



SageELFTM

Sample Fractionator

Operations Manual:

Size Fractionation of Protein Samples

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The SageELF instrument and gel cassettes are covered by U.S. patents **9,599,590** and **10,473,619**.

For research use only.

Made in the USA.

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1 Introduction

Thank you for purchasing the SageELF from Sage Science.

We urge you to read this manual to familiarize yourself with the system's capabilities and precautions.

System Overview

There are three components to the SageELF system:

- **Instrument** – The instrument contains an epifluorescence detectors, an electrophoresis power supply, two electrode arrays, and a single-board computer. The system computer is accessed by an external LCD monitor, mouse, and keyboard.
- **Software** – System software allows the user to enter parameters that predict the DNA or protein fraction distributions that can be expected to be collected in 12 elution wells. The software also logs fluorescence and electrical current data which can be analyzed in a review screen.
- **Gel Cassettes** – Pre-cast, disposable agarose cassettes are manufactured by Sage Science. Cassettes are supplied in kits that contain 10 cassettes and sufficient reagents to complete all collections. Kits have been developed to address DNA or protein collections within specific size ranges.

2 Safety and Precautions

Icons Used

In this manual, the following icons will be used to provide the user with information pertinent to the use of the SageELF.



Caution! Warns the user that injury or instrument damage may occur if the contents of the warning are not properly followed.



High Voltage! Warns of the risk of electrical shock if the contents of the warning are not properly followed.



Important! Provide important information about the proper use of the system that may influence the quality of the result.



Information. Provides additional information regarding the function of the system or applications for which is used.

Safe Use Guidelines

The SageELF system is designed to operate under the following environmental conditions:

- Pollution Degree 2
- Installation category 2
- Altitude 2000m
- Indoor use
- Ambient temperature 17-25°C
- Humidity 10-80%, non-condensing



Caution! The SageELF was designed to be operated on a flat surface. Do not operate on a tilted surface or tilt during operation.

3 Unpacking and Installation

3.1 Unpacking the SageELF

The SageELF instrumentation is shipped in two boxes: one will contain the SageELF and Accessories and the second box will contain the computer monitor in the manufacturer's original packaging. With the boxes in the upright position, open and confirm that the following items are enclosed:

Monitor

- LCD computer monitor
- Video cable
- Power cord

SageELF

- SageELF Instrument
- Accessory box
 - Computer keyboard, USB
 - Computer mouse, USB
 - Power supply
 - Power cord
 - Rinse cassette (for maintenance of electrodes)
 - Two calibration fixtures (for setting optical baseline before each run)

3.2 Installing the SageELF

1. Open the LCD monitor box, and assemble it according to the manufacturer instructions
2. Using a box cutter, open the top seal on the SageELF box, and open.
3. Remove the accessory box located just inside, atop the instrument. Remove the keyboard, mouse, and instrument power supply and cords from their packaging.
4. Firmly grip both sides of the bottom of the instrument and lift it along with the foam packaging inserts. The SageELF weighs approximately **20 lbs**. Place the unit onto the bench top.
5. Connect the LCD monitor (VGA port) to the SageELF (HDMI port, back panel) using supplied video cable. Connect monitor to the power outlet using the power supply supplied with monitor (**Figure 3.2**).
6. Insert USB connector from the computer **keyboard** into any USB port located on the back panel of the SageELF (**Figure 3.2**).
7. Insert USB connector from the computer **mouse** into any USB port located on the back panel of the SageELF (**Figure 3.2**).
8. Connect SageELF instrument to the power outlet using the SageELF power supply and power cable. The power input connector is in the lower left hand corner on the back panel of SageELF, below the power switch (**Figure 3.2**).
9. When Powered on, the blue LED detector on the cassette nest will be on, and the blue light on the front panel of the Instrument will be on (**Figure 3.3**). The LEDs and front panel light are on at all times when attached to power, regardless whether the software has been launched.
10. Press power switch located on the rear of the instrument, and wait for software to launch (approximately 30 seconds)

The SageELF is ready for use. The software should automatically launch – allow 30 seconds.

Figures 3.2 and 3.3 on the next page show the rear and front panels of the SageELF.



Figure 3.2. Rear Panel of the Sage ELF

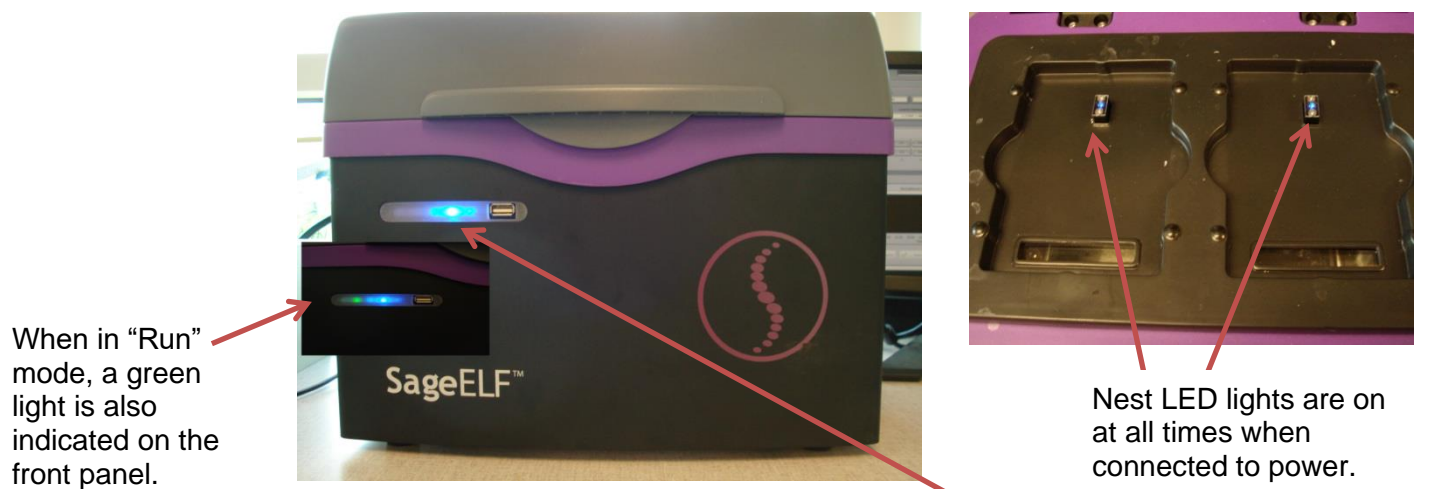


Figure 3.3. Front Panel of the SageELF and the Cassette nest

After the power button is pressed, the front panel LED will light, and the software will launch.

3.3 Unpacking gel cassettes

Gel cassettes are shipped in boxes of 10 cassettes. Ensure boxes are in the upright position and confirm that following contents are present:

- 10 foil-sealed gel cassettes (**store at R.T.**)
- 1 package of adhesive tape for sealing elution wells
- 1 tube of fluorescent marker [contains loading solution] sufficient for 10 cassettes (**store at 4 °C**)
 - 200µl, for size-based fractionation protocols
- 1 tube of loading solution sufficient for 10 cassettes (**store at 4 °C**)
 - 500µl, for time-based fractionation protocols
- Spare SDS-electrophoresis buffer (200ml)

3.4 Storage conditions for cassettes and reagents

The following storage requirements should be observed:

- **Gel Cassettes**- Store at room temperature in the foil bags until ready for use.
- **Electrophoresis buffer** - Store at room temperature.
- **Loading solution** – store at 4 °C. Bring to room temperature before use
- **Fluorescent marker (labeled)** – store at 4°C. Bring to room temperature before use.

4 Sage ELF Workflow Summaries

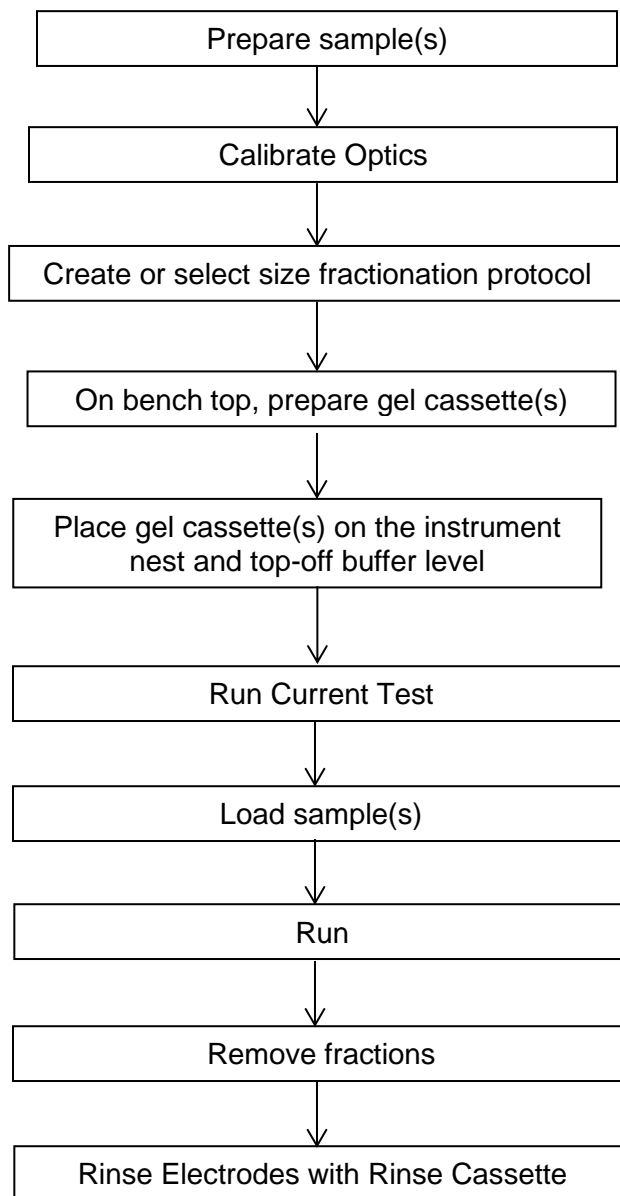
4.1 Recommended user-supplied laboratory equipment

