

# Phoenix Manual



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## Safety

For safe operation of this instrument, you must:

1. Study these safety instructions before you use the instrument.
2. Heed all warning and caution statements in the manual and on labels on the instrument.
3. Know how to shut down the instrument in an emergency.
4. Practice good laboratory techniques to minimize hazards.
5. Contact your Art Robbins representative to receive instructions for any maintenance or service procedure before you attempt to service the instrument.

### ***Caution and Warning Symbols Used in This Manual***

Warnings describe actions that can result in severe injury or death.

Cautions describe actions that can result in minor injury or in damage to equipment or data.

The following symbols might be used in the manual to alert you to warnings and cautions:



General caution or warning



Pinch point hazard—Moving parts: protect hands



Electrical caution or warning




Chemical caution or warning



Eye caution or warning

## Stopping the Instrument in an Emergency

To stop the system in an emergency, click the Stop button (  ) in the main screen of the software.

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.
  - If any containers have been misplaced or dropped, replace them as needed.
  - Ensure that the stage area is free of obstructions.
3. If the system components are not damaged and it is safe to run the instrument, restart the run in the Phoenix software.

## Following Good Laboratory Practice



**Caution:** Do not attempt to adjust or correct system containers or the instrument during operation.



**Caution:** Keep hands free of any moving parts on the instrument.



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



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



**Caution:** Set your aspirate and dispense heights so that the needle tips never touch the bottom of the receptacle. If needle tips contact the bottom of the receptacle, there is

danger that backpressure will build in the syringes and could cause the glass syringe barrels to crack or break. (*Exception:* You can safely dispense volumes of less than 1.0 $\mu$ L onto a dry plate with the needle tips touching the plate.)

## ***Moving the Phoenix Instrument***



**Caution:** The Phoenix instrument weighs 63.5 kg (140 lbs) (see

Table 1). If you must move the instrument, do the following to prevent the possibility of injury or of damage to the instrument:

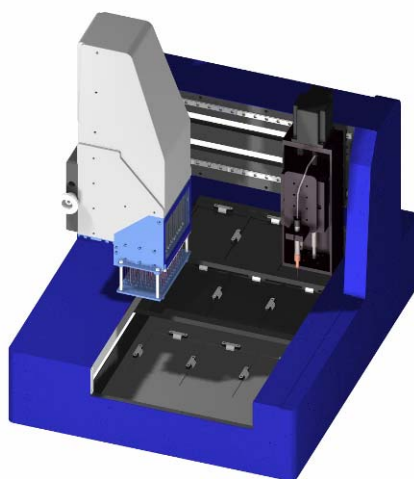
- Lift the Phoenix only from the front and sides. Do not lift the Phoenix from the rear.
- When lifting the Phoenix, be sure that you are lifting the unit by the base and not just by the enclosure.

**Table 1. Phoenix Dimensions and Weight**

Width	64 cm / 25 in
Depth	69 cm / 27 in
Height	74 cm / 29 in
Weight (base and head)	63.5 kg / 140 lbs

## **Introduction to the Phoenix**

The **Phoenix** is a multiposition, high-throughput liquid-handling platform that incorporates a space-saving design with flexible dispensing options. By using a combination of interchangeable multiple syringe heads and non-contact dispensers, the competitively priced Phoenix provides increased efficiency and cost-effectiveness. The Phoenix minimizes the amount of reagent volume needed and performs many microtiter plate assays. It is designed to be used in a bench-top lab environment. With all its capacity, the Phoenix is only 25 inches by 27 inches—a remarkably capable machine for its size that will greatly enhance the throughput of your laboratory.



### ***Features of the Phoenix system***

- Nine assay positions: 6 source or destination plate positions, 2 reagent positions, 1 wash station position
- Multiple-syringe drive: can accommodate 48, 96, or 384 interchangeable syringe head configurations
- Two needle types available: Teflon coated stainless steel needles or flexible Nitinol needles
- Multiple dispense options: dispenses into 48-, 96-, 384-, or 1536-well plates and into many protein crystallography plates
- Non-contact nano-dispenser drive: can accommodate single- or 8-channel dispenser to deliver volumes as low as 50nL
- Bench-top lab use: footprint of 25 x 27 in
- Microsoft Windows-based software
- Closed-loop motion control on each axis: verification of task completion via encoders on each motor

## Getting Started

To use the Phoenix system, you will need to

1. Calibrate the stage
2. Define labware
3. Write protocols

This guide shows you how to complete those tasks and then how to run the protocols.

You will only need to calibrate the stage one time, at first use of the system. Thereafter you only need to calibrate the stage if you move the system.

You define labware for your first use of the system and at any time thereafter when you want to use a new type of labware in your protocols.

You can save the protocols you create and use them over and over again. You can edit existing protocols as well as create new ones.

**Note:** A sample protocol is provided on page 31.

## Calibrate the Stage

Your Art Robbins Instruments representative will calibrate the stage when the system is installed.

