



Addendum to Gryphon Setup Guide: LCP Setup

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1. Introduction to the Gryphon LCP

The Gryphon LCP is a small, fast and affordable option for setting up Lipidic Cubic Phase (LCP) sandwich plates and bicelle assays, as well as traditional protein crystallography plates. The dedicated syringe based LCP dispenser is used for the protein and a 96 head is used for the screening solutions. A 96 well sandwich plate can be sealed in less than 2 minutes. LCP drops are set up in 45 seconds. An optional non-contact Nano dispenser can be added at anytime to set up vapor diffusion sitting drop and hanging drop plates. The non contact dispenser can also be used for additive experiments.



Gryphon Specifications

Dispense Volumes

96 Head: 100 ul syringes deliver 100 nL to 100 μ L

LCP Syringe: 100 μ L syringe delivers 10 nl + with short LCP needle, optional long tip is available for serial dilution work.

Size

Base: 20 in (51 cm) H x 23 in (59 cm) W x 23 in (59 cm) D
68 lbs (31 Kg)

Wash: 13 in (33 cm) H x 6.5 in (17 cm) W x 14 in (36 cm) D
5.5 lbs, 2.5 (kg)

Nano: 9.5 in (24cm) H x 6 in (15 cm) W x 16 in (41cm) D
18.5 lbs, 8.4 Kg

Electrical

Base: 120/240 Vac, 3 A, 50/60 Hz

LCP Arm: Integrated Power

Software

Windows .NET

Features of the Gryphon LCP system

- Two assay positions—source and destination plate positions; 8 position serial dilutor extension; LCP Mixing Station; LCP Pre-dispensing Station; 1 96 way Head wash Station.
- Multiple-syringe drive Head selection—accommodates 24, 96, or 384 interchangeable syringe head configurations
- Two 96 channel needle types available—Teflon coated stainless steel needles or flexible Nitinol needles
- Multiple dispense options: dispenses into 96-, 384-, or 1536-well plates into commercial protein crystallography plates
- Microsoft Windows-based software
- Closed-loop motion control on each axis—task completion verified via encoders on each motor

2. Safety

Study these safety instructions **before** you use the instrument.

1. Heed all **Caution** statements in this manual and adhere to the **Danger** and **Warning** labels on the instrument:
2. Know how to shut down the instrument in an emergency.
3. Practice good laboratory techniques to minimize hazards.
4. Contact your ARI representative for instructions before you attempt any maintenance or service procedures.

Stopping the Instrument in an Emergency

To stop the system in an emergency, click  on the main screen.

To restart the system after an emergency shutdown:

1. Inspect the system components. If any are physically damaged, recovery is not possible.
2. Verify that it is safe for the run to continue:
 - Clean up any spills.
 - Replace any containers have been misplaced or dropped.
 - Ensure that the stage area is free of obstructions.
3. If the system components are not damaged and it is safe to continue, power off the Gryphon; then turn it back on.

4. Disconnect and reconnect the software.
5. Verify LCP Syringe is disengaged.
6. Restart the run in the Gryphon software.

Following Good Laboratory Practices

Turn off the system and unplug the power cord before you perform any service or maintenance tasks.

Handle chemicals carefully, following manufacturers' safety instructions for their use, storage, and disposal.

Set your aspirate and dispense heights so that the needle tips never touch the bottom of the receptacle. If this occurs, backpressure building in the syringes could cause the glass syringe barrels to crack or break.

Exception: You can safely dispense volumes of less than 1.0µL onto a dry plate with the needle tips touching the plate.

Moving the Gryphon Instrument

The Gryphon instrument weighs 92 lbs (41.9 Kg). If you must move the instrument, prevent injury to yourself or damage to the instrument by taking the following precautions:

- Lift the Gryphon only from the front and sides. Do not lift the Gryphon from the rear.
- Be sure that you are lifting the unit by the base and not just by the enclosure.

3. Getting Started

The Gryphon system—as delivered—has the basic parameters installed, but you will need to understand the following concepts.

Configuration Settings

1. Configuring the Motor Engine. These fields have been correctly calibrated prior to installation.
2. Configuring Global Settings. Although these fields are also correctly calibrated at installation; you may need to edit this screen to suit your specific configuration.
3. Calibrating the Stage. The system will be correctly calibrated when installed. Thereafter, recalibrate only if you move the system, or if you need to remove the head or have damaged the LCP syringe tip.

4. Defining Labware. The system is preconfigured with a large number of commercially available labware options. You can edit existing categories or create new ones whenever you want to use a new type of labware in your protocols

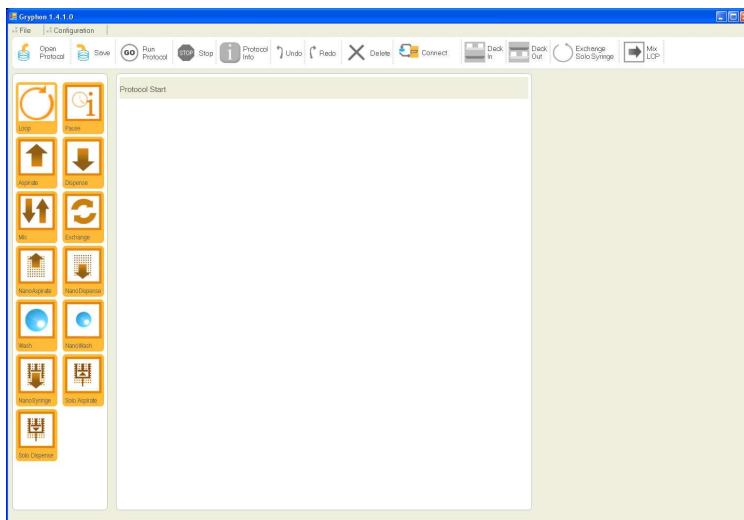
Writing Protocols

The Gryphon system is protocol-based. You can save the protocols you create and use them over and over again. You can also edit existing protocols, as well as create new ones.

This guide describes how to complete these tasks and how to run the protocols.

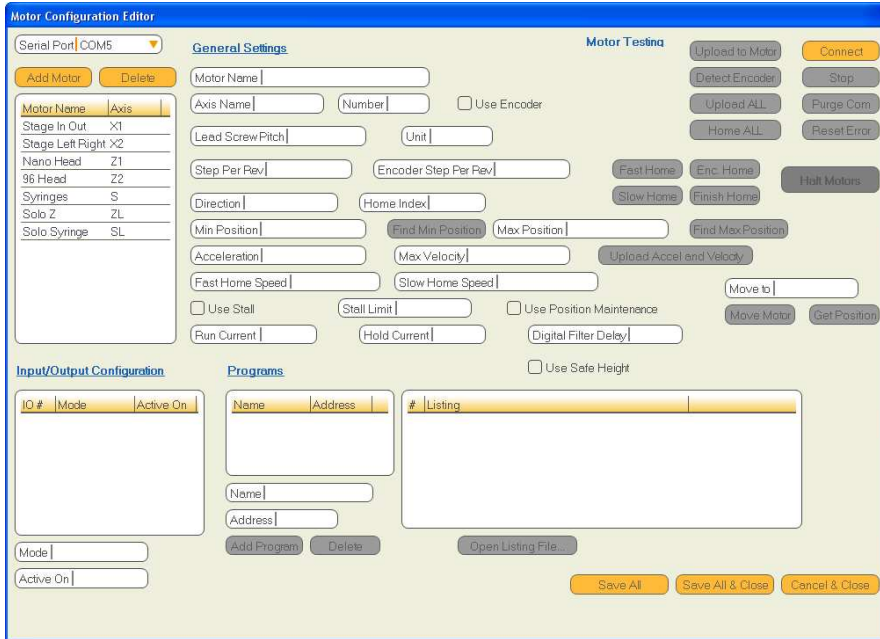
Launching the Program

Turn on the Gryphon LCP system and computer and click the Gryphon icon to launch the Gryphon program. Begin by accessing the Configuration menu at the top left of the screen.