- P100 single-channel pipette
- P100 8- or 12-channel pipette*
- Buffer trough
- 0.2 ml tubes
- 0.2 ml 12-strip tubes or 96-well microplate
- TCEP (tris(2-carboxyethyl)phosphine), 500mM, (1.43 g/10ml H₂O)
- Heating block, preferably a thermal cycler

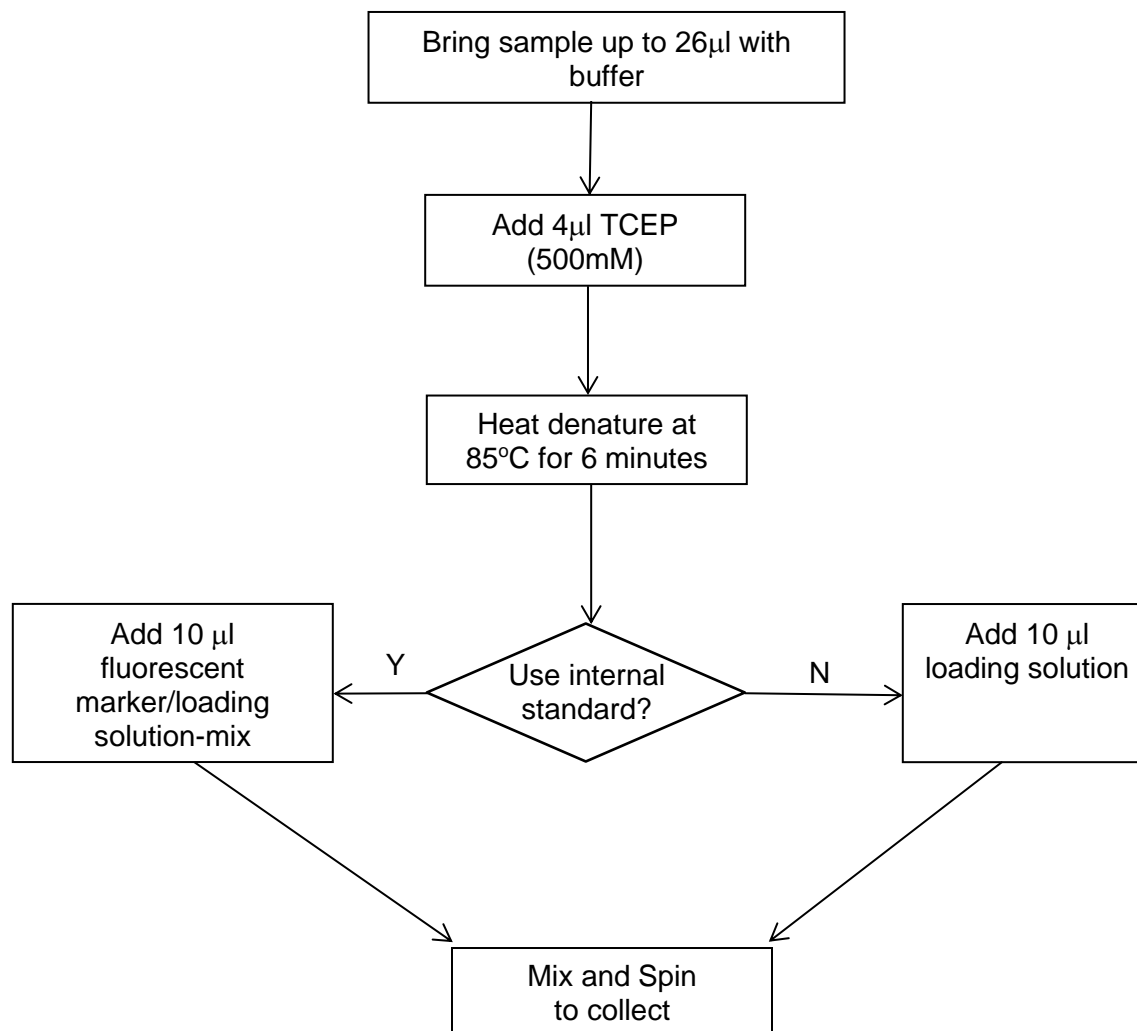


Elution wells are on a 4.5mm spacing. Equip an 8-channel pipette with 6 tips to aspirate every other well. An adjustable 12-channel pipette is convenient for transferring 12 samples between a 4.5 mm and 9 mm (strip tubes or microplate) spacing. We recommend the Viaflo Integra 125 Voyager [PN:4132] or similar.

4.1 Recommended fractionation workflow



4.2 Recommended sample prep workflow



5 Sample Preparation

5.1 Input Sample Characteristics

When running the SageELF, characteristics of input samples can affect separation resolution and efficiency of product recovery. The following general guidelines should be followed:

- **Ionic strength**
If sample is in high salt buffer (e.g. 7M Urea or 8M guanidine), buffer should be exchanged by dialysis or gel filtration into the provided SDS-electrophoresis buffer. Sample and buffer composition will influence sample migration. Results may vary when using alternative buffers. Using a reductant such as TCEP or DTT is recommended.
- **Recommended Buffer**
10-30 mM Tris, pH 7.4 – 8.0, 0-10 % glycerol, up to 50mM TCEP
- **Sample Amount***
Total protein in a sample load should be between 100ng (minimum) and 350µg (maximum).

* These max and min limits have not been rigorously tested. The values have worked for many sample types we have tested so far.

5.2 Materials supplied by users (recommended)

- P100 single-channel pipette
- 0.2 ml tubes
- TCEP* (tris(2-carboxyethyl)phosphine), 500mM (1.43g/10 ml or water) - 5 µl/sample, 100µl/kit
- Vortex mixer
- Microcentrifuge
- Heating block, preferably a thermal cycler

* DTT, 1M, may also be used (1.54g/10ml)

5.3 Reagents supplied by Sage Science:

- Spare SDS-electrophoresis buffer (200ml)
- Loading solution / fluorescent marker mix (200 μ l, for size-based fractionation)
- Loading solution (500 μ l, for time-based fractionation)

5.4 Sample preparation procedure

1. Bring the loading solution/marker-mix (or loading solution for time-based fractionation) to room temperature.
2. Bring each protein sample up to 26 μ l with buffer (the SDS-electrophoresis buffer can be used).
3. Add 4 μ l of 0.5M TCEP** to each sample
4. Heat-denature the samples at 85°C for 6 minutes.
5. Combine the 30 μ l protein sample with 10 μ l of loading solution/marker-mix (or loading solution for time-based fractionation). The total sample volume is 40 μ l.
6. Mix the samples thoroughly (vortex mixer). Briefly centrifuge to collect.

** Alternatively, 14 μ l of loading solution with 4 μ l of DTT may be used

6 Optical Calibration Procedure

The SageELF uses an LED/photo diode optical assembly for detection of internal markers. If internal markers are used, the SageELF optics should be calibrated prior to running. If internal markers are used regularly, the optics should be calibrated once per week. Optical calibration adjusts for the gradual drift that can occur with LED lights.

1. Select the “Main Screen” Tab.
2. Place the calibration fixture(s) over the optical detector(s). The calibration fixtures are supplied with the SageELF. There is a dark film strip in between orange plexiglass plates. Ensure that this is over the LED detector.



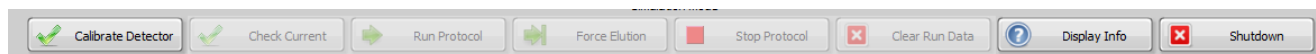
Proper placement of the calibration fixtures

3. **Close** the SageELF Lid



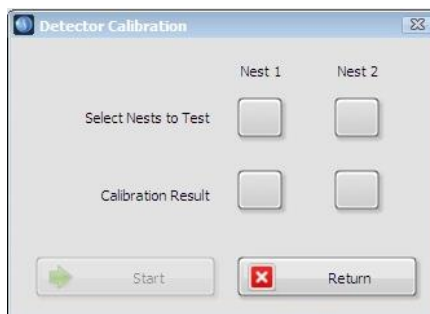
Important! The instrument lid must be closed to successfully calibrate the optical detectors. Ambient light will cause a failed or incorrect test.

4. If necessary, clear the “Protocol” Field.
5. On the Main Screen, at the bottom, select the “Calibrate Detector” button.

