GELFREE® 8100 Fractionation System

Molecular weight fractionation with liquid phase recovery amplification success

expedeon

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ORDERING INFORMATION

PRODUCT	CAT. NO.
GELFREE 8100 Fractionation System	48100

KIT CONTENTS

DESCRIPTION
GELFREE 8100 Fractionation Station
Power Cord
User Manual

SPECIFICATIONS

Dimensions	33 cm W x 14 cm H x 33 cm D
Clearance & Ventilation	38 cm vertical clearance for cartridge
Loading	20 cm back clearance for airflow
Weight	4.5kg (10 lbs)
Max Power	450 VA
Temperature Limits	Storage: 10-60°C, 30-80% relative humidity
Ingress Prot. Rating	IPXo

HANDLING

During operation, the GELFREE 8100 Fractionation Station lid must always remain closed to ensure the safety of the operator. This product is safe to use when operated in accordance with this instruction manual. If this unit is used or modified in a manner not specified in this manual, then protection afforded by the unit will be impaired. Alteration of this unit will:

- Void the warranty
- Create potential safety hazards

Protein Discovery is not responsible for any injury or damage caused by use of this device when operated for purposes for which it is not intended. All repairs and services should be performed by Protein Discovery.

Note: The GELFREE 8100 Fractionation Station is capable of supplying up to 300 V per channel. However, the voltage recommended for best results is specific to each cartridge type, as more fully described on the Cartridge Kit Reference Card.

The GELFREE 8100 Fractionation System is manufactured in the USA from domestic and international components.

To ensure safe, reliable operation, always operate the GELFREE 8100 Fractionation System according to the instructions provided in this manual. Wear personal protective equipment (e.g. gloves and safety glasses) when working in a laboratory environment.

Expedeon products are intended for in vitro use only.

Expedeon is not responsible for injuries or damages caused by improper use.

UNPACKING AND PLACEMENT

Unpacking and Inspection of Parts

Upon receipt of your GELFREE 8100 Fractionation Station, open the box, carefully remove the foam packaging, and remove the instrument and power cord from the box.

Remove the tape securing the lid, along with the protective plastic, so that the lid can operate freely. The instrument could have been damaged during shipment. Inspect the instrument case, lid and hinges, and electrodes for any obvious signs of damage. If damage has occurred, please notify Customer Service immediately.

Instrument Placement

The GELFREE 8100 Fractionation Station includes a high voltage power supply and electronics that are sensitive to moisture, extreme temperature, and electromagnetic interference. Heat generated when using the instrument is dissipated using a series of fans in the rear of the instrument. Proper airflow must be ensured to avoid overheating of the instrument during operation. As such, the instrument should be placed on a level table or laboratory bench removed from routine handling of liquid reagents. The minimum recommended space is 24" W x 24" D x 18" H allowing for 8" of unobstructed space behind the instrument.

DESCRIPTION OF PARTS

GELFREE 8100 Cartridge Kit
The GELFREE 8100 Fractionation Station is an independently-controllable, eight channel electrophoretic power supply designed specifically for use with GELFREE 8100 Cartridge Kits. It features a touch screen display, power supply capable of delivering constant voltage (20-300 V) for pre-programmed time intervals, and retractable electrode arrays. The primary instrument components referred to in this manual are labeled below.

GELFREE 8100 Cartridge Kit

The GELFREE 8100 Cartridge is a patented, single use cartridge designed for insertion into the GELFREE 8100 Fractionation Station. The cartridge is supplied with storage buffer and specially designed, precast gels, ensuring maximum seperation reproducibility. The primary cartridge components are labeled below.

Included with each Cartridge Kit is the Running Buffer (500 mL) and (5x) Sample Buffer (1.9 mL), which comprises all the buffer necessary to run all eight channels in the cartridge.

Note: Do not refrigerate the catridge. The cartridge must be stored at room temperature (20-25°C) at all times.

OPERATING PROCEDURES

Installation and Power Up

To power on the instrument, insert the provided power cord into the socket on the back of the instrument, move the instrument to its final position, and plug the cord into a suitable power supply (100240 VAC, 50-60 Hz). Power on the unit using the switch located on the back of the instrument above the power cord.