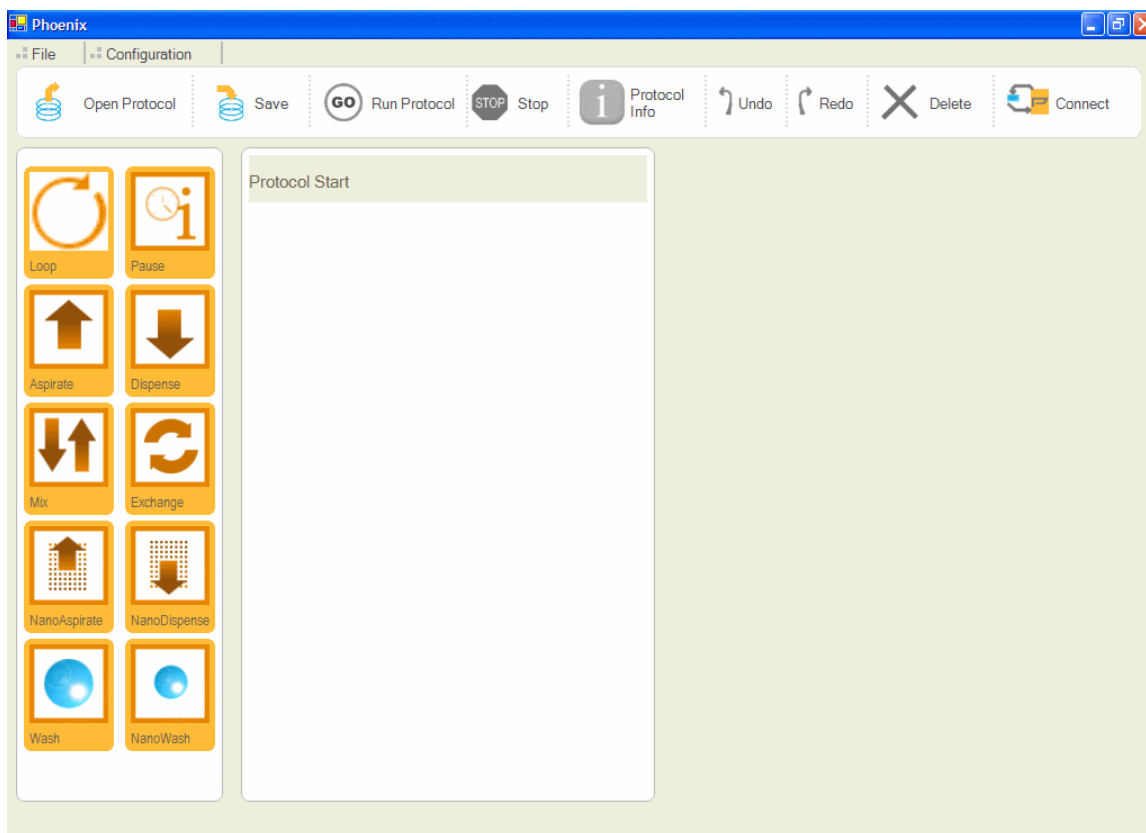


**Caution:** Study the safety information (see Safety on page 4) for this instrument before you operate it and always follow the cautions and warnings, both in this manual and on the instrument's labels, regarding the instrument's operation.

To calibrate the Phoenix:

1. Turn on the Phoenix system and computer and click the Phoenix icon to start the Phoenix program. The Phoenix screen appears.





2. Select Configuration > Calibrate Stage. The Calibrate Stage screen appears.

**Calibrate Stage**

Trays Edition

1	empty	2	empty	3	empty
4	empty	5	empty	6	empty
7	empty	8	empty	9	empty

Number	Level
9	3
8	3
7	3
6	2
5	2
4	2
3	1
2	1
1	1
10	3
...	-

Number |

X |

Y |

Z |

Nano X |

Nano Y |

Nano Z |

Color |

Current Position

0.0

0.0

0.0

0.0

0.0

0.0

0.0

Start Teaching

☐ Teach 96 Syringes Head

☐ Teach Nano Dispenser Head

Stop Teaching

Engine Control

Connect

Stop

Step Jog

STOP

Step Size (mm) | 9.0

Continuous Jog

STOP

Go Above Position

Go To Z Level

Update XY Tray Position

Update Z

Save

Save & Close

Cancel & Close

- Under the screen subsection Engine Control, click Connect.



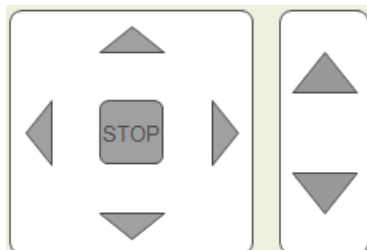
**Caution:** If you need to stop the calibration movements at any time, click **Stop** under Engine Control.

- IMPORTANT: Read the following *BEFORE*** you begin the calibration, so that you will know how the screen controls move the head and tray.

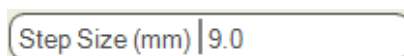
At the bottom of the screen, there are four “control” areas containing arrows.

The two control areas on the left contain the **Step Jog** arrows:

## Step Jog



The Step Jog arrows move the head and tray in a stepwise mode. Each mouse click moves the 96-syringes head or the nano dispenser 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 heads are far above or far to the left or right of the calibration holes, and then use a smaller step size when the heads and calibration holes are closer to one another.

Use the arrows in the larger (first) control area to move the head left and right:



And to move the tray forward and back:

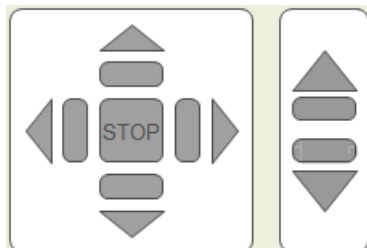


Use the arrows in the smaller (second) control area to move the head up and down:



The two control areas on the right contain the **Continuous Jog** arrows:

### Continuous Jog



The Continuous Jog arrows move the head and tray continuously as long you press and hold down the left mouse button. The outer arrows move the head more slowly and the inner capsule-shaped “arrows” move it more quickly.

Use the arrows in the larger (third) control area to move the head left and right:



And to move the tray forward and back:



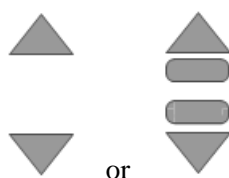
Use the arrows in the smaller (fourth) control area to move the head up and down:

5. **To begin the calibration:** Click Number 1 in the table at the top middle of the screen:

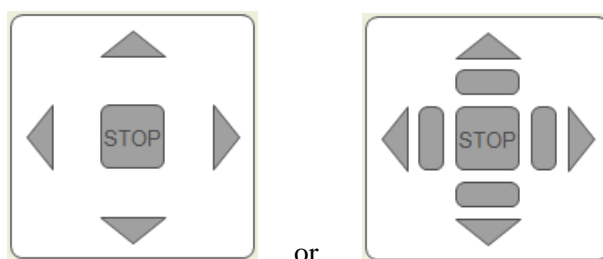
Number	Level
9	3
8	3
7	3
6	2
5	2
4	2
3	1
2	1
1	1

This action starts the calibration at the Number 1 position (see the tray layout at the top left of the screen).

6. Click Start Teaching and then click the control box next to either Teach 96 Syringes Head or Teach Nano Dispenser Head. (You will need to “teach” both heads to the system; you can start with either head.)
7. Now use the vertical up/down arrows in Step Jog or Continuous Jog (as described in Step 4) to adjust the space between needle tips and top of plate so you can clearly see whether the A1 needle tip is centered over the A1 well. Find the small hole in the A1 nest position (there is an A1 nest position hole for both head types) and then position the needle tip so that it covers that hole when you lower the needle.



8. Use the arrows in the large control areas under either Step Jog or Continuous Jog (as described in Step 4) to move the head and the tray so the needle tip is centered over the hole in A1. You are adjusting the XY Tray Position.