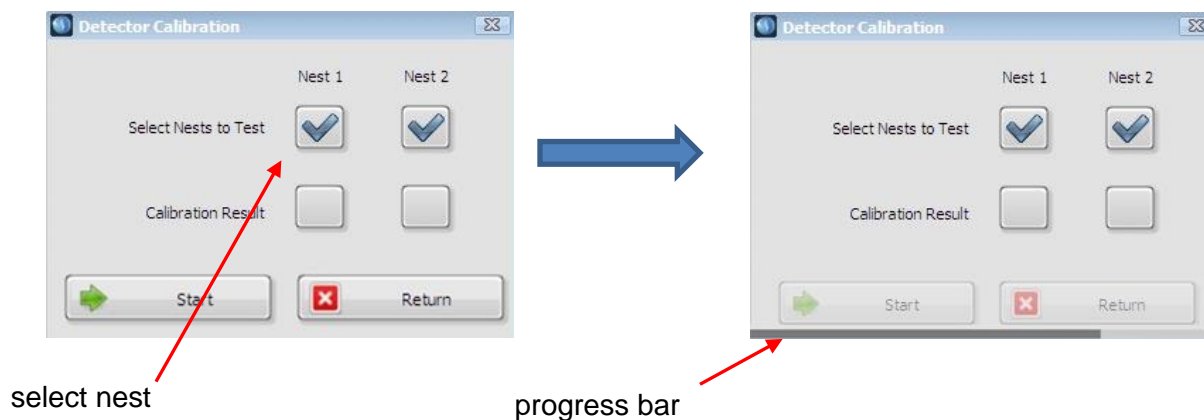


Calibrate Detector

A pop-up window will appear.



6. Select the nest(s) to be calibrated. And press “Start”. A progress bar will appear until the routine is completed.



7. When complete, the “Calibration Result” will indicate a green check mark, and the optics will be properly calibrated.



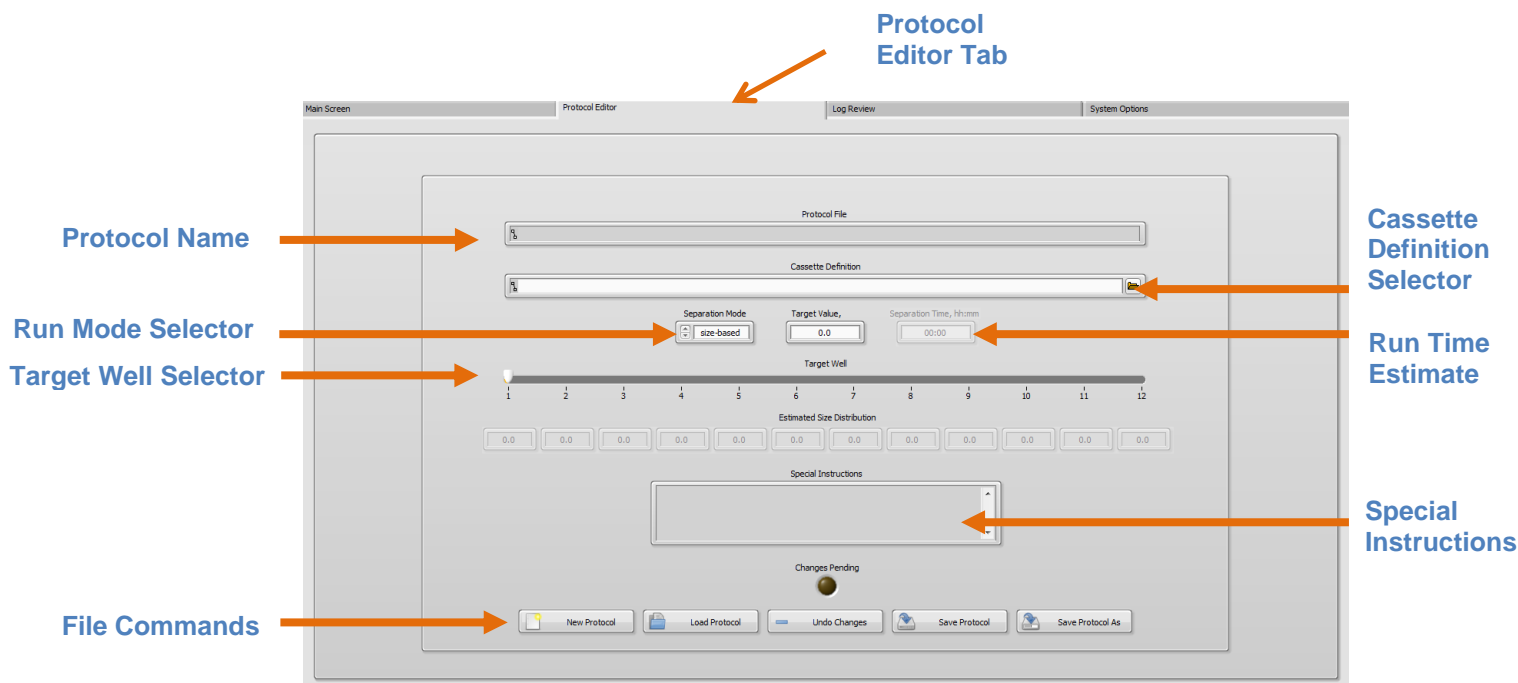
8. Press “Return” to close the window and return to the Main Screen.

7 Protocol Editor – Creating a Protocol

The default software screen on the SageELF has a tabbed format. The Protocol Editor is the second tab.

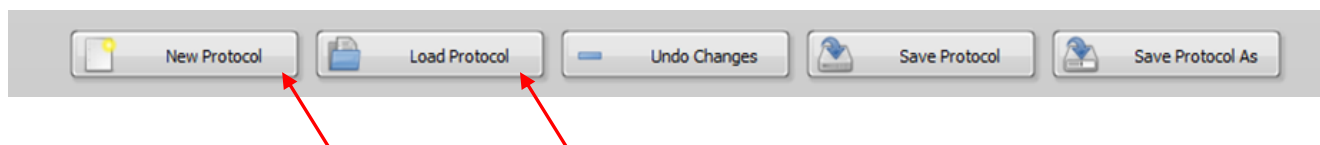
7.1 Overview

The protocol editor tab is the screen with which users create new protocols, or edit existing protocols, for sample fractionation. A protocol is created for a cassette type (within the applicable target range) and is saved as a named protocol file with a “.ppprot” file extension. When running a cassette, an appropriate protocol file is selected and applied to the run. A protocol file may be applied to any run, provided the cassette type is correct.

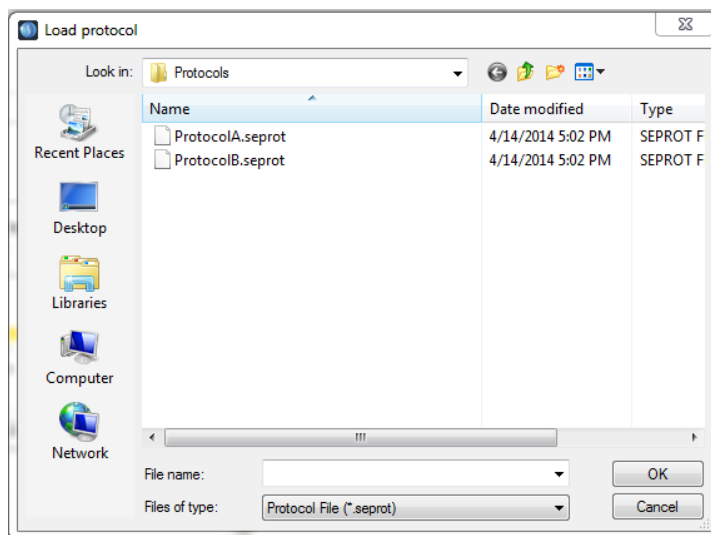


7.2 Creating a Protocol

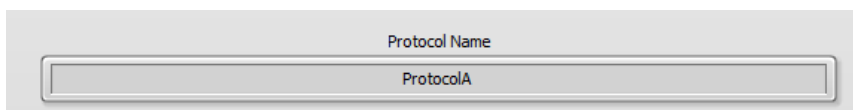
1. Click the “Protocol Editor” tab along top of the screen. File Commands are at the bottom of the screen:



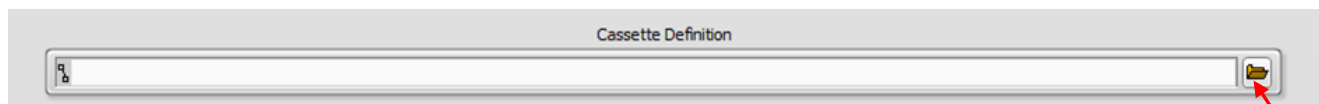
- **New Protocol** – clears all fields, and requires the user enter new protocol parameters
 - **Load Protocol** – opens a previously saved protocol, which has been named by a user, and allow the protocol edited and resaved. A saved protocol that is open in the protocol editor screen will applied to the samples that are run.
2. Select “New Protocol”, to create a new protocol. This will clear all populated fields in the Protocol Editor Tab (this can be undone using the “Undo Changes” button). Skip to Step #4.
 3. To edit an existing protocol, press the “Load Protocol” button (unless the currently loaded protocol is going to be edited) to select the previously saved protocol. A list protocols will appear in a pop up window, select the appropriate file from the “Protocols” folder, and select “Okay”:



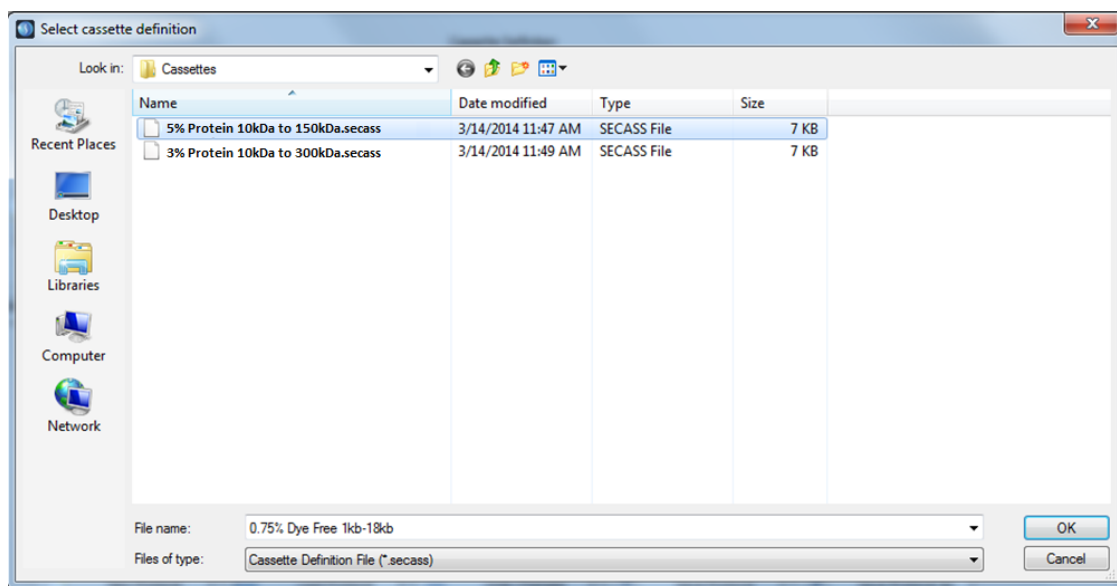
The protocol name will appear in the “Protocol Name” field:



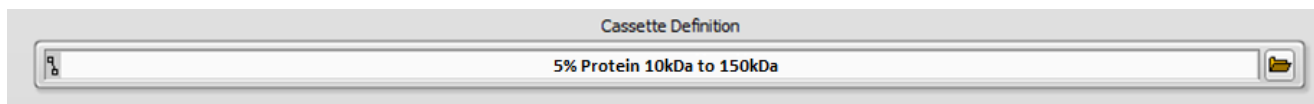
4. Select a cassette definition from the “Cassette Definition” menu (if necessary), by clicking the folder icon. Protocol fields cannot be edited unless a cassette type has been selected.



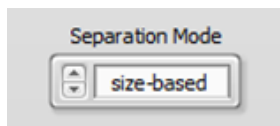
A list of available cassette choices will appear in a pop-up window, select the appropriate definition from the “Cassettes” folder, and press “Okay”.



The cassette definition will appear in the “Cassette Definition” field. Note that the definitions include the range of target values for which the cassette definition can be used:



5. Select the separation mode with the “Separation Mode” selector:

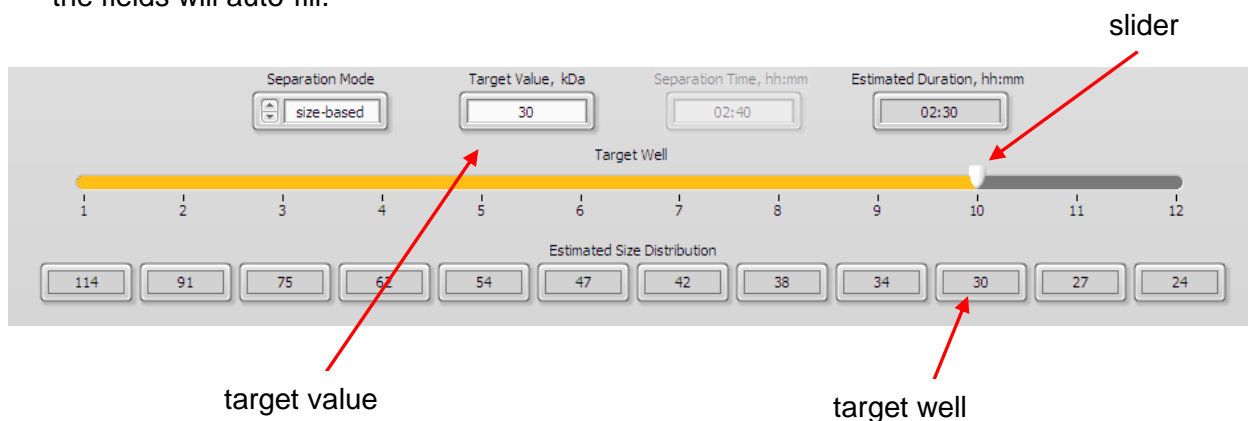


- **size-based** – allows users to program by editing entering a target value for a given elution well.
- **timed** – allows the user to set the separation run time.

Important. For best reproducibility, use the size-based mode and use an internal standard.

Important. The two separation modes are different approaches to programming a fractionation range, but use the same calibration algorithm. However, the running operations may be different, if an internal standard is used.

6. To program in “size-based” mode, use the slider to select a target well, and enter a value in the “Target Value” field. The target value for the remaining wells will be calculated and the fields will auto-fill.



7. To program in “timed” mode, edit the “Separation Time” field directly, and the target values for each well will be calculated and the fields will auto-fill.

Important. Timed mode will ignore data from internal standard and therefore will be less reproducible than size-based mode. In addition, the predicted size distribution in the protocol editor may not be achieved.

8. Press “Save As” button, enter a name for the protocol.

8 Preparing a Cassette

8.1 Recommended Laboratory Equipment

- P100 single-channel pipette
- P100 8- or 12-channel pipette*
- Buffer trough
- Spare electrophoresis buffer (included with the cassette package)
- Elution well sealing tape ((included with the cassette package)

* Elution wells are on a 4.5mm spacing. Equip an 8-channel pipette with 6 tips to aspirate every other well. An adjustable 12-channel pipette is convenient for transferring 12 samples between a 4.5 mm and 9 mm (strip tubes or microplate) spacing. We recommend the Viaflo Integra 125 Voyager [PN:4132] or similar.

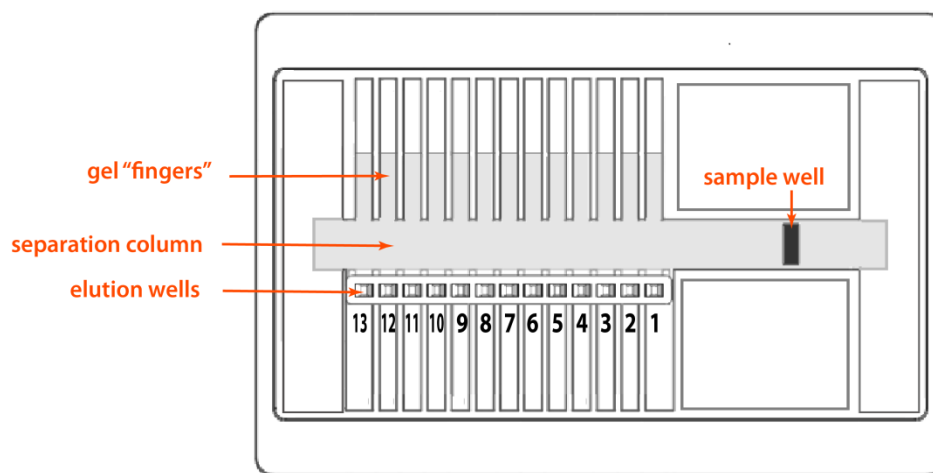


Important! Note that there are 13 wells on the cassettes, although the software calibrates for 12. The last well is necessary to ensure the integrity of the electrophoretic field lines for well 12.

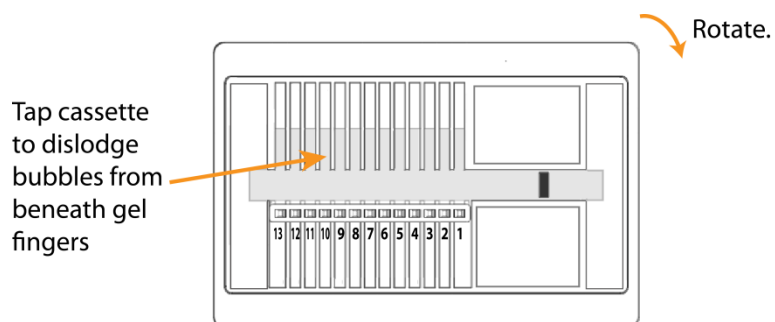
8.2 On the Bench Top



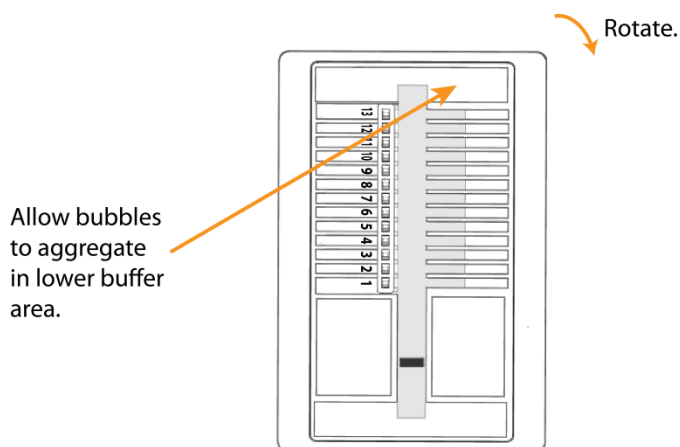
Important! Gel cassettes are designed with continuous buffer (there are no contained buffer chambers). With the exception of the area directly behind the elution ports, electrophoretic current contacts underneath the agarose. Gel cassettes are shipped with some air pockets, and the cassette preparation requires dislodging bubbles and shunting the air toward the sample well, and then displacing the air with extra buffer.