During the power up procedure, the Protein Discovery logo will be displayed on the touch screen. Once powered up, the main control screen foill be displperation of the high vor interlock by oosing the lid of the instrument. In the lower right corner of the main screen, the 'Lid State' indicator will switch from green (closed) to red (open) to indicate engagement of the interlock.

Loading the Cartridge and Electrode Placement

Opening theaccess to the cpartment for loading and unloading the cartridgcting fractions during the experiment. The cartridge is loaded such that the well numbers imprinted on the cartridge can be read by the operator and the arrows imprinted on the cartridge point to the right. The cartridge positioning key (Figure 3) prevents the user from loading the cartridge in the wrong orientation. The loaded cartridge should rest fully on the bottom of the cartridge compartment. With the cartridge in place, the electrode arrays are lowered in place by the user. The arrows in Figures 5 and 6 indicate the direction of the applied force when raising and lowering the electrodes. Do not force the electrodes into position – this can permanently damage the mechanism. Very little resistance should be met when raising and lowering the electrodes into position.

Operating the Software

The GELFREE 8100 Fractionation Station is controlled by an onboard computer with a touch screen interface. The primary operating modes are the following:

- Method Development/Editing
- Method Execution
- Method Monitoring

Once the device is powered up, the central navigation and operating screen will be displayed on the touch screen interface. Teh 'Main Screen' includes the key featurees necessary to navigate the control software and develop/edit, execute and monitor seperation methods (Figure 7).

Method Development/Editing

Note: Be sure to refer to the Cartridge Lit Reference CArd provided with each Cartridge Kit when setting up and running the fractioation method.

To create a new method, follow thses steps:

1. On the Main Screen, touch the 'Method' button displayed in the uppe This will take you to the Meth r right hand corner (Figure 7). ods Screen (Figure 8).
2. Touch the 'Retrieve' button. This will bring up a dialogue screen (Figure 9) requesting a method number.
3. Begin by pressing the "C" button on the number pad to clear the existing input. Next, enter a method number that has not been used and press OK. This will leas to the screen shown in Figure 10. Touch the step number to be modified.
4. This leads to the input screen shown in Figure 11. The green cell indicates an active cell where a value for voltage or time can be added or modified by following these simple steps:

- Cancel the value in the cell using the 'C' on the number pad.
- Enter the desired value using the keypad.
- Touch the inactive cell to activate it. Then repeat steps a and b.
- The 'Next' and 'Previous' buttons allow the user to edit the next step or the previous step, respectively. A maximum of 30 steps may be included in any single method.
- When editing is complete, touch the OK button.

5. Review the voltage/time profiles for each step in the new method. Press 'Save' on the Method Screen (Figure 10). A pop-up box will appear with a default method number of 1. Clear or type over the method number to give the newly created method a different number.

6. Next press 'Apply' onthe same screen to go back to the main screen (Figure 7).

To edit an existing method, follow the steps outlined above, withthe following exception. Rather than using a number for a new method in step 3, enter the number of an existing method. Edit the method as described above and then save the method.

Important: It is critical that evth the GELFREE 8100 Fractionation Station incorporate a first step to allow the user to add an additional 2 mL of Running Buffer to the Cathode Buffer Reservoir (bringing the total volume to 8 mL) and to thoroughly rinse the Sample Loading Chamber.

For standard methods, the first step parameters are shown on the Quick Reference Card provided with each Cartridge Kit. For a custom method, the first step should be applied once the dye front is visible in the gel tube (16 minutes at 50 V, 8 minutes at 100 V).