9. Use the large vertical arrows again (the ones you used in Step 7) to adjust the Z height. Set Z so that the needle tips just touch the stage.
10. Click Update XY Tray Position and Update Z.
11. Click Save and then click Stop Teaching.



**Caution:** Put the tube holder or wash receptacle in place in tray position 12 BEFORE you set the Z height for that position. When calibrating tray position 10, put the chiller in place BEFORE you set the Z height.

12. Repeat the calibration steps for the remaining positions, for both heads.

**Note:** To move to an existing X,Y position quickly—for example, when you want to check the setting or modify it—click Go Above Position.

13. Click Save & Close.

## Define Labware

Defining labware is “teaching” the system the physical parameters of the labware pieces you will be using in your protocols. Most labware manufacturers can provide you with the required dimensions.

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 those single definitions is called a “layer.”



**Caution:** Study the safety information (see Safety on page 4) for this instrument before you operate it and always follow the cautions and warnings, both in this manual and on the instrument's labels, regarding the instrument's operation.

To define labware for the Phoenix:

1. Have a measuring tool ready to measure the dimensions of your labware.
2. Turn on the Phoenix system and computer and click the Phoenix icon to start the Phoenix program. The Phoenix screen appears.
3. Select Configuration > Open Labware Editor. The Labware Editor screen appears.

Name	Maker	Type
96 Flat Bottom	Greiner	96 Wells
Greiner 1536		1536 Wells
384	Greiner	384 Wells
Protein vial(LLNL)		96 Wells
96 Lid		96 Wells
Greiner96 (LRC)	Greiner	96 Wells
96 round		96 Wells
Intelliplate	Robbins	96 Wells
Reservoir	Generic	96 Wells
PCR		384 Wells
Deep Well 2 ml - 96	Beckman	96 Wells
384 second		384 Wells
384 - Polystyrene - Square	Greiner	384 Wells
Wash Station	ARI	96 Wells
96 square		96 Wells
Teflon Film Frame	Neuro Probe	96 Wells

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

- Select a format from the Format drop-down list.
- 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.
- Click on the "Corner" option that applies to your plate (the corner that is notched).

- Click Add Layer.
- Enter Name and Depth for that layer ("Depth" = distance from top of plate to 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 [zero]).
12. Enter Layer Width and Height and then select the shape of the layer.

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 ▼

## Edit Liquid Classes

The Phoenix software uses a *liquid class* to set dispenser parameters that are used to define the properties of a liquid. The selection of the correct liquid class is an important factor in ensuring good dispense accuracy.

Editing liquid classes is setting parameters to fine-tune the aspiration or dispense of various types (“classes”) of liquids. You will specify which of your pre-specified liquid classes you want to use when you set up aspirate and dispense commands in your protocols.

To edit liquid classes on the Phoenix:

1. Select Configuration > Edit Liquid Classes. The Edit Liquid Classes screen appears.



**Liquid Classes**

Liquid Class Name

Water(asp)

Water(dsp)

Screen

djw

Name

**Syringe Head Settings**

Plunger Speed (ul/sec)

Dwell Time (sec)

Head Speed (mm/sec)

**Nano-dispenser Settings**

Pressure (mbar)

Vacuum (mbar)

Viscosity (centipoise)

Add New Class

Delete Class

Save & Close

Cancel & Close

Predefined and recommended user defined liquids are listed in the left hand pane of the dialog. Parameters for the selected liquid appear in the right hand pane.

2. Click Add New Class.
3. In the upper right, enter a name for the class.
4. Enter values for plunger speed, dwell time, and head speed.

When you set the liquid class for the Syringe head, it is also necessary to set the acceleration for the plungers. The table below gives the recommended acceleration for each plunger speed.

Speed (μL/sec)	Acceleration
1	10000
2	10000
3	20000
4	50000
5	50000
6	50000
7	100000
8	200000
9	200000
10	200000
11	200000
12	200000
13	200000
14	200000
15	200000

5. If you will use the nano dispenser, you will next enter values for pressure, vacuum, and viscosity. **IMPORTANT:** *BEFORE* you set these values, study the information and instructions under Define Liquid Classes for the Nano Dispenser, starting on page 18, and *FOLLOW THOSE INSTRUCTIONS CAREFULLY.*
6. When you have finished entering values, click Save & Close.

**Note:** You can also delete liquid classes from this screen, and if you make changes to a liquid-class definition that you don't want to save, you can click Cancel & Close (thereby not saving changes).

## ***Define Liquid Classes for the Nano Dispenser***

For the nano dispenser, three parameters define a liquid class: pressure, vacuum and viscosity.



**Caution:** Do NOT adjust the vacuum without consulting the factory.

Definitions of those configurable parameters follow:

### **Pump Pressure:**

Pressure, in mBar, which is applied to the liquid in the tip during dispensation. You might have to increase the dispense pressure to prevent the formation of hanging drops with high-viscosity liquids. The dispense pressure must lie in the range of 100 to 4500 mBar.

### **Vacuum Pressure:**

Level of vacuum, in mBar, which is applied to the tip during liquid aspiration. Do NOT alter this value.

### **Viscosity:**

Viscosity, in centipoises, defined for this liquid.

New liquid classes will be required for liquids not already contained in the standard configuration provided with the system. Unless the liquid is very viscous, the only parameter that you will need to change is the *viscosity constant*. This value controls the amount of time the valve remains open; the greater the viscosity constant, the longer the valve remains open.

To determine the viscosity constant for a given liquid, you will need to dispense the liquid with the nano dispenser to determine the actual volume dispensed for a given request volume. You then will use this value to alter the viscosity constant to account for the viscosity of the liquid and ensure that the correct volume of all liquids is dispensed. To determine the needed viscosity constant for a given liquid, follow these steps:

1. Place the liquid to be assessed in the reservoir or in selected wells of a 96 well plate in plate position 1 of the Phoenix.
2. Place a 96 well plate in position 2.

3. Create a protocol with 10 loops to dispense the desired volume into plate position 2. Use the liquid class for Water.
4. Assess the volume of liquid dispensed either gravimetrically or by using a hand pipette.

This will allow the determination of the viscosity constant for this liquid in the flowing manner:

$$Z = \frac{\text{Volume requested}}{\text{Actual volume dispensed}}$$

$$\text{Viscosity}_{\text{New}} = \text{Viscosity}_{\text{Water}} \times Z$$

For example:

You have a master mix solution for PCR.

You want to know the liquid class.

You dispense the customer assay volume (20µL) 10 times = 200µL.