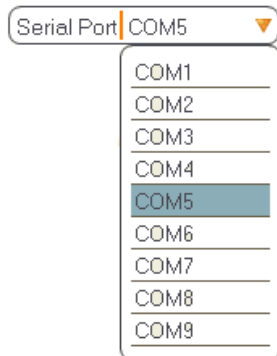
4. Configuring the Motor

The Gryphon motor engine is appropriately configured at installation. To reach this screen, click **Configuration → Configure Motor Engine**.



The Gryphon deck motors are usually configured on COM 5 at ARI. This teach field is used to define the minimum and maximum positions.

Note that the Serial Port in the upper left of the screen is set to 1 to 9, depending on the computer that shipped with the system. Of the 9 possible settings, 6 through 9 are reserved for a Nano Dispenser. The correct Com Port assignments can be found in the Windows Device Manager.



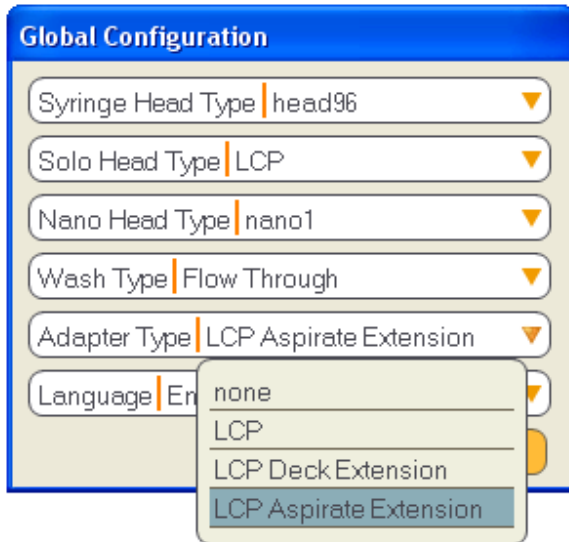
The Gryphon main deck motor engines are usually on COM 5. Please contact ARI prior to making any changes to the preconfigured Serial Port settings.

5. Configuring Global Settings

The Global Settings are also preconfigured, but there are a number of edits you may want to make, depending on your system. LCP specific settings are outlined here.

Global Settings comprises three areas. Click **Configuration** → **Configure Global Setting**.

Adapter Type describes the deck layout of the LCP option.



None means no LCP adapter is selected.

LCP means an adapter plate, no longer in the current system

LCP Deck Extension is the metal, bolt-on adapter for the glass slide pre-dispense location

LCP Aspirate Extension includes the Deck Extension locations plus the White Vial holder

6. Calibrating the LCP Syringe to the Stage

Your ARI representative will calibrate the stage when the system is installed.



Study the [safety](#) information for this instrument before you operate it and always follow the **Cautions** in this guide as well as the **Dangers** and **Warnings** on the instrument's labels.

We recommend that teach points are checked if the system is moved or if new equipment is installed.

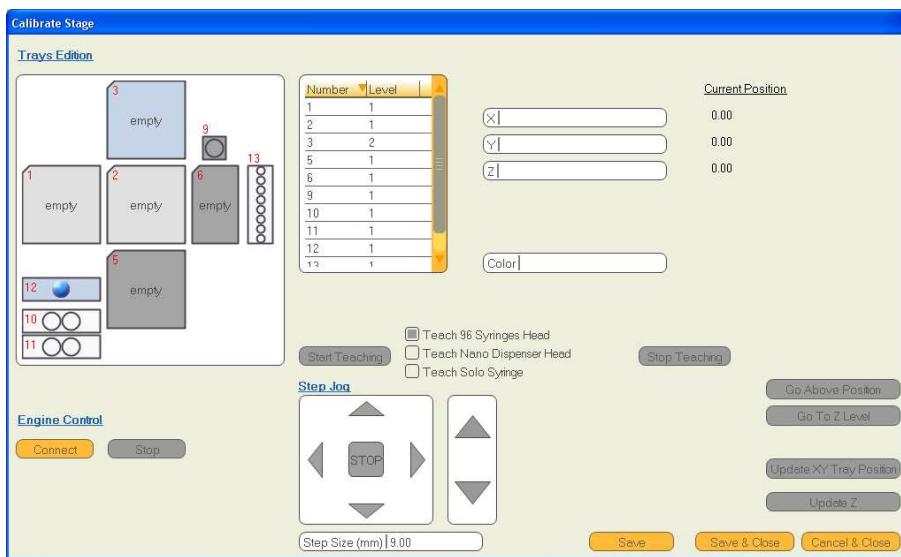
Training the deck involves the following sections:

The syringe with short needle to deck positions 2 (Gryphon right deck), 5 (LCP adapter stage placed on right deck), 6 (LCP predispense slide).

Position 9 for coupled mixing syringes for mixing processes.

Position 13 accessed with ARI long needle on 8-way diluter stage.

1. Select **Configuration** → **Calibrate Stage** to reach the Calibrate Stage screen.



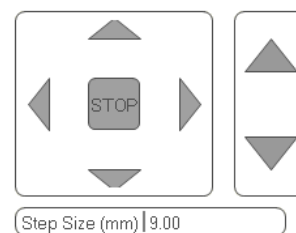
2. Under Engine Control at the bottom left of the screen, click **Connect**.

CAUTION	<p>Make sure the LCP syringe is disengaged before homing.</p> <p>If you need to stop the calibration movements at any time, click Stop under <u>Engine Control</u>.</p>
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Beginning Calibration

The arrows at the bottom of the screen move the head and tray shelf in a step-wise mode.

- Use the arrows in the larger control area to move the stage left and right and forward and back.
- Use the arrows in the control area on the right to move the Z height up and down.



Use large steps to begin adjusting the XY and Z positions, and then smaller step sizes selected in the pull-down menu as you approach the nesting position.

To move to an existing XY position quickly—for example, if you want to check the setting or modify it—click **Go Above Position** or **Go to Z Level**.

⚠ CAUTION	Because going to Level Z is the mostly like time that a crash can occur, we recommend using the step function to reach this level.
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Each mouse-click moves the head one step. You can set the size of the step in the Step Size (mm) control box below the Step Jog arrows.

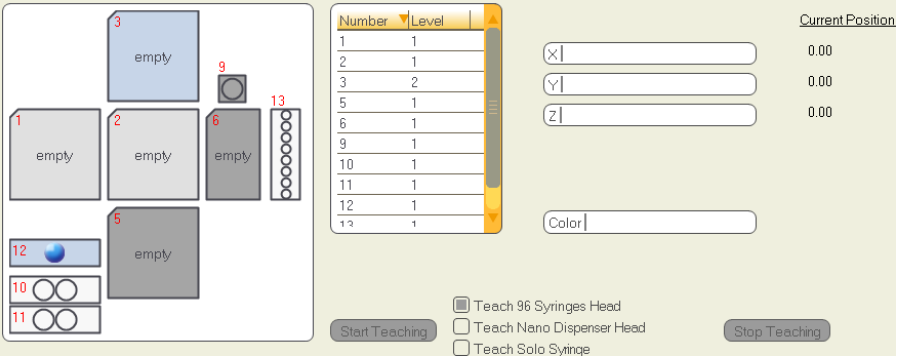
Use a larger step size when the syringe is far above or far to the left or right of the calibration holes; use a smaller step size when calibration target and needle are closer together.