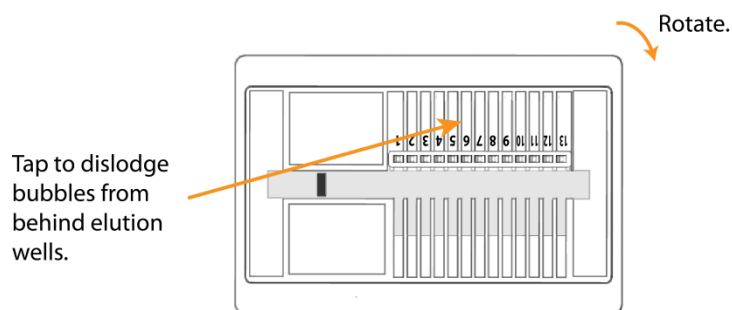
1. Remove the gel cassette from the foil bag.
2. **While still taped closed!** – Hold the cassette sideways with the gel fingers side up and elution port side down. Tap to remove air bubbles from beneath the gel fingers. The bubbles will aggregate in the right-side buffer area.



3. Rotate the cassette in the clock-wise direction. Allow the bubbles to collect in the lower buffer area. Gently tap if necessary.

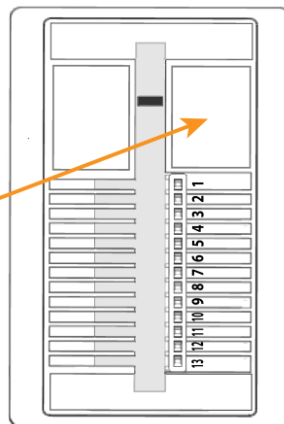


4. Continue to rotate cassette clockwise, moving as many bubbles as possible to the side buffer area. Tap the cassette firmly to dislodge any bubbles behind elution wells.



5. Continue to rotate the cassette until most of the bubbles have accumulated in the upper buffer area.

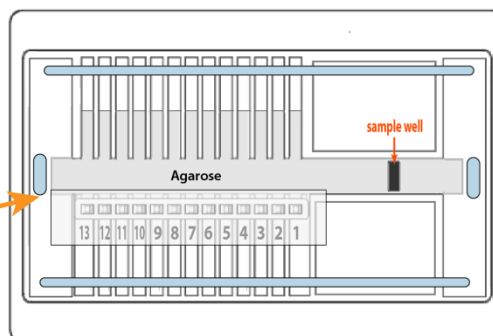
Continue to move the bubbles until most of the air is accumulated in the upper buffer area.



Important! The SageELF cassette nest is part of a moving mechanism. It is very important to refrain from removing the sealing tape from a gel cassette while seated in the nest. The applied pressure that is required could adversely affect this mechanism, and affect the quality of the run or possibly damage the instrument.

6. Place cassette on flat bench surface, hold firmly on the bench, and peel off the adhesive tape. Grab the tape tab, and pull the tape firmly and with a steady motion.
7. Remove all buffer from all 13 elution wells (set pipette to 40 μ l to completely empty the wells). Keep pipette tips vertical in the well to avoid damage to the membranes.
8. Taking care not to introduce additional bubbles into the elution modules:
 - For DNA cassettes: add 30 μ l of buffer to all 13 wells
 - For Protein (SDS) cassettes: add 28 μ l of buffer to all 13 wells
9. From left-to-right, seal the elution modules with the adhesive tape provided - take care not to obstruct lower electrode port with the tape. Firmly rub the tape with a smooth round plastic object (like a lab marker pen) to firmly seal the elution elution wells.

Make sure adhesive strip does not obstruct electrode port.



 = electrode ports

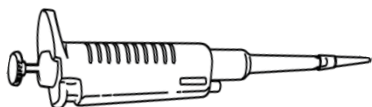
10. Carefully move cassette to instrument nest, while keeping cassette level.

8.3 On the Instrument Nest

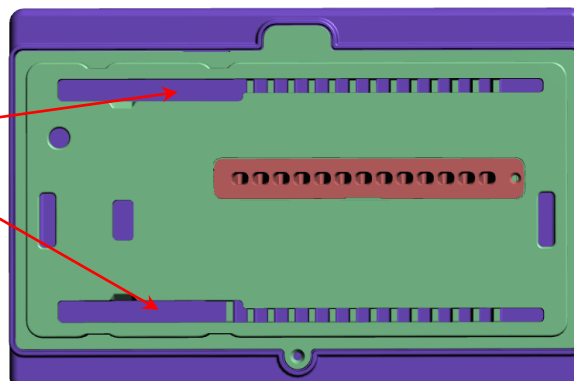


Important! All the electrode reservoirs are connected by a continuous “moat”, rather than discreet buffer chambers. The reproducibility of any procedure with the ELF system is critically dependent on filling the cassette properly. If the cassette is not filled properly before setting electrode heights instrument performance will be severely affected.

1. Top up the liquid level in the upper buffer area. Setting the correct buffer level is a critical step for achieving best results from the SageELF. The following illustrations describe this procedure:



Add buffer to either of these openings. The buffer level will equilibrate across the cassette.



An illustration of a cassette showing the top ports.

There will be airspace in the upper buffer area resulting from previous steps.



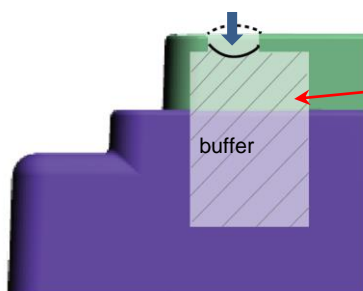
Cut away view of the front of the cassette, upper buffer area.

Add buffer to the edge of the cassette top, forming a convex meniscus, as shown.

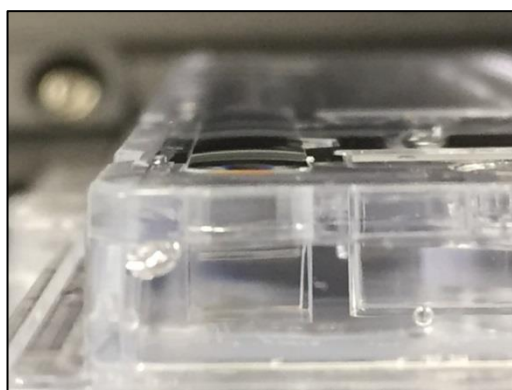


2. Set the proper volume by removing buffer from the upper buffer area.

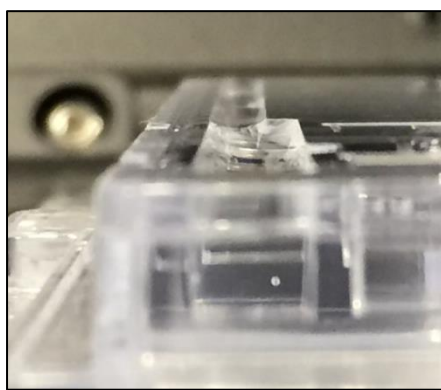
- For DNA cassettes: remove 1.0 ml
- For protein (SDS) cassettes: remove 1.2 ml



remove 1.0 ml for DNA
remove 1.2 ml for SDS-protein



End view of a completely filled moat



End view after removal of 1ml buffer

3. Continue to Current Test.

9 Electrophoresis Current Test Procedure

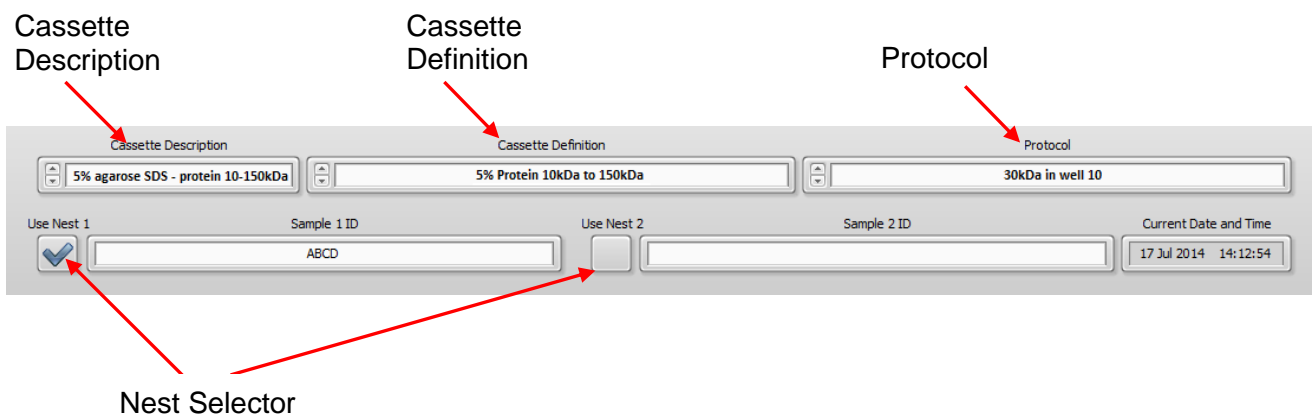
Prior to loading a sample and running a cassette, the current test should be run to ensure that the electrophoretic properties of the cassette, based on measuring the current, are within expected limits.