You use a hand pipette to assess how much is in the tip and find that there is only 150µL.

200/150 = 1.3333

1.333x1000 (viscosity constant of water) = 1333

This is the new viscosity constant for the PCR mix.

You can now enter the parameters for the nano dispenser for the liquid class:

1. Enter pressure 1198.
2. Enter Vacuum 676.
3. Enter the just calculated viscosity.
4. Click Save & Close.

Verify the liquid class settings by repeating the above test with the new liquid class and confirming that the correct volume of liquid is dispensed.

## **More About Setting Parameters for Nano Dispenser Liquid Classes**

As mentioned before, normally the only setting that will need alteration is the viscosity constant; however, for very viscous liquids you might need to alter the pump pressure. Increase the pressure until hanging drops are eliminated from the end of the tip.

Although the liquid class settings are normally used to define parameters specific to the liquid being dispensed, sometimes certain plates require that different dispense parameters be used. For example, some plates have very steep sides and as a result the liquid sometimes hits the bottom and some splashing occurs. In this case, lowering the pressure of dispense will prevent the liquid from exiting the well. You can change the dispense pressure in an existing liquid class or create a new class to effect this pressure change. Lowering the dispense pressure will normally not have a large effect on the amount of liquid dispensed, but it is always a good idea to check this, either with a hand pipette or gravimetrically.

## **Optimize Dispense Precision for Viscous Solutions**

To determine the optimum dispense pressure setting for obtaining the best CV% for the new liquid:

1. Set up and run a dispense that has the system dispense a volume close to the desired operational volume required from the liquid, using the standard calibrated system settings for water.
  - If a drop is dispensed, the pressure of the system can be deemed appropriate for that fluid.
  - If a hanging drop occurs or if the droplet is not “cleanly” dispensed, increase the system pressure. Increase the pressure in steps of 500 mBar until an acceptable drop is dispensed.
2. Measure the volume of the fluid dispensed and ensure that the CVs from all tips are acceptable to the user or are below 10%. This method is a scientific measurement of the “cleanliness” of the dispensed drop.
  - Perform this task using a fluorescent or absorption dye and a well plate reader.
  - If the CVs are above 10% or are unacceptable to the user, increase the pressure in steps of 200 mBar until the CVs become more reliable.

## **Optimize Dispense Accuracy**

To adjust dispense accuracy after the CVs are at an acceptable level:

If the drop volume measured is different than the requested volume, you need to alter the viscosity constant of the liquid.

1. The viscosity constant is proportional to the volume dispensed. As a first approximation, increase the viscosity constant by the ratio of the volume dispensed to the volume desired. For example:
  - If the dispensed volume is 100nL and the desired volume is 200nL, double the viscosity constant.
  - If the dispensed volume is 327nL and the desired volume is 600nL, multiply the viscosity constant by 1.84.
2. After this alteration, measure the dispense performance again using a standard fluorescent or absorption dye and a well plate reader.
  - If the well plate provides satisfactory CVs for both precision and accuracy, save the derived system settings derived as the settings for that fluid’s liquid class.
  - If the well plate readings are not accurate in terms of volume dispensed, there are two options:
    - Variation of the viscosity constant or the volume offset.
    - Further minor variation of the viscosity constant should allow easy tuning of the accuracy. This can be verified by repeating the above steps.

## Create Protocols

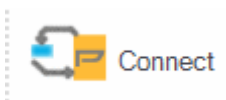


**Caution:** Study the safety information (see Safety on page 4) for this instrument before you operate it and always follow the cautions and warnings, both in this manual and on the instrument's labels, regarding the instrument's operation.

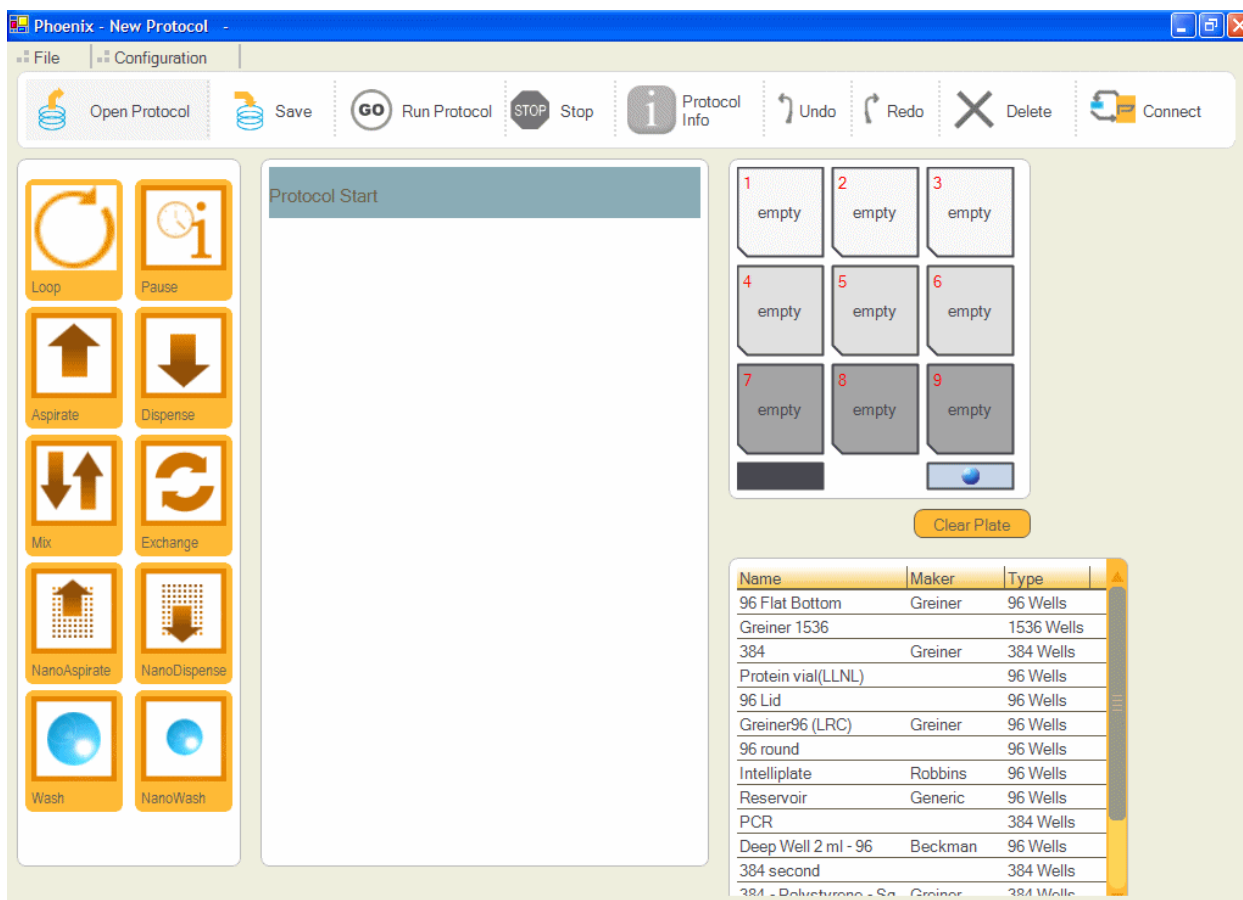
Note: A sample protocol is provided later in this manual, beginning on 31.