Calibrating the Plate Position

Calibrating Plate Deck Position 2

Gryphon deck position 2 can be used for typical plate setups on the Gryphon that could contain normal protein mixtures, bicelle mixtures or LCP mixtures to be used with typical protein crystallization plates. It is generally not recommended for LCP highly hydrophobic plates.

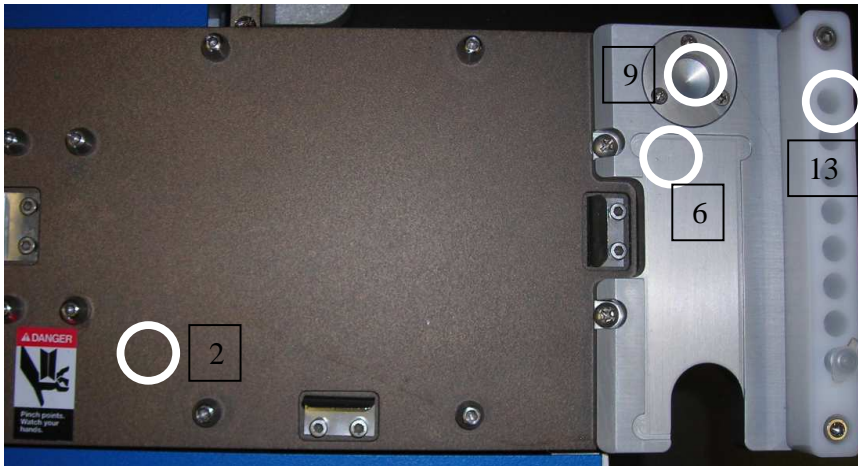
- Click **Number 2/Level 1** in the table at the top middle of the screen:

4. 

Number	Level
1	1
2	1
3	2
5	1
6	1
9	1
10	1
11	1
12	1
13	1

- Center-screen, select the Teach Solo Syringe (LCP) on the system.. Then click **Start Teaching**.

6. Now use the vertical up/down Step Jog arrow to adjust Z height and the XY cross-hair adjustments to maneuver the tip and find the small hole in the deck nest position.
7. The teach points are the origin for all the instrument's movements. For deck position 2 the teach point is located towards the front of the deck as indicated in the above picture. Deck positions 5,6,9 and 13 use teach points toward the rear of the unit.



8. Using the included .002"/.05mm feeler gauge, drive the head down in Z until the tip is as close to the shim as possible, but the shim is free to slide.
9. Change the Z motion step size to 0.05mm, move Z until the tip just pinches the feeler gauge. Do not over pinch, or let the shim be free to slide.
10. Move the Z axis up 1x 0.05 mm click, remove feeler gauge, and then move it back down into the trained position.
11. Click **Update XY Tray Position** and **Update Z**.

12. Click **Save** → **Stop Teaching**.

Repeat the calibration steps for Deck Positions 5, 6, 9, and 13 as follows.

Calibrating LCP Adapter Position 5

The LCP plate adapter is generally recommended for LCP highly hydrophobic plates.

To teach LCP adapter position 5, place the adapter on the right deck over position 2.

NOTE

To teach position 5, place the slide adapter plate onto position 2, making sure it is flat and securely positioned before teaching.

1. Click **Number 5/Level 1** in the table.
2. Repeat the same motions as configured above for position 2, with the syringe LCP tip and the feeler gauge.
3. The teach point position is now located to the rear of the LCP dispense adapter platform.



4. Maneuver the XY stage and Z height and check with feeler gauge as explained above.
5. Click **Update XY Tray Position** and **Update Z**.
6. Click **Save & Close**.

Calibrating LCP Adapter Predispense Stage position 6:

This is the pre-dispense slide useful to initiate the LCP dispense process.

1. Position 6 teach point is in the upper left corner of the adapter.
2. Click **Number 6/Level 1** in the table.
3. Follow the same calibration instructions as for deck positions 2 and 5.
4. Teach position with feeler gauge as explained above.
5. Click **Update XY Tray Position** and **Update Z**.
6. Click **Save & Stop teaching to continue or alternatively** Click **Save & Close** to close the teaching module.

Calibrating LCP Coupled Syringe Mixing Position 9

1. Before starting teaching, make sure LCP top bracket is far enough away from syringe plunger. See section called Exchange Solo Syringe.



2. Assemble an LCP syringe pair, making sure to securely tighten all fittings.

3. Place syringe assembly into position. Coupling goes onto “middle” bracket.



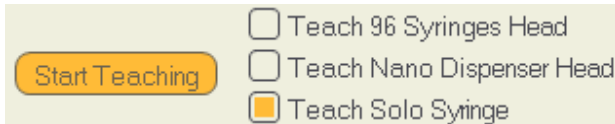
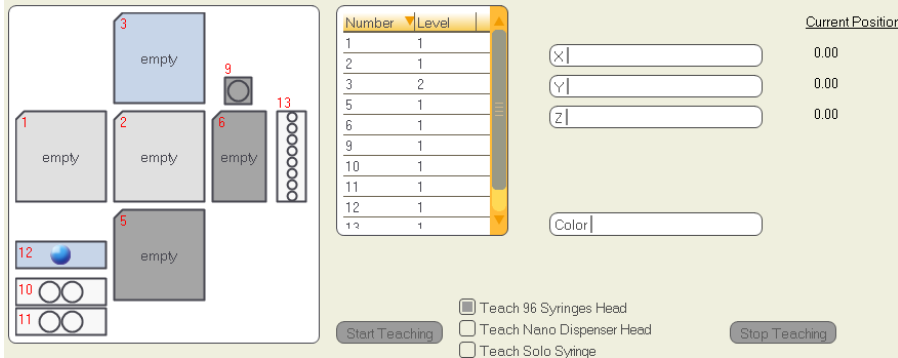
4. Push bottom syringe plunger all the way up, so that all of the mixture is located in the upper syringe body raising the upper syringe plunger by doing so.





Failure to teach mix position correctly may cause the syringe pair to break in actual use.

5. Make sure top and bottom syringes are visually aligned vertically.
6. Click **Number 9/Level 1** in the table at the top middle of the screen:



7. Center-screen, select Teach Solo Syringe on the system. Then click **Start Teaching**.
8. Now use the XY cross-hair adjustments to maneuver the syringe plunger end and find the mixing nest position in XY.
9. Vertically adjust until the syringe plunger end is just touching the spring loaded mixing position.
10. Change Z Step Jog size to 1.0mm.
11. Move down 1.0mm in Z height depressing the spring loaded stage position 1mm.
12. Click **Update XY Tray Position** and **Update Z**.
13. Click **Save → Stop Teaching**.
14. Click **Save & Close**.



You will notice the spring is depressed 1 mM on each cycle during the mixing process. If the coupling stops depressing the spring and begins to move above position 9, the syringe coupling is likely leaking.

Calibrating LCP Deck Position 13

Deck position 13 is a vial rack that contains 8 positions. This module is effective for performing aspirating and dispensing solutions from, mixing solutions, performing serial dilutions, and it is useful for crystal seeding experiments.