1. When cassette(s) is on the instrument nest(s)
2. Select the “Main Screen” Tab.
3. Clear the previous run data by pressing the “Clear Run Data” button:

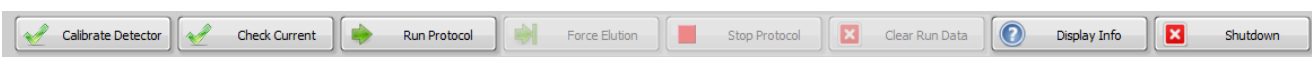


Clear Run Data

4. Select the following cassette and protocol fields from drop down menus and select the nests on which the current test is to be performed. The current test cannot proceed until these fields are populated.
 - a. **Cassette Description:** The description of the physical cassette type that was purchased. Provided in the drop-down menu.
 - b. **Cassette Definition:** The calibration and electrophoresis routine developed Sage Science and provided in the drop down menu.
 - c. **Protocol:** The fractionation range created by users (in the Protocol Editor screen). When saved, the protocol appears in the drop-down menu.
 - d. **Nest Selector** – Determines which nest or nests the cassette(s) will be run.

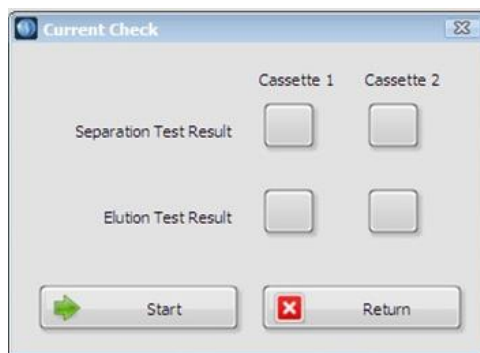


5. Close the SageELF lid.
6. Once the lid is closed, the “Check Current” button will activate on the command button menu.

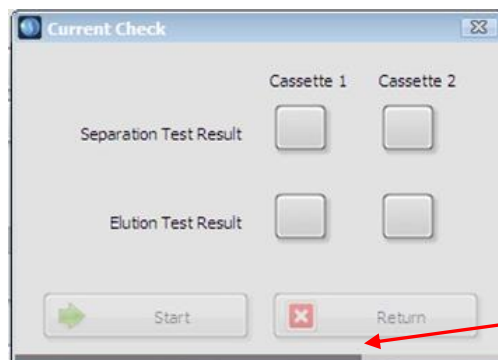


Check current

7. A pop-up window will appear:

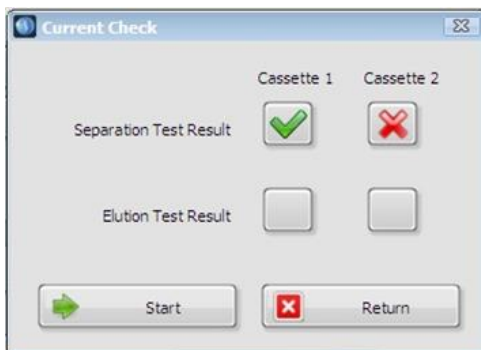


8. Press “Start”. The lid lock mechanism will engage, and the nest will motor will activate. A progress bar will appear at the bottom of the pop up window:



progress bar

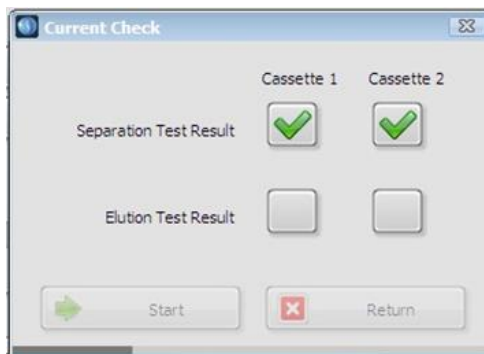
9. The current test routine will check the separation current first. If the test fails, the nest will automatically lower, and the lid will disengage until the issue is rectified.



Important! There are two likely reasons for a failed separation current test;

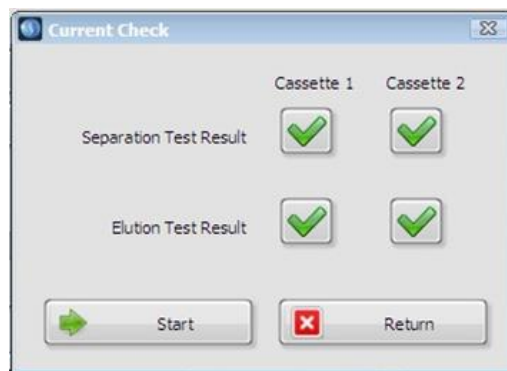
- 1) The buffer level in a cassette is low and needs to be filled
- 2) The "Cassette Type" field is populated, but no cassette has been placed in that nest.

10. Once the routine for the separation current test is complete and successful, an elution current test will commence. Once again, a progress bar will appear.



Important! The most likely reason for a failed elution current test, is if the elution module buffer was not replaced or mis-pipetted, and several elution wells are not filled.

11. When the current test is complete, a green check mark will appear in the test result box for each cassette. If a cassette is not loaded on the nest (or if the “Cassette Type” field is blank), the boxes will be empty:



12. Press “Return” and proceed to the Loading Samples.

10 Loading Samples

10.1 Loading Guidelines

Proper sample loading is critical for best performance of SageELF cassettes. For maximum reproducibility and accuracy, the sample should travel through the central section of the gel column, and should be bounded on all four sides by uniformly conductive media – either gel or electrophoresis buffer. The goal of the loading procedure is to produce this geometry in the sample loading well, as illustrated in the bottom section of **Figure 10.1**. Properly prepared samples will be 40 μ l in total volume. Loading solution used during sample prep contains concentrated Ficoll as a densifying agent (see following chapter on Sample Preparation for details), and therefore the samples will sink and form a high density layer beneath the electrophoresis buffer when pipetted slowly into the sample wells. If there is insufficient conductive buffer over the sample, the electrophoretic forces lines will curve upward as the sample exits the well (see **Figure 10.1**, upper section), and the sample will be drawn to the top of the cassette where it can travel out of the gel into the gap between the gel column and the plastic top of the channel. Sample moving in this gap will travel at a different rate than the sample inside the gel column, and will lead to elution of undesired size fractions in the eluted material. In such cases, the contaminating sample will usually (but not always) be higher in molecular weight than the anticipated fractions.

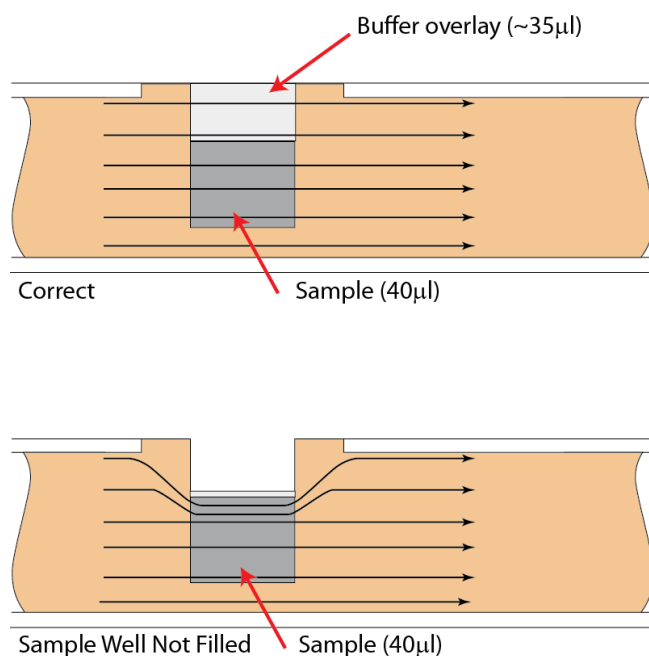


Figure 10.1. An illustration of the electrophoretic effect on a sample when a sample well is not completely filled.