Executing a Method

1. Retrieve a defined method by pressing the 'Method' button on the Main Screen as previously described. Once the method has been retrieved, press 'Apply' to apply the method and return to the Main Screen.
2. Select the channels to run in the cartridge by pressing the 'Channel' button on the Main Screen. This will bring up the Channel Screen (Figure 12). Select the combination of channels to run during the experiment by pressing the corresponding channel button (e.g. 'Channel 1'), or press 'Set All Channels' to apply the method to all eight channels. Once the desired combination of channels has been selected, press 'Done' to return to the Main Screen.
3. Press the polarity button on the Main Screen to set the polarity of the field. For all experiments using SDS, the polarity should be 'Negative.'
4. Once the method has been applied and the channels selected, press the 'Start' button on the Main Screen to begin a run. Once running, the 'Start' button becomes the 'Pause' button, which allows the user to pause the run.
5. To completely stop an experiment once it has been started, press the 'Abort' button on the Main Screen. A confirmation screen will appear (Figure 13). To abort, touch 'Yes' or touch 'No' to return to the Main Screen.

Monitoring a Method

1. Channel Status: THe circles shown in the box on the left side of the Main Screen indicate the status of each channel. A green circle indicates that the channel is active. The applied coltages and currents are displayed to the right of the channel status circles. When the method is paused, the circles will turn yellow. If the method fails to start or fails during the run, the circles will turn red. When the circles are either yellow or red, the voltage readout is zero.
2. Time Monitor: By touching the button above the digital time counter, the user can toggle between 'Time Elapsed', 'Time Remaining', or 'Time To Pause'. The progress bar provides a visual indication of all time metrics.
3. Active Method: At the bottom left corner of the Main Screen, the method that is currently selected is shown.
4. Method Status: At the bottom center of the Main Screen, the current step that is active is shown (e.g. 'Step 1 of 12 (50 V, 16 min)).
5. Lid Status: At the bottom right corner of the Main Screen, a green circle indicates that the lid is closed (ready/safe to operate) and a red circle indicates that the lid is open (will not operate).
6. Software Release Information: Touch the PDI icon found in the top left of the screen to view the current software version running the GELFREE 8100 Fractionation Station.

Running the Cartridge

It isperative that the steps below are followed in sequence:

1. Prepare the sample(s) and create or modify existing method.
2. Prepare the cartridge and load the sample(s).
3. Start the run.
4. Collect the fractions.

Preparing the Sample(s)

Important: The presence of low molecular weight contaminants, such as detergents, urea, and excessive salts will negatively impact the fractionation results. Before you start, be sure to fully desalt and remove any known contaminants. If you sample has particulates, we highly recommend passing the sample through a 0.8 µm syringe filter to remove any loose particulates prior to further preparation.

1. Once the sample is ready for preparation, please follow these steps:

In a 500 µL vial, combine the following:
Up to 112 µL Sample
- + 30 µL Sample Buffer (5X)
- + 8 µL 1M DTT (optional, not provided in the kit)
- + x µL H_2O
- = 150 µL

Note: If the sample volume is less than 112 µL, add 18MΩ H2O to bring the final volume to 150 µL.

2. Heat the vial for 10 minutes at 50°C

Note: Overheating the sample will cause protein degradation.

3. Allow sample to cool to room temperature (22°C).

Note: Changes in teh sample, buffer, cartridge, or room temperature will cause changes in elution time. For best results, run the system with all the solutions at 22°C

Preparing the Cartridge and Laoding the Sample

1. Remove the cartridge from the foil package and remove the plate sealer. If all 8 channels in the cartridge will be utilized in one experiment, dump out the storage buffer found in all compartments. If only some of the 8 channels will be utilized, use a Pasteur or transfer pipette to remove only the Storage Buffer from the reservoirs associated with the active channels.
2. Add 8.0 mL of Running Buffer to the Ancode Buffer Reservoir for all active channels.
3. After rinsing out the Storage Buffer with two 200 µL aliquots of Running Buffer, add 150 µL of Running Buffer to the Sample Collection Chambers for all active channels.
4. Add 6.0 mL of GELFREE Running Buffer to the Cathode Buffer Reservoirs for all active channels.

Important: Take care to avoid introducing and trapping bubbles in the Cathode and Buffer Reservoirs, as they will block electrical current and impact elution time reproducibility. To dislodge bubbles potentially trapped against the membrane in the Buffer Reservoirs, simply flush the membrane with Running Buffer using a pipette. Note: Overfilling the reservoir at this point may result in irregular sample migration and separation.