NOTE	Position 13 A1 is the vial location furthest away from the user. This location is intended to be used with the long nozzle version syringes.
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1. Place a 0.2 mL PCR tube that the rack is defined for in position 13 A1.
2. Click **Number 13/Level 1** in the table.
3. Train the long ARI needle tip to the center in XY and bottom of the PCR tube for Z height using the same movements specified for the other teach positions. Ensure the needle is near the tube bottom, clearly enough to slightly move the tube.
4. Click **Update XY Tray Position** and **Update Z**.
5. Click **Save & Stop teaching to continue or alternatively** Click **Save & Close** to close the teaching module.

7. Defining New Labware

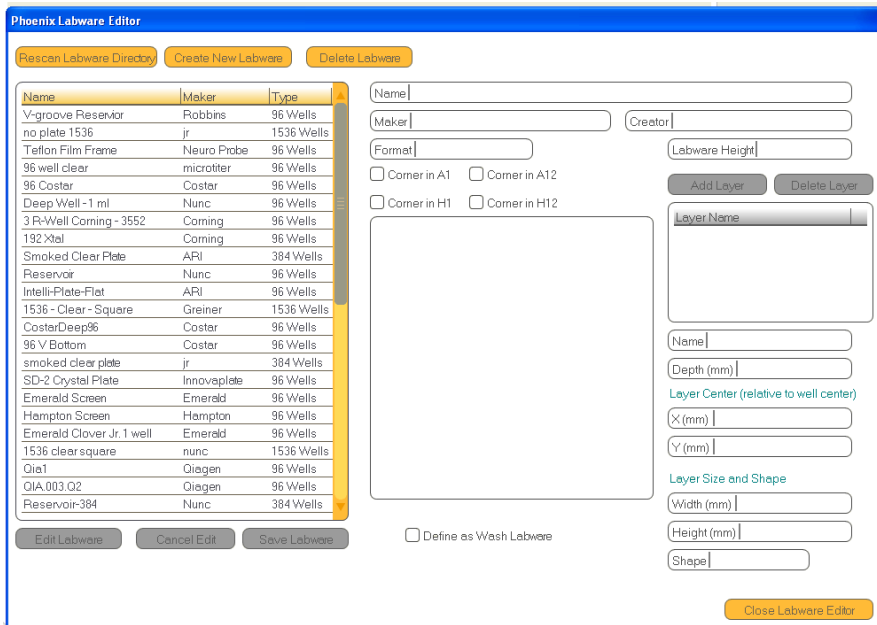
Defining labware is “teaching” the system the physical parameters of the labware pieces you will use in your protocols. Most labware manufacturers provide the required dimensions. ARI can also provide this information.

This is the same operation as in the general section as already in the manual.

You can define not only the parameters for different well types on the same plate, but also different depths and needle-tip-to-well-center orientations for the same well. Each of these single definitions is called a “layer.”

To define labware for the Gryphon:

1. Have calipers ready to measure the dimensions of your labware.
2. Launch the Gryphon program.
3. On the main menu, click **Configuration** → **Open Labware Editor**.



4. Click **Create New Labware**.
5. In the upper right, enter a Name, Maker and Creator for the labware. (Maker and Creator values are optional.)

Name | Intelliplate

Maker | Robbins Creator | David Wright

6. Select a format from the Format drop-down list.

Format | 96 Wells

- 96 Wells
- 384 Wells
- 1536 Wells

7. If you did not receive the labware's exact height measurement from the manufacturer, measure the overall height of your plate. Enter the value in Labware Height.

Labware Height | 15

8. Click the *Corner* option that applies to your plate (the corners that are notched).

Format | 96 Wells

Corner in A1 Corner in A12

Corner in H1 Corner in H12

9. Click **Add Layer**.

Add Layer

Delete Layer

10. Enter Name and Depth for that layer. (Depth = distance from the top of the plate to the bottom of well.)
11. Enter the distance from the center of the layer to the center of the well. If the layer and well center are the same, then values for X and Y will be 0.)
12. Enter Layer Width and Height; then select the shape of the layer. The plate definition creates a graphic illustrating the values you have entered.

Name | Reservoir

Depth (mm) | 12

Layer Center (relative to well center)

X (mm) | 2.25

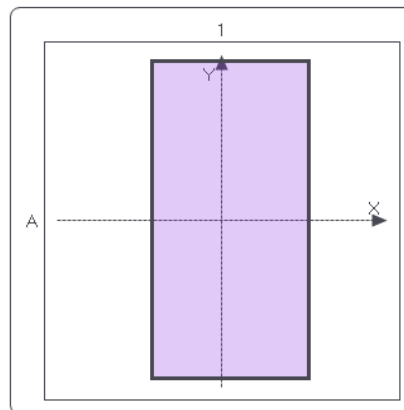
Y (mm) | 0

Layer Size and Shape

Width (mm) | 4

Height (mm) | 8

Shape | Square



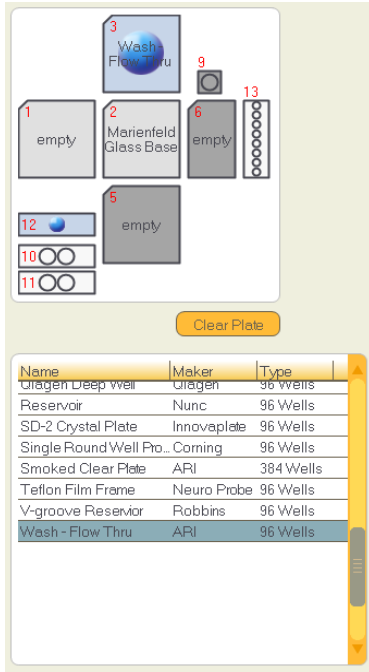
NOTE

LCP labware may need modified height settings. The software is constrained to dispense within the well depth, which for an LCP slide may only be the depth of the tape (e.g. ~0.15mm). A dispense height higher than this may be desired, especially for larger volume dispenses.

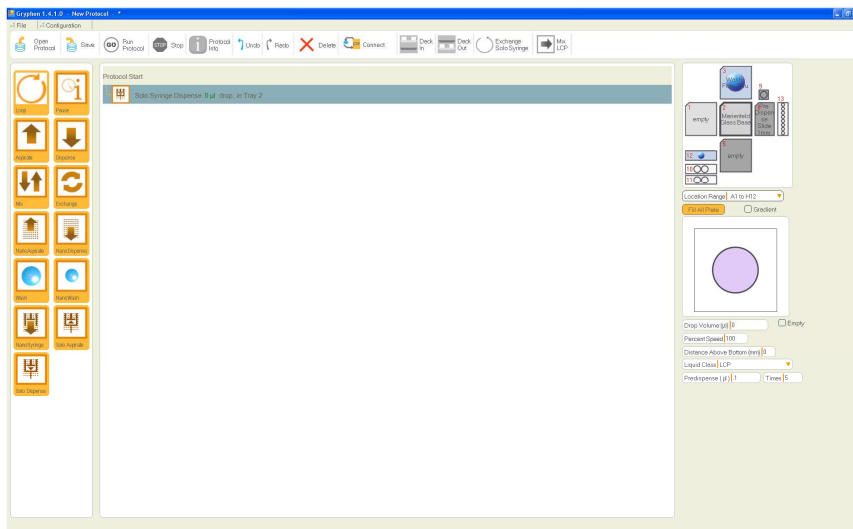
To increase the available dispense height the Labware Height and Well Depth may be modified. For example, a 1mm glass slide has a tape layer that is 0.15mm deep. The nominal Labware Height and Well Depth are therefore 1.15mm and 0.15mm respectively. Changing the Labware Height and Well Depth to 1.5mm and 0.5mm respectively will maintain the correct 1mm glass thickness and allow additional dispense height adjustability. The Labware Height and Well Depth **MUST** be adjusted together.