To create protocols for the Phoenix:

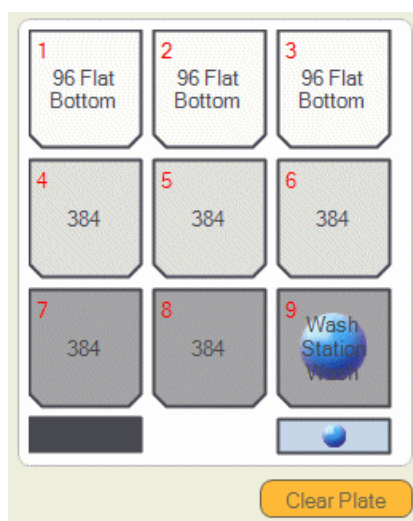
1. Turn on the Phoenix system and computer and click the Phoenix icon to start the Phoenix program. The Phoenix screen appears.
2. Click Connect on the top right of the screen.



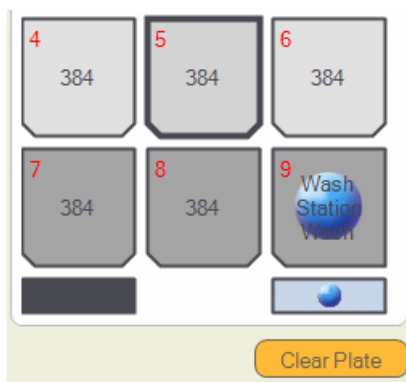
3. Select File > New Protocol.



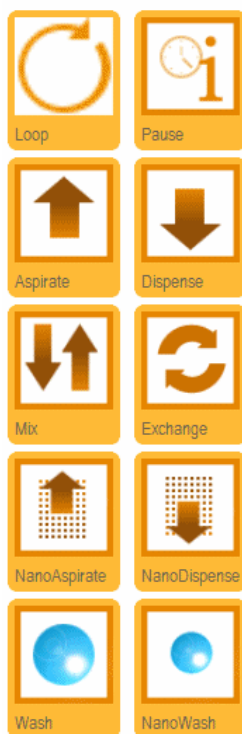
- Click a plate name in the table on the lower right and drag it onto the plate position on the deck where you will place that plate. Continue to click and drag plate names until the deck is populated with the labware you need for your protocol.



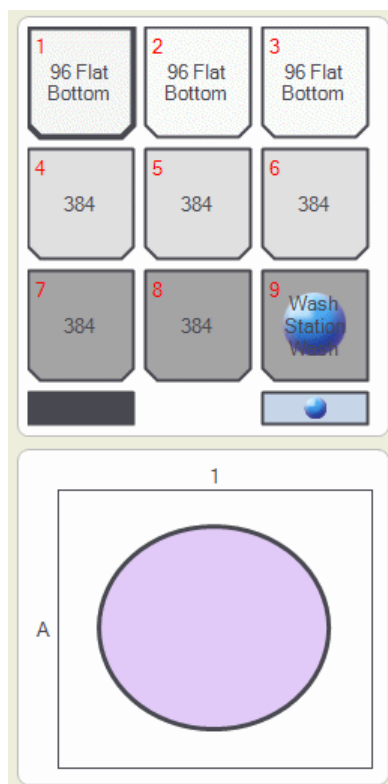
- If you accidentally drag a plate into an incorrect deck position, click on the deck position to select it (5 is selected below) and then click Clear Plate.



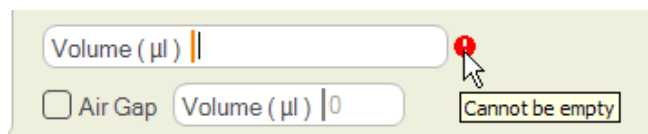
- Click the first command you want to use in the protocol in the command area on the left side of the screen and drag it into the Protocol Start area in the middle of the screen. See the Command Descriptions starting on page 25 for details about the commands.



- For each of the commands, click on the plate location on the deck where the command is to be executed. A representation of the plate's well or wells will appear below the deck. The plate in position 1 is selected in the following example:



8. Fill in the parameter values needed for that command. If an alert symbol appears next to a command value field, move the cursor over the symbol to see the alert message. Generally the alerts appear if the value provided for the command is out of range.



9. When you have finished entering commands, click the Protocol Info button at the top middle of the screen and fill in the protocol information. Then close the Protocol Information window.

Protocol Information	
Protocol Title	First Protocol
Creator	First User
Description	AspDisp plus ProteinDisp



10. Click the Save button on the Toolbar to save the protocol.

**Note:** The software automatically validates the protocol. If there is a problem with any of the commands, you will see a red symbol pop up, as described in Step 8 above.

11. Close the protocol's window if you are finished creating protocols.

See Run Protocols on page 31 for information about running the protocols you've created. See page 31 for a sample protocol.

## Command Descriptions

### Loop

—Runs the commands contained in the loop, in order, for the number of loops specified. A Loop command must be followed by at least two subordinate commands. You can disable the loop for particular protocol runs if you wish.

Number of Loops: Number of times the loop of subordinate commands is to repeat.



Number of Loops | 3

☐ Disable Loop

### Pause

— Waits for specified number of seconds or displays message and waits for user to press Enter.

Use Pause: Optional. If this box is selected, specifies that the system will pause for the specified amount of time before continuing to the next command.

Pause Time: If Use Pause is selected, you must enter a value in this field; specifies amount of time in seconds that the system will pause before continuing to the next command.

Message: Optional. When the protocol runs, displays message to operator that was typed into the message space when the command was inserted in the protocol. Can be used with or without a specified Pause Time value. If no Pause Time value is specified, the protocol run will pause until you press Enter.