10.2 Sample Loading Procedure

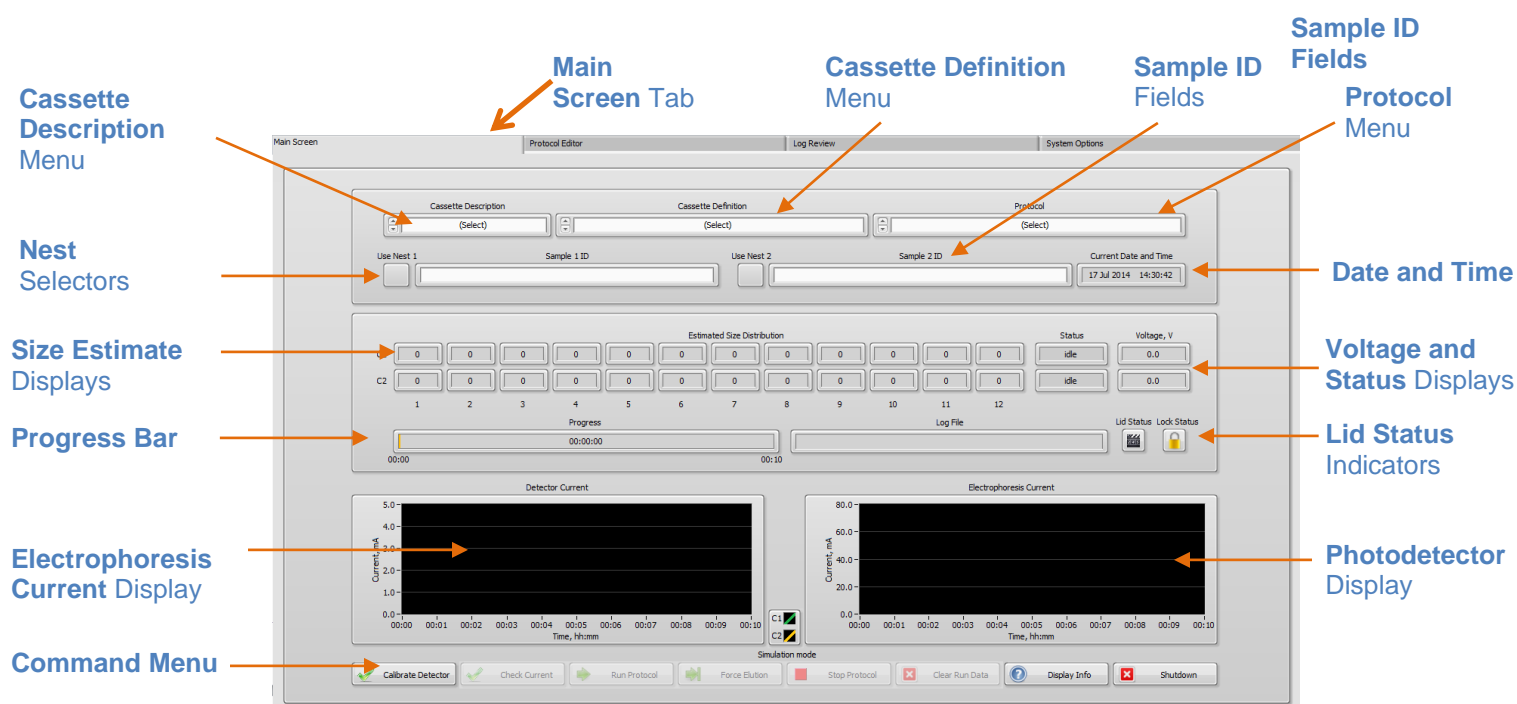
1. **Re-check the buffer level in the sample well(s).** Make sure that sample well(s) are completely full to the top with electrophoresis buffer. Top off with additional buffer, if necessary. The total volume of the sample well is 75 μ l.
2. **Remove 40 μ l of buffer from the sample well, and load 40 μ l of sample into that well.** Take care not pierce the agarose with the pipette tip. There is gel on all sides and bottom of the sample well. In addition, there is an agarose “chimney” surrounding the top of the sample well that protrudes up through the cassette cover (see **Figure 10.1** on the previous page). When removing buffer, some users find it useful to immerse the pipette tip just below the surface of the buffer and follow the liquid level down with the tip as the buffer is removed. When buffer removal is completed, there will be ~35 μ l of buffer left in the well. When adding sample, place tip of pipette just below the surface of the buffer, and follow the liquid level up with the tip as the well fills. Don’t be concerned if the sample well slightly overfills. The density of the sample will allow it to sink before it can flow out of the well.

11 Running the SageELF

The default software screen on the SageELF has a tabbed format. The Main Screen is the first tab.

11.1 Overview

The main screen is the interface with which users initiate and monitor a fractionation run. One or two cassettes may be run simultaneously and the same cassette type and protocol must be used. This interface is used to setup a run (see Section 8) and to initiate and monitor a run (below).



11.2 Initiating a Run

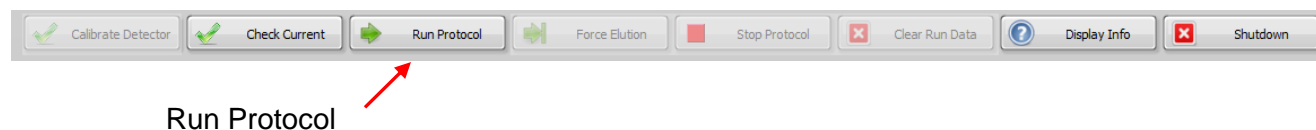
1. Confirm that the following fields have been populated with the correct information:

- Cassette Description:** The description of the physical cassette type that was purchased. Provided in the drop-down menu.
- Cassette Definition:** The calibration and electrophoresis routine developed Sage Science and provided in the drop down menu.
- Protocol:** The fractionation range created by users (in the Protocol Editor screen). When saved, the protocol appears in the drop-down menu.
- Nest Selector** – Determines which nest or nests the cassette(s) will be run.
- Sample ID (optional):** Sample information that may be manually entered by users that will be saved to the log file.

The screenshot displays the SageELF Protein Operations configuration window. It includes the following fields and controls:

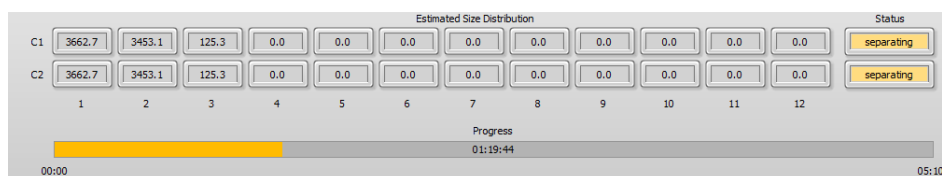
- Cassette Description:** A dropdown menu showing "5% agarose SDS - protein 10-150kDa".
- Cassette Definition:** A dropdown menu showing "5% Protein 10kDa to 150kDa".
- Protocol:** A dropdown menu showing "30kDa in well 10".
- Nest Selectors:** Two checkboxes labeled "Use Nest 1" (checked) and "Use Nest 2" (unchecked).
- Sample ID (optional):** Two text input fields. The first is labeled "Sample 1 ID" and contains the text "ABCD". The second is labeled "Sample 2 ID" and is empty.
- Current Date and Time:** A display showing "17 Jul 2014 14:12:54".

2. Close the Lid and press the “Run Protocol” button on the Command Menu:



11.3 Monitoring a Run

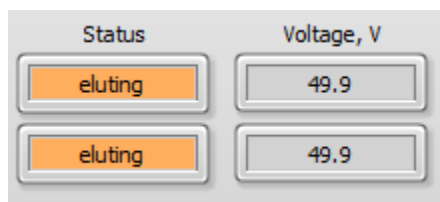
- After the run begins, a yellow progress bar will track the elapsed time, and size estimate indicators will calculate the expected size fractions in each well(s). The Status window will turn yellow and display “separating”:



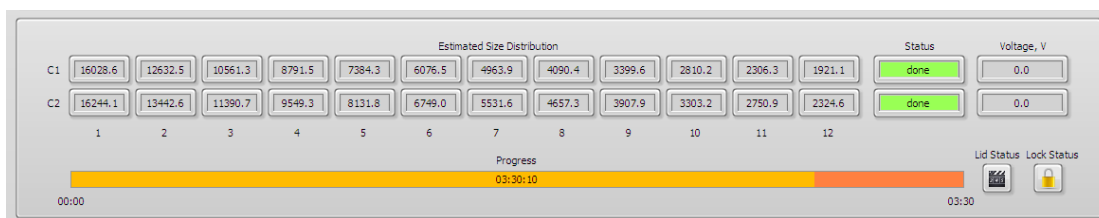
- The value in the middle of the progress bar indicates the elapsed time of the run. The value at the end of the progress bar indicates either:
 - In size-based mode, the maximum allowable run time (before buffer depletion)
 - In time-based mode, the input separation time plus the elution time (total run time).



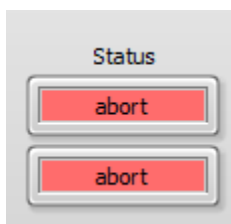
- When elution commences, the progress bar color becomes orange and the Status window will turn orange and display “eluting”. The elution voltage will be displayed in the Voltage window:



- At the end of the run, the Status window will turn green and display “done”.



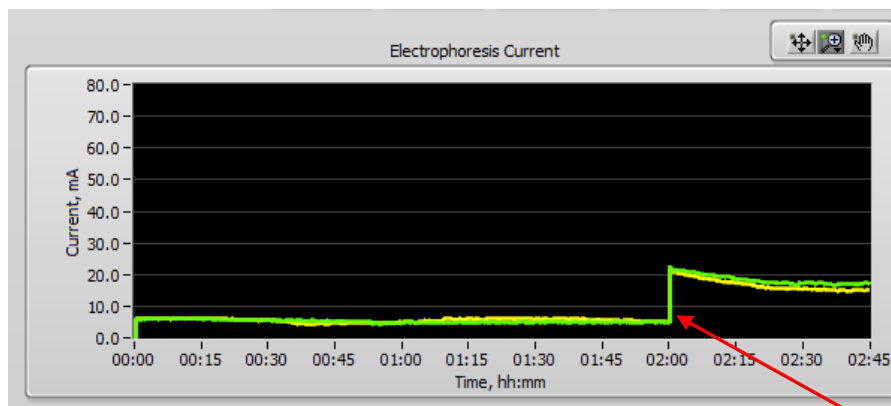
5. If a run has been aborted by pressing the “Stop” button on the Command Menu, then the Status window will turn red and display “abort”:



6. If all fields have been cleared by pressing the “Clear Run Data” button on the Command Menu, then the status window will turn gray, and display “idle”.