CAUTION

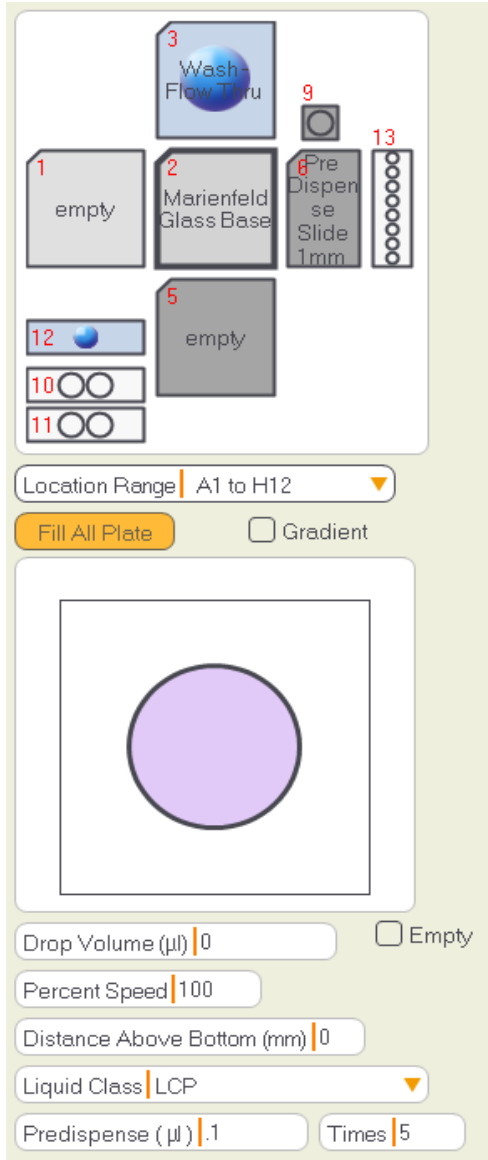
Failure to adjust both Labware Height and Well Depth together may result in a plate definition that will damage the needle.



- Click the first command you want to use in the protocol in the command area on the left side of the screen and drag it into the Protocol Start area in the middle of the screen. In this case, it is the Solo Dispense command. Refer to the [Command Descriptions](#) for details.




- For each command, click on the plate location on the deck where the command will be executed. A representation of the plate's well or wells will appear below the deck. The plate in position 2 is selected in this example.



NOTE

The software automatically validates the protocol. If there is a problem with any of the commands, a red alert symbol will pop up next to the command field. Move your cursor over the symbol to see the alert message. Generally the alerts appear if the value provided for the command is out of range.

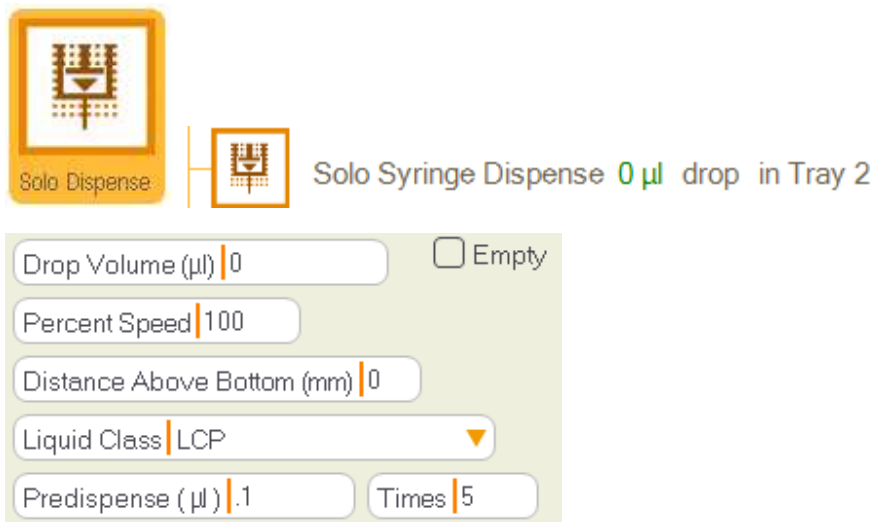
- Click **Save**  on the toolbar to save the protocol. Other options are available under the File Menu.

Command Descriptions

The Gryphon provides commands, as shown at the left on the main menu. To execute protocol functions, drag-and-drop commands below the Protocol Start bar. We describe the LCP and Nano Syringe specific commands here:

Solo Dispense

The ARI Solo Dispense uses a positive displacement syringe to dispense LCP mixtures.



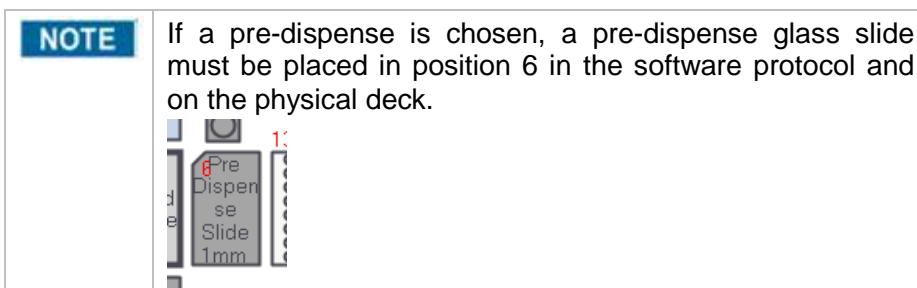
Drop Volume—Volume to be dispensed in μL ., if Empty is checked, syringe will empty its contents.

Percent Speed—Speed of the deck between dispenses.

Distance Above Bottom (mm)—Height of the tip from the well bottom during dispense.

Liquid Class—Set to LCP by default.

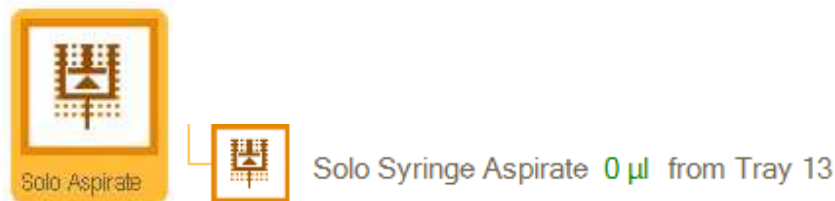
PreDispense (μL)—Specifies the volume to be pre-dispensed onto the pre-dispense glass slide in position 6, default is 0. PreDispense specifies the volume, the same value as intended to dispense, and the number of times you wish to prime the syringe tip. We recommend 4-5 x during normal operation.



Times—Specifies the number of pre dispenses.

Solo Aspirate

The ARI Solo Aspirate uses a positive displacement syringe to dispense LCP mixtures.



Volume (µl) 0	
Distance Above Bottom (mm) 0	
Liquid Class LCP ▼	
Predispense (µl) 0	Times 0
<input type="checkbox"/> Air Gap	Volume (µl) 0

NOTE

A long nozzle syringe is required to aspirate from the 8 vial positions in the sample diluter rack position 13.

