.5–6.7 Buffer, includes only ampholytes, 1 ml
163-2095	ReadyStrip 100x pH 6.3–8.3 Buffer, includes only ampholytes, 1 ml
163-2099	ReadyStrip Instruction Manual, free upon request with ReadyStrip purchase

ReadyStrip IPG Strips, 12 per package.

	7 cm	11 cm	17 cm	18 cm	24 cm
pH 3–10	163-2000	163-2014	163-2007	163-2032	163-2042
pH 3–10 NL	163-2002	163-2016	163-2009	163-2033	163-2043
pH 3–6	163-2003	163-2017	163-2010	163-2035	163-2045
pH 4–7	163-2001	163-2015	163-2008	163-2034	163-2044
pH 5–8	163-2004	163-2018	163-2011	163-2036	163-2046
pH 7–10	163-2005	163-2019	163-2012	163-2037	163-2047
pH 3.9–5.1	163-2028	163-2024	163-2020	163-2038	163-2048
pH 4.7–5.9	163-2029	163-2025	163-2021	163-2039	163-2049
pH 5.5–6.7	163-2030	163-2026	163-2022	163-2040	163-2050
pH 6.3–8.3	163-2031	163-2027	163-2023	163-2041	163-2051

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