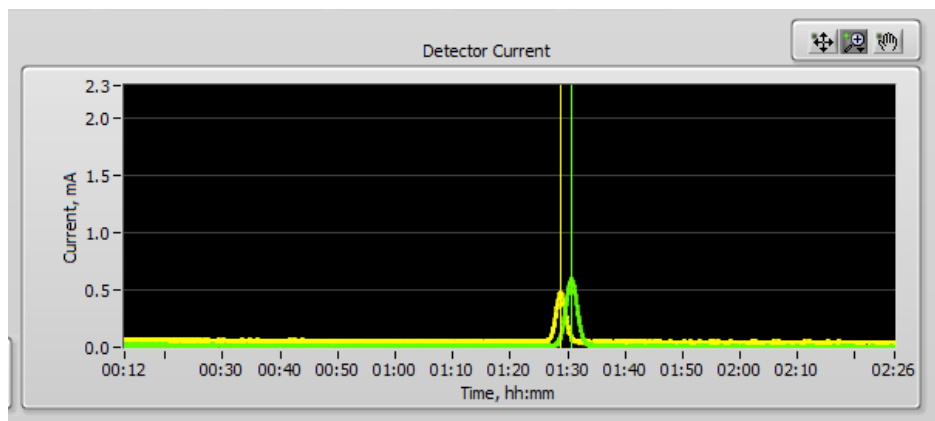


7. During a run, the electrophoresis current is displayed on the lower left hand side of the Main Screen Tab:



Current changes during elution

8. During a run, the photodetector signal is also displayed. If a fluorescent marker is used, the peaks will be detected and displayed:



9. The Main Screen Tab also displays the Lid status (open or closed) and the Lock Status (unlocked or locked). The Locked status also indicates that the nest is engaged with the lid. When running, the Lid is closed and locked.:



12 Removing Sample Fractions

1. After a run has been completed, remove the cassette from the nest(s).
2. Place the cassette on the bench top to remove the adhesive tape that is covering the elution wells.
3. Press the cassette firmly against the bench top and grab the white tab.
4. Pull the tape back, in a quick and even motion.
5. Remove the samples using a pipette or multi-pipetter

12.1 Using the well guide

Cassettes are shipped with disposable well guide that can be used to visually identify the well numbers.

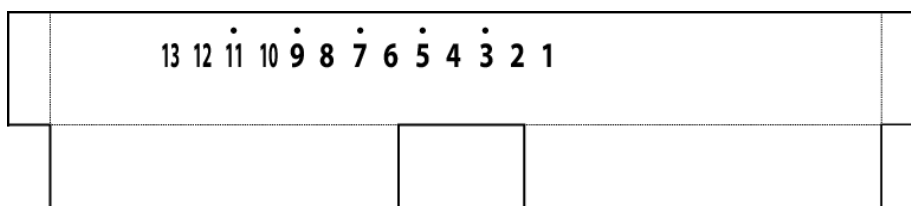


Figure 12.1 An illustration of the SageELF well guide

1. Bend the flaps on the well guide along the perforations.
2. Loosely align the notched portion of the guide over the feature at bottom (well side) of the cassette. The folded flaps should align around the perimeter of the cassette top:

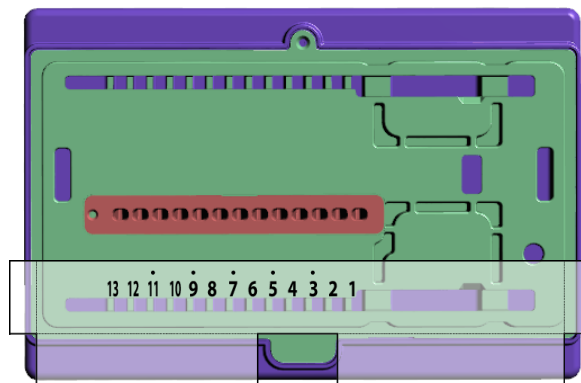


Figure 12.2 The Well Guide fitted over a cassette

13 Rinsing Electrodes

Rinsing the SageELF Electrodes is an important maintenance function. It is recommended that they be rinsed after every run, or at the end of a day of use.

1. Place the blank SageELF cassette bodies (provided by Sage Science) onto the Nests.
2. Completely fill the cassettes with deionized water.
3. Close the instrument Lid.
4. In the Main Tab, press the “Clear Run Data” button on the Command Menu Bar.
5. In the cassette and protocol fields, enter the pre-programmed “Rinse Cassette” and “Rinse Protocol” options from the drop-down menus:

The screenshot displays the SageELF software interface for setting up a rinse protocol. It features three main input fields at the top: 'Cassette Description' with a dropdown menu showing 'R - Rinse Cassette', 'Cassette Definition' with a dropdown menu showing 'Rinse Cassette', and 'Protocol' with a dropdown menu showing 'Rinse Protocol'. Below these fields are two sections for sample identification: 'Use Nest 1' with a checked checkbox and an empty 'Sample 1 ID' text box, and 'Use Nest 2' with a checked checkbox and an empty 'Sample 2 ID' text box. On the far right, a 'Current Date and Time' field displays '17 Jul 2014 14:54:37'.

6. Press “Run” on the Command Menu. The protocol will take about 6 minutes to run.
7. Open the lid. Remove the rinse cassettes, and pour out the wash into a drain.

14 Cassette Definitions

14.1 About Cassette Definitions

Cassette definitions describe the electrophoretic performance of a sample (DNA or protein) with a particular cassette type and a particular set of instrument settings (separation voltage, separation waveform, elution voltage, internal standard type, photodetector settings, etc.). Cassette definitions contain empirically determined calibration constants that allow users to program specific product size distributions. Calibration of ELF cassette definitions involves analyzing fraction sizes as a function of time. For good reproducibility, sample electrophoretic mobility and separation time are normalized using a fluorescent DNA internal standard, which is designed to migrate slightly faster than sample fractions of interest.

Cassette definitions can be viewed and edited using the “Cassette Editor” screen in the SageELF system Advanced Tools. Contact Sage Science if you would like to use the Advanced Tools feature to create custom cassette definitions.

14.2 Reference Guides

When using an internal standard, the actual average size will be within 15% of the estimated fraction size. The marker run times are used to normalize these values. The actual run time will vary based on ambient temperature, small variation in the cassette manufacture, and input sample characteristics.

The following pages provide estimated product size distributions for cassette definitions that are currently available.

Note: Native agarose cassettes are also available for time-based fractionation only. To order:

Native Agarose Type	Cassette Kit Part No.
2%	ELD2010
0.75%	ELD7510

Reference Guide 1: 5% SDS Agarose 10-150kDa

Cassette Kit part No. **ELP5010**
 Cassette Description: **5% agarose SDS – protein 10 – 150kDa**
 Cassette Definition: **5% Protein 10kDa to 150kDa**
 Software accuracy vs. actual average: **± 15% (with internal marker)**
 Approximate internal marker run time (Tmrk): **1.21 hr**

Trun/Tmark	Est. Trun (hrs)	Elution Wells 1 (top) to 12 (bottom), Estimated Median Size (kDa)											
		1	2	3	4	5	6	7	8	9	10	11	12
1.1	1.5	17.6	14.2	11.7	9.9	8.6	7.4	6.3	5.2	4.1	3.0	2.0	1.3
1.5	2	28.3	22.6	18.4	15.4	13.2	11.6	10.3	9.3	8.3	7.4	6.5	5.8
1.9	2.5	31.0	29.3	26.8	24.0	21.2	18.6	16.4	14.6	13.3	12.3	11.4	10.4
2.2	3	39.1	34.8	31.1	27.9	25.1	22.7	20.5	18.5	16.7	15.1	13.9	12.9
2.6	3.5	84.5	60.6	45.3	36.1	30.9	28.0	26.2	24.5	22.6	20.3	18.1	16.8

Reference Guide 2: 3% SDS Agarose 10-300kDa

Cassette Kit part No. **ELP3010**
 Cassette Description: **3% agarose SDS – protein 10-300kDa**
 Cassette Definition: **3% Protein 10kDa to 3000kDa**
 Software accuracy vs. actual average: **± 15% (with internal marker)**
 Approximate internal marker run time (Tmrk): **2.14 hr**

Trun/Tmark	Est. Run Time (hrs)	Elution Wells 1 (top) to 12 (bottom), Estimated Median Size (kDa)											
		1	2	3	4	5	6	7	8	9	10	11	12
0.9	1.1	30.0	23.0	14.1	9.0	2.3	2.5	0.9	0.0	0.0	0.0	0.0	0.0
1.1	1.3	39.0	30.0	20.1	14.6	9.0	7.1	5.1	3.4	2.4	1.5	1.4	1.0
1.1	1.4	45.0	40.0	26.1	20.3	15.7	11.8	9.3	7.0	5.7	4.4	3.7	2.9
1.2	1.5	51.0	42.0	32.0	26.0	22.3	16.5	13.4	10.5	9.0	7.4	5.9	4.7
1.3	1.7	55.0	48.0	38.0	31.7	29.0	21.2	17.6	14.0	12.3	10.3	8.2	6.6
1.5	1.8	60.0	55.0	43.9	37.3	35.7	25.9	21.8	17.6	15.7	13.3	10.4	8.5
1.6	1.9	68.0	64.0	49.9	43.0	42.3	30.6	25.9	21.1	19.0	16.2	12.7	10.4