Volume—Volume to be aspirated in µL.

Distance Above Bottom (mm)—Height of the tip from the well bottom during aspirate.

Liquid Class—Set to LCP by default.

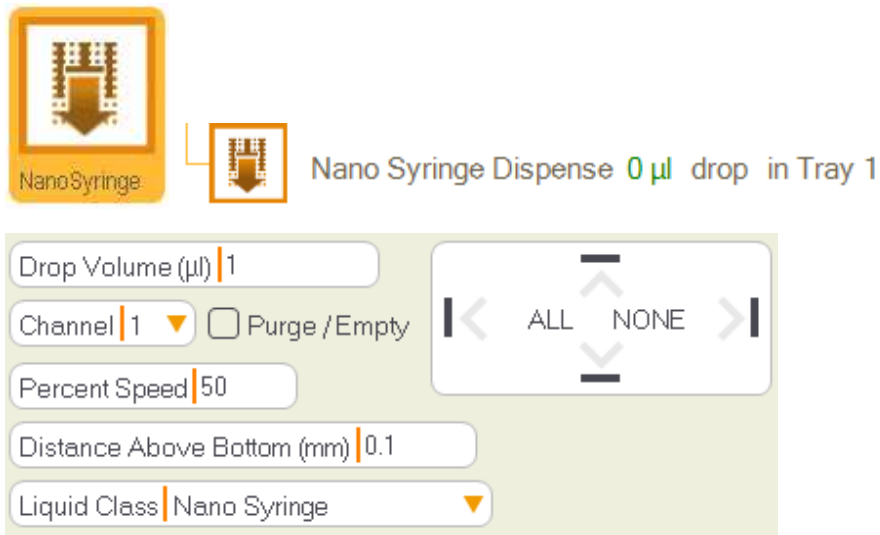
PreDispense (µL)—Specifies the volume to be pre-dispensed back into the aspirate vial.

Times—Specifies the number of pre dispenses.

Air Gap—If checked, Volume specifies the amount of air gap to be aspirated.

NanoSyringe

The ARI NanoSyringe uses the Nano (if present) as a positive displacement syringe to dispense samples in a touch off mode.



Drop Volume—Volume to be Dispensed in uL.

Channel—Selected tip of multi tip systems, default is 1. If Purge/Empty is checked, that tip will Purge syringe contents.

Percent Speed—Speed of the deck between dispenses.

Distance Above Bottom (mm)—Height of the tip from the well bottom during dispense.

Liquid Class—Set to Nano Syringe by default.

Gradient Checkbox—If gradient checkbox is checked, unit will autocalculate a volume based linear gradient and dispense per row, as specified in Edit Volume fields.

Row	Begin Vol	End Vol
A	0.1	0.4
B	0.1	0.4
C	0.1	0.4
D	0.1	0.4
E	0.1	0.4
F	0.1	0.4
G	0.1	0.4
H	0.1	0.4

Tip Touch—Tip will touch off on the well sides as specified by all, none, or selecting the appropriate arrows.

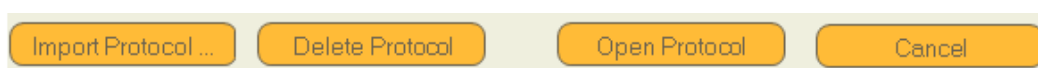
9. Run Protocols



Protocols are the Gryphon Instrument's user interface and comprise the sequence of commands that allow the device to carry out useful automated work in the laboratory.

Protocols can be developed to do simple tasks—like wash routines—or conduct complex experimental maneuvers. They can be developed and saved, or modified and resaved.

[Appendix A](#) contains a sample protocol showing how the Gryphon “thinks” as it completes an experimental setup.

1. Launch the Gryphon program. Click **Open Protocol** at the top left of the screen and then select the protocol you want to run. Click **Open Protocol** at the bottom of the screen. Note that you can also import or delete protocols, as well as cancel the transaction.



2. Ensure that the correct labware is on the deck and that the solutions to be aspirated are in the labware.
3. Click  at the top of the screen to run the protocol.
4. To stop the protocol at any time, click  at the top of the screen.

10. LCP Functions

There are two LCP specific function buttons available at the top of the screen, Exchange Solo Syringe and Mix LCP. Exchange Solo Syringe is used to allow the removal and replacing of an LCP syringe. The Mix LCP function controls the built in mixing function of the LCP unit.



Exchange Solo Syringe

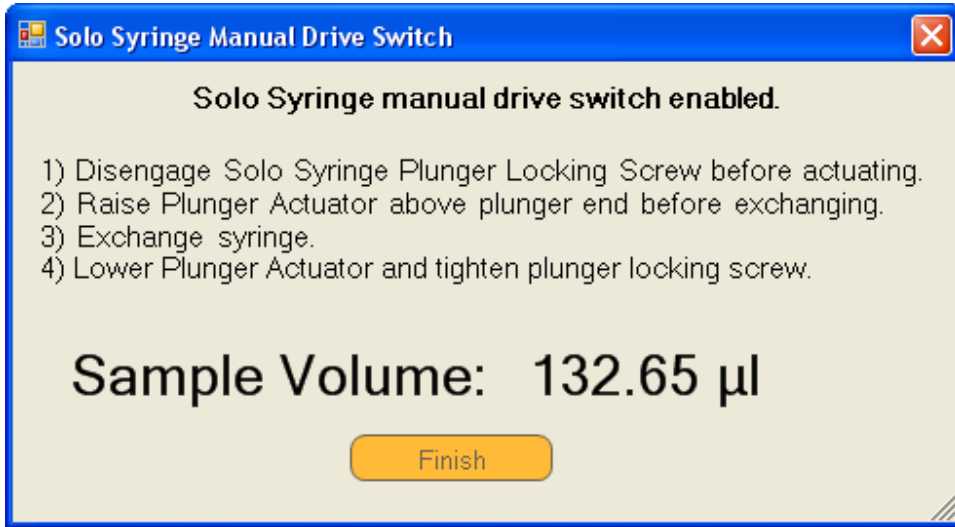
The Exchange Solo Syringe function is used to set up a prefilled syringe for LCP dispensing, or to move the actuating arm out of the way if required, and placing or replacing a syringe onto the LCP drive.



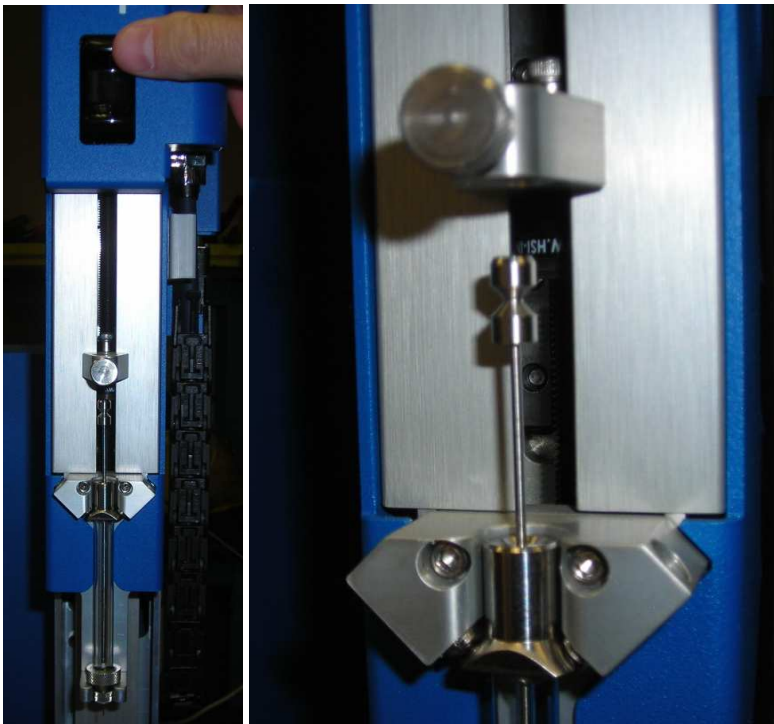
Connecting to the system with an LCP syringe plunger locked in place may lead to a damaged/broken syringe, and loss of

sample.