## Aspirate

—Aspirates specified volume from specified plate into the syringes.

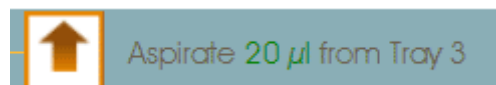
Volume: Amount of solution to be aspirated.

Air Gap (optional): Aspirates an air gap of specified volume before aspirating solution, so that during dispense the plunger pushes both solution volume and air out of the syringe.

Pre-dispense (optional): Aspirates extra specified amount of solution into the syringes. That amount will be dispensed into the plates the specified number of times before the dispense volume is dispensed.

Above Bottom: Specifies the aspirate height—that is, the vertical position in the well where the aspirate is to occur.

Liquid Class: Specifies a set of pre-set parameters, including plunger speed, dwell time, and head speed, that will be used by the system when this command is performed.



## Dispense

—Dispenses specified volume from syringes into specified plate.

Volume: Amount of solution to be dispensed.

Distance Above Bottom: Specifies the dispense height—that is, the vertical position in the well where the dispense is to occur.

**Tip Touch (optional):** Specifies dispense to occur with needle tip almost touching any of 4 tip touch positions in the well: left and right sides, front and back.

**Liquid Class:** Specifies a set of pre-set parameters, including plunger speed, dwell time, and head speed, that will be used by the system when this command is performed.

**Empty:** When this box is selected, specifies that the entire contents of the syringe are to be emptied into the well in one dispense action.

## Mix

—Aspirates specified volume from specified plate location into the syringes and then dispenses the aspirated volume back into the same location. Aspirate/dispense sequence occurs the specified number of times.

**Volume:** Amount of solution to be aspirated.

**Distance Above Bottom:** Specifies the aspirate/dispense height—that is, the vertical position in the well where the aspirations and dispenses are to occur.

**Number of Cycles:** Number of times the aspirate/dispense sequence will be repeated.

**Air Gap (optional):** Aspirates an air gap of specified volume before aspirating solution, so that during dispense the plunger pushes both solution volume and air out of the syringe.

**Liquid Class:** Specifies a set of pre-set parameters, including plunger speed, dwell time, and head speed, that will be used by the system when this command is performed.

Volume (μl) | 5

Distance Above Bottom (mm) | 4

Number of Cycles | 3

☐ Air Gap Volume (μl) | 0

Liquid Class | Water(dsp) ▼

## Exchange

— Stops the protocol, moves the deck forward, and prompts the user with one of:

Remove plate: Remove plate  $x$  from position  $x$ .

Replace plate with selected plate below: Remove plate  $x$  from position  $x$  and replace it with plate  $y$ . You can select any plate from the plate list to be the replacement plate.

**Note:** The changes are reflected on the plate layout when the screen is refreshed.



☐ Remove plate

☒ Replace plate with selected plate below:

Name	Maker	Type
Protein vial(LLNL)		96 Wells
Deep Well 2 ml - 96	Beckman	96 Wells
Wash Station	ARI	96 Wells
Greiner96 (LRC)	Greiner	96 Wells
384	Greiner	384 Wells
Intelliplate	Robbins	96 Wells
96 Flat Bottom	Greiner	96 Wells
Reservoir	Generic	96 Wells
96 square		96 Wells

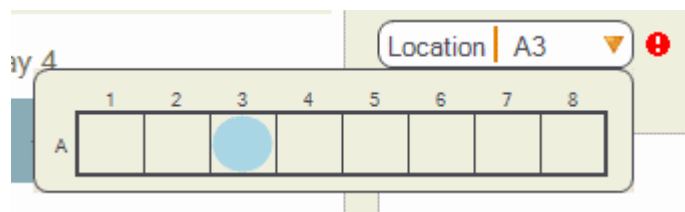
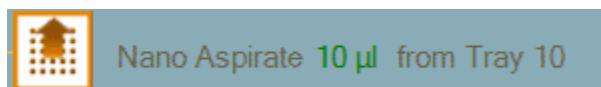
## Nano Aspirate

— Nano syringe aspirates specified volume from specified plate into nano syringe. Select the location in the specified plate from which to aspirate using the Location drop-down.

Volume: Amount of solution to be aspirated.

**Distance Above Bottom:** Specifies the aspirate height—that is, the vertical position in the well where the aspirate is to occur.

**Liquid Class:** Specifies a set of pre-set parameters, including pressure, vacuum, and viscosity, that will be used by the system when this command is performed.



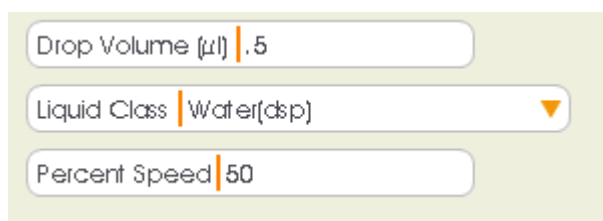
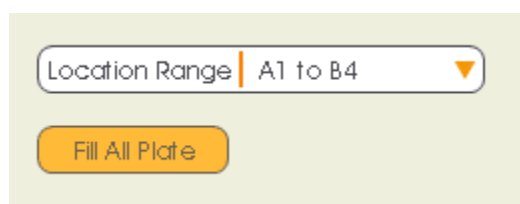
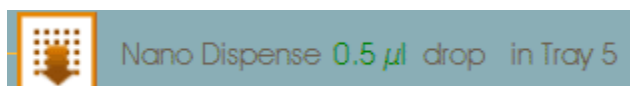
## Nano Dispense

—Nano syringe dispenses specified volume from nano syringe into specified plate locations. Select the locations in the specified plate into which to dispense by using the Location drop-down or click Fill All Plate.

**Drop Volume:** Amount of solution to be dispensed.

**Liquid Class:** Specifies a set of pre-set parameters, including pressure, vacuum, and viscosity, that will be used by the system when this command is performed.

**Percent Speed:** Specifies the speed at which the stage moves during dispense.



## Wash

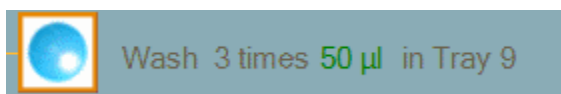
—Aspirates specified wash volume from specified Wash location into the syringes and then dispenses the aspirated wash liquid back into the same location; sequence occurs the specified number of times.

Number of Cycles: Specifies number of times syringes will aspirate and dispense wash solution (one aspirate + one dispense = one cycle).