5. Using a fine-tipped transfer or multi-channel pipettor, remove and discard any buffer that has flowed into the Sample Loading Chambers from the Cathode Buffer Reservoirs for the channels being loaded. Immediately load 150 µL of the prepared sample into the Sample Loading Chamber of the corresponding channels. Be careful to avoid introducing bubbles into the Sample Loading Chambers.

Note: Buffer passes freely through the membrane separating the Cathode Buffer Reservoir and the Sample Loading Chamber. Be sure to load the sample immediately after removing Running Buffer in the Sample Loading Chamber and immediately before running the cartridge.

Performing the Run and Collecting the Fractions

1. Place the cartridge in the instrument, lower the electrodes, and close the lid.
2. Start the method by touching 'Start' on the Main Screen.

Note: Proper operation is indicated by a green circle next to each channel number on the Main Screen.

<u>If operation fails, check these items:</u>

- Lid properly closed (indicated by green circle at bottom right of screen).
- Correct method selected (indicated at bottom left of screen).
- Correct voltage and current displayed for all channels (as set by user).
- Buffer in all Cathode and Anode Buffer Reservoirs.
- All electrodes contacting buffer.

- Buffer in all Sample Collection Chambers.
- No bubbles around the membranes restricting current flow.

3. At the end of the first step, as specified on the Quick Reference Card for the specific Cartridge Kit used, the instrument will pause for rinsing of the Sample Loading Chambers with Running Buffer and addition of an extra 2 ml of Running Buffer in the Cathode Buffer Reservoirs. The electrodes can be left in place during this step. Add 2 mL Running Buffer to the Cathode Buffer Reservoir of each of the active channels and resume the Method.

Note: At this point, the sample will have migrated into the gel and a band of dye will be visible near the left end of the gel.

Note: Upon resuming the method, check for proper operation as in Step 2 above.

4. The Station will pause for collection of fraction 1 at the second pre-defined pause interval. At that point, use a single or multi-channel pipette to transfer the contents of the Sample Collection Chambers to the first column of a 96-well pl

Note: To maximise recovery, pipette up and down twice before removing the sample.

5. Using fresh pipette tips, wash the Sample Collection Chambers twice with 200 µL fresh Running Buffer, Pipette up and down at least twice before removing and discarding the wash buffer. Fill the Sample Collection Chambers with 150 µL of fresh Runnig Buffer, and resume the Method.

6. At each pre-programmed pause interval, repeat the collection, wash, and fill steps (4-5), placing fraction 2 into column 2 of the 96-well plate, fraction 3 into column 3, and so on.

7. To ensure proper buffering capacity, Running Buffer must be replaced in the Anode and Cathode Buffer Reservoirs after approximately 30 minutes of cumulative run time at 100V or 60 minutes of cumulative run time at 50 V, according to the following procedure:

- Remove the cartridge from the instrument and discard the buffer.
- Fill each Anode Buffer Reservoir with 8 mL Running Buffer.
- Fill each Cathode Buffer Reservoir with 8mL Running Buffer.
- Fill the Sample Loading Chambers with 150 µL Running Buffer.
- Fill the Sample Collection Chambers with 150 µL Running Buffer.
- Return the cartridge to the instrument and resume the Method.

8. Repeat the Sample Collection Chamber collection, wash and fill steps for each subsequent fraction.

9. Once all the Method's steps have been completed, the display screen will indicate that the experiment has ended. Collect the final fraction and enjoy the benefits of reproducible, high yield, liquid-phase fractionation in your protein research.

MAINTENANCE AND TECHNICAL SERVICES

Cleaning Specifications

General maintenance for the GELFREE 8100 Fractionation System includes wiping the unit with a soft dry or slightly dampened cloth. Do not handle or clean electrodes. Do not use solvents or detergents.

Replacement Parts

Please contact Expedeon for any replacement parts for the GELFREE 8100 Fractionation System. The use of parts, buffer solutions, or cartridges that are not supplied by Protein Discovery, Inc may cause damage to the unti and cause the warranty to become null and void.

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