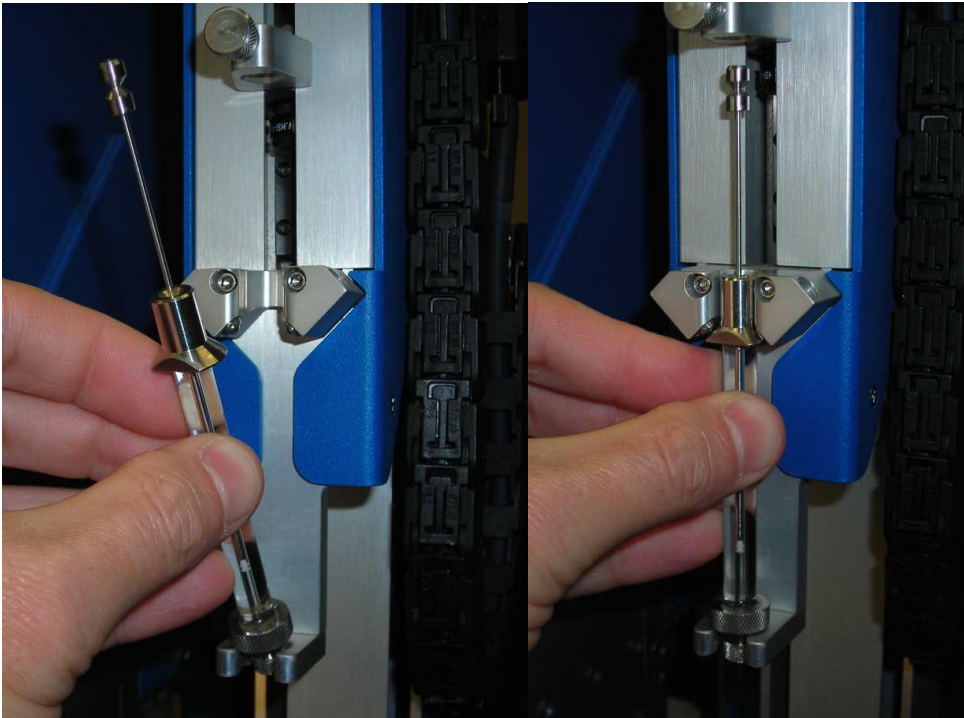
1. Click **Exchange Solo Syringe** at the top of the screen to run the function.
2. Ensure that the thumb screw is loose and not able to contact the syringe plunger
2. A control window will open outlining the steps to perform.



3. To remove a syringe already in place, disengage the thumb screw holding the Solo Syringe Plunger. If a syringe is being placed in an empty unit, use the black rocker switch to be sure the plunger lock is backed off far enough to allow the plunger through when mounting the syringe.



4. Place the syringe in the mating mount locations, point down if dispensing, and rock top into spring loaded ball stop mounts, notice flat on syringe is facing out.



5. Lower plunger actuator until even with plunger end.

NOTE

A long nozzle syringe is required to aspirate from the 8 vial positions in the sample diluter rack position 13.

Note: it is better to drive the plunger slightly down during the tightening process than to allow the teflon seal to be lifted above the sample creating an air pocket.

- 6.
- 7.
8. Tighten actuator onto plunger end.



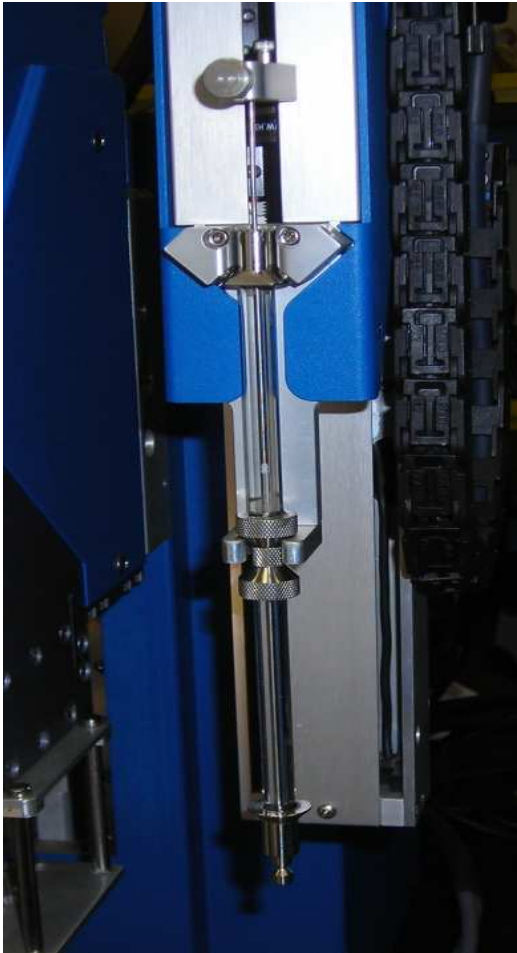
9. You may choose to manually actuate the LCP syringe slightly to make sure sample is at the tip, or you may perform a pre-dispense in the LCP protocol to accomplish the same purpose.
10. Press Finish when done.

Mix LCP

The Mix LCP function is used to mix a prefilled syringe for LCP dispensing

⚠ CAUTION	Starting the system with an LCP syringe plunger locked in place may lead to a damaged/broken syringe, and loss of sample.
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1. The rocker switch controlling the syringe motion is active in this command, allowing you to exchange or place a new syringe if required.
2. See exchange Solo Syringe section on how to mount syringe assembly.
3. The mounted syringe assembly should look like this:

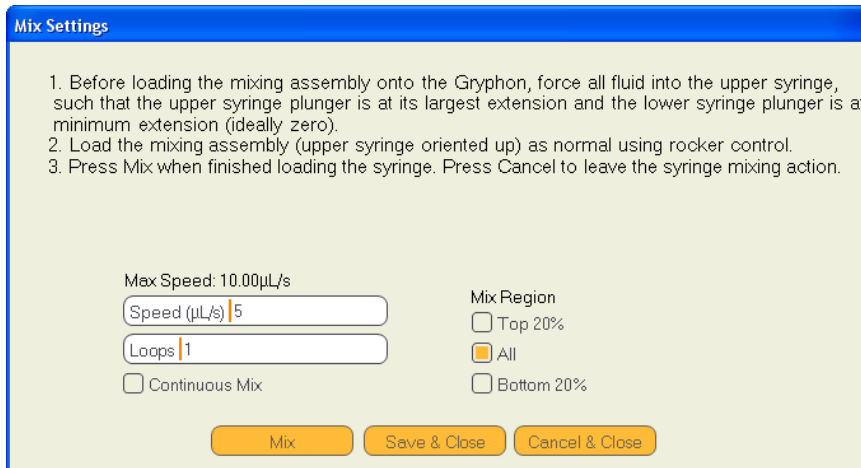


CAUTION

Bottom syringe plunger of mixing assembly should be fully depressed prior to mixing or it may lead to a damaged/broken syringe, and loss of sample.