Volume: Specifies amount of wash solution to be aspirated.

Distance Above Bottom: Specifies the wash height—that is, the vertical position in the well where the aspirate/dispense of the wash solution is to occur.

Liquid Class: Specifies a set of pre-set parameters, including plunger speed, dwell time, and head speed, that will be used by the system when this command is performed.



Wash 3 times 50 µl in Tray 9

Number of Cycles | 3

Volume ( µl ) | 50

Distance Above Bottom (mm) | 5

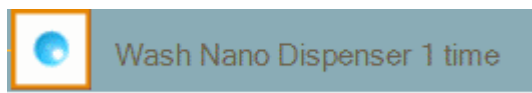
Liquid Class | Water(dsp) ▼

## Wash Nano

—Aspirates specified wash volume from specified Wash location into the nano dispenser and then dispenses the aspirated wash liquid back into the same location; sequence occurs the specified number of times.

Number of Cycles: Specifies number of times nano dispenser will aspirate and dispense wash solution (one aspirate + one dispense = one cycle).

Volume: Specifies amount of wash solution to be aspirated.



Wash Nano Dispenser 1 time


Number of Cycles | 1

Volume ( µl ) | 200

## Run Protocols



**Caution:** Study the safety information (see Safety on page 4) for this instrument before you operate it, and always follow the cautions and warnings, both in this manual and on the instrument's labels, regarding the instrument's operation.

1. Turn on the Phoenix system and computer.
2. Click the Phoenix icon to start the Phoenix program. The Phoenix screen appears.
3. Click Open Protocol on the top left of the screen and then click on the protocol you want to run.
4. Ensure that the correct labware is on the deck and that solutions to be aspirated are in the labware.
5. Click the GO icon at the top of the screen to run the protocol.
6. **To stop the protocol run at any time:** Click STOP (  ) at the top of the screen.

## Sample Protocol



**Caution:** The following sample protocol is provided as an example only. Do not run this or any other protocol on your system without first testing and validating the protocol on your system.

## Screen 1: Deck layout

Drag and drop plates from the plate list onto the desired deck locations.

The screenshot displays the Phoenix 1.9 software interface. The top menu bar includes File, Configuration, and a toolbar with icons for Open Protocol, Save, Run Protocol, Stop, Protocol Info, Undo, Redo, Delete, and Connect. The main workspace is divided into three sections:

- Left Panel:** A vertical toolbar with icons for Loop, Pause, Aspirate, Dispense, Mix, Exchange, NanoAspirate, NanoDispense, Wash, and NanoWash.
- Center Panel:** A protocol editor titled "Protocol Start" showing a sequence of steps:
  - Loop: 3 loops
  - Aspirate 80 µl from Tray 5
  - Dispense 60 µl in Tray 2 in location A,1
  - Nano Aspirate 50 µl from Tray 6
  - Nano Dispense 0.5 µl drop in Tray 2
  - Dispense 0.5 µl in Tray 2 in location A,1
  - Dispense 0.5 µl in Tray 2 in location A,1
  - Empty in Tray 2 in location A,1
  - Replace plate in tray 2
  - Replace plate in tray 5
  - Wash 30 times 90 µl in Tray 7
  - Wash Nano Dispenser 3 times
- Right Panel:** A deck layout grid with 9 positions:
  - 1: Intelliplate
  - 2: Intelliplate
  - 3: Intelliplate
  - 4: Hampton Screen
  - 5: Hampton Screen
  - 6: Protein Chill Block
  - 7: Wash Station
  - 8: Reservoir
  - 9: Reservoir
 Below the grid is a "Clear Plate" button.
- Bottom Right:** A "Plate List" table with columns Name, Maker, and Type.
 

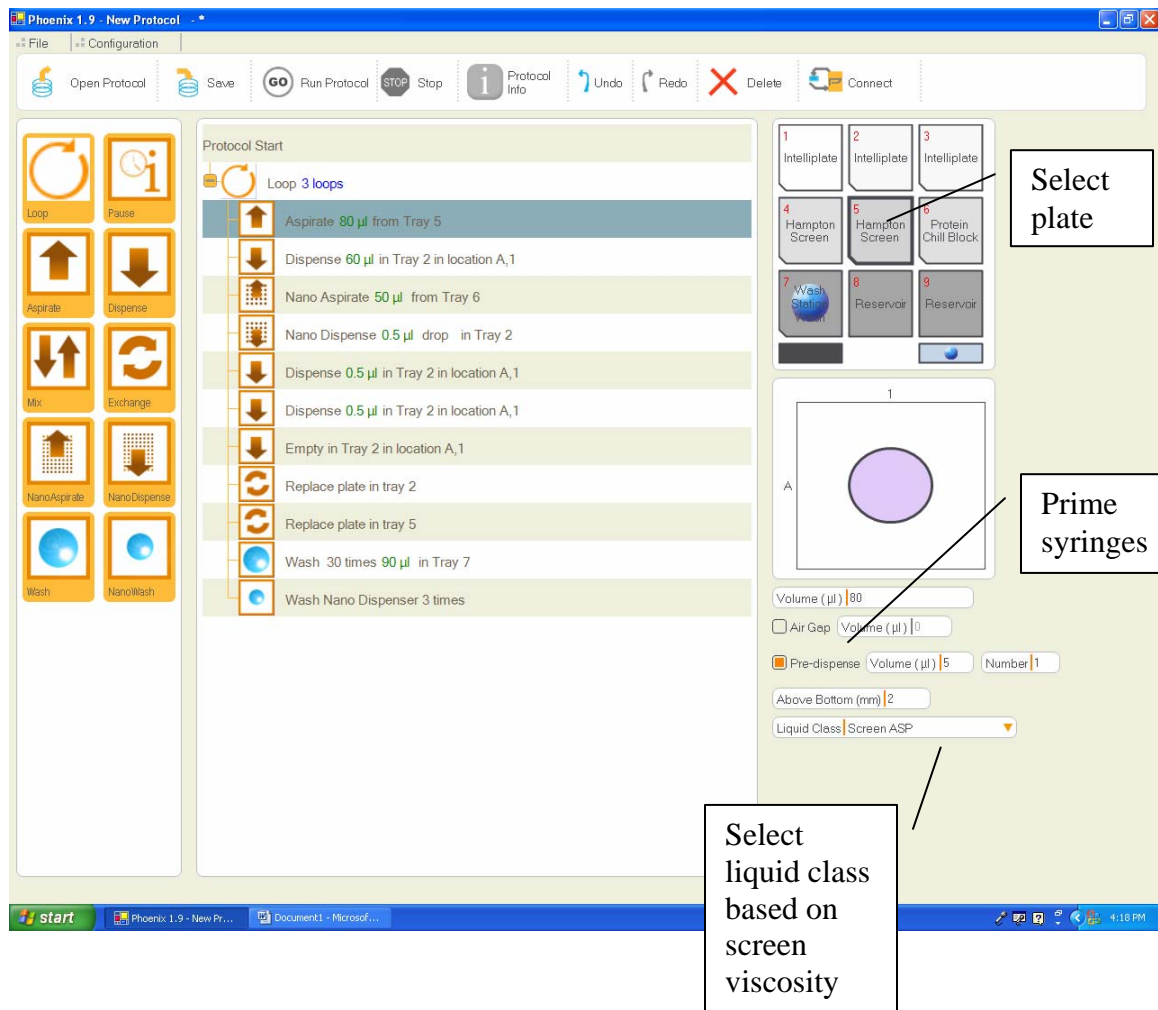
Name	Maker	Type
1536 - Clear - Square...	Greiner	1536 Wells
192 Xtal	Corning	96 Wells
384	Greiner	384 Wells
384 - Polystyrene - Sq...	Greiner	384 Wells
384 Black/Clear	Costar	384 Wells
96 Black/Clear	Greiner	96 Wells
96 Block 1/2 Height	Greiner	96 Wells
96 Costar	Costar	96 Wells
96 Flat Bottom	Greiner	96 Wells
96 Lid - offset		96 Wells
Costar 96	Jaime	96 Wells
Deep Well 2 ml - 96	Beckman	96 Wells
djwPCRholder	djw	96 Wells