4. Click on **Mix LCP** button.





5. The mixing protocol parameters are set here.

Speed—The mixing speed in microliters per second.

Loops—The number of full syringe strokes, up and down, to perform.

Mix Region—This setting allows mixing of only certain portions of the sample. Usually it is used if the ends of a sample fail to mix well, in which case you can set the unit to focus on the appropriate end..

Continuous Mix—This setting will continuously run the syringe until explicitly told to stop by pressing the button marked Change. After pressing Change, the unit will continue to move until the cycle it is running is completed.



6. Press **Save and Close** if you'd like to save the parameters you've set.

7. Press **Cancel and Close** to cancel any changes you've made.

8. Press **Mix** to perform the Mix protocol.

NOTE The system will finish the cycle it is working on when requested to stop. Therefore choosing very small values of mixing speed may lead to long waits for the system to complete its action.

Appendix A

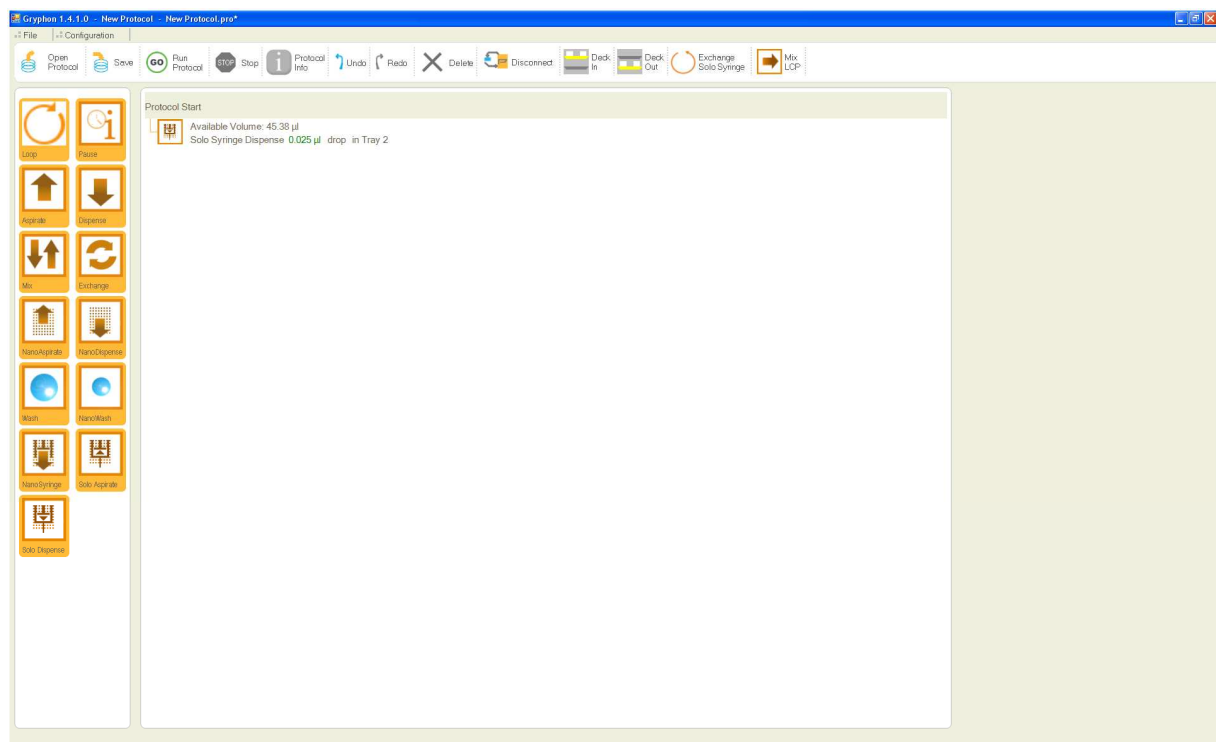
Sample Protocol

The following protocol describes an LCP protein dispense. This procedure will show how to set up an LCP dispense onto a glass plate..



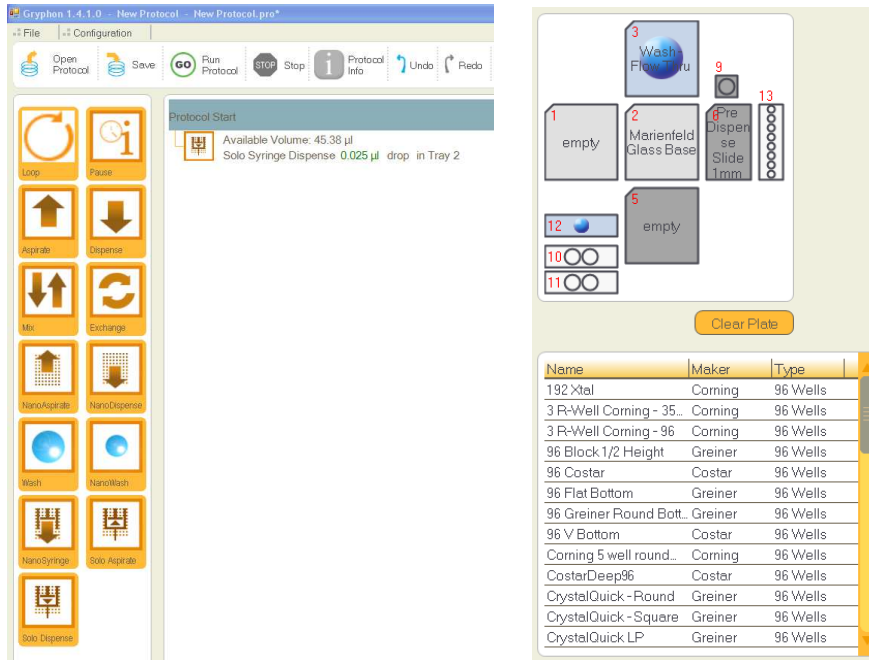
This is provided as an example only. Do not run this—or any other protocol—on your system without first testing and validating it.

When you have completed all the steps, your screen should look like this.

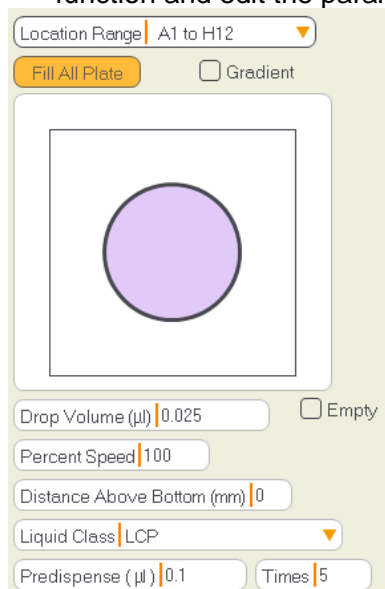


Refer to the [Command Descriptions](#) section at any time for more detailed information on each step.

1. Launch the Gryphon System and Click **Protocol Start** to populate the screen. Drag-and-drop plates from the Plate List onto the desired Deck locations. In this example, the Wash Flow Thru station is in position 3, a Marienfeld Glass base is in position 2, and a Pre dispense Slide 1mm is in position 6.



2. This protocol only has one function: to dispense LCP onto the glass slide. Select the function and edit the parameters.



3. Press Run Protocol.