Annotations with arrows point to the "Deck" grid and the "Plate List" table.



## Screen 2: Aspirate Screen

Select the plate, prime the syringes, and select the liquid class based on the viscosity of the screen.



### Screen 3: Initial Screen Dispense

Select the plate, select the well, and select the liquid class based on the viscosity of the screen; recommended dwell time: 2 sec. The screen will be dispensed in 2 steps. The needle should not touch the bottom of the plate.

Phoenix 1.9 - New Protocol

File Configuration

Open Protocol Save Run Protocol Stop Protocol Info Undo Redo Delete Connect

Protocol Start

Loop: 3 loops

- Aspirate 80  $\mu$ l from Tray 5
- Dispense 60  $\mu$ l in Tray 2 in location A,1
- Nano Aspirate 50  $\mu$ l from Tray 6
- Nano Dispense 0.5  $\mu$ l drop in Tray 2
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 30 times 90  $\mu$ l in Tray 7
- Wash Nano Dispenser 3 times

Loop Pause

Aspirate Dispense

Mix Exchange

NanoAspirate NanoDispense

Wash NanoWash

Select plate

Select well

First of 2 dispense steps

Needle tip should be above plate bottom

Select liquid class based on screen viscosity; rec. dwell time: 2 sec

1 Intelliplate 2 Intelliplate 3 Intelliplate

4 Hampton Screen 5 Hampton Screen 6 Protein Chill Block

7 Wash Station 8 Reservoir 9 Reservoir

Volume ( $\mu$ l) 60

Distance Above Bottom (mm) 0.5

Tip Touch

Liquid Class Screen DSP-2

## Screen 4: Aspirate Protein

Select plate, location, xxx, and volume. Select liquid class based on sample viscosity. The needle should be above the plate bottom.

The screenshot shows the Phoenix 1.9 software interface for creating a new protocol. The interface includes a menu bar (File, Configuration), a toolbar (Open Protocol, Save, Run Protocol, Stop, Protocol Info, Undo, Redo, Delete, Connect), and a main workspace. The workspace is divided into a left sidebar with icons for various actions (Loop, Pause, Aspirate, Dispense, Mix, Exchange, Nano Aspirate, Nano Dispense, Wash, Nano Wash), a central 'Protocol Start' list, and a right sidebar with configuration options.

The 'Protocol Start' list contains the following steps:

- Loop 3 loops
- Aspirate 80  $\mu$ l from Tray 5
- Dispense 60  $\mu$ l in Tray 2 in location A,1
- Nano Aspirate 50  $\mu$ l from Tray 6
- Nano Dispense 0.5  $\mu$ l drop in Tray 2
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 30 times 90  $\mu$ l in Tray 7
- Wash Nano Dispenser 3 times

The right sidebar contains the following configuration options:

- Select plate:** A grid of 9 options: 1 Intelliplate, 2 Intelliplate, 3 Intelliplate, 4 Hampton Screen, 5 Hampton Screen, 6 Protein Chill Block, 7 Wash Station, 8 Reservoir, 9 Reservoir.
- Select location:** A dropdown menu showing 'Location A,1'.
- Select ???:** A diagram of a plate with a purple circle in the center.
- Select volume:** A text input field showing 'Volume ( $\mu$ l) 50'.
- Select liquid class based on sample viscosity:** A dropdown menu showing 'Liquid Class Protein 10% Glycerol'.
- Distance Above Bottom (mm):** A text input field showing '2'.

Annotations with arrows point to the following elements:

- Select plate:** Points to the grid of plate options.
- Select location:** Points to the 'Location A,1' dropdown.
- Select ???:** Points to the plate diagram.
- Select volume:** Points to the 'Volume ( $\mu$ l) 50' input field.
- Select liquid class based on sample viscosity:** Points to the 'Liquid Class Protein 10% Glycerol' dropdown.
- Needle should be above plate bottom:** Points to the 'Distance Above Bottom (mm) 2' input field.

## Screen 5: Dispense Protein

Select plate, well location range, and well. Set the volume. Set the liquid class based on sample viscosity. Recommended speed: 20% – 50%.

**Phoenix 1.9 - New Protocol**

File Configuration

Open Protocol Save Run Protocol Stop Protocol Info Undo Redo Delete Connect

**Protocol Start**

Loop 3 loops

- Aspirate 80  $\mu$ l from Tray 5
- Dispense 60  $\mu$ l in Tray 2 in location A,1
- Nano Aspirate 50  $\mu$ l from Tray 6
- Nano Dispense 0.5  $\mu$ l drop in Tray 2
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 30 times 90  $\mu$ l in Tray 7
- Wash Nano Dispenser 3 times

**Plate Selection:**

1 Intelliplate	2 Intelliplate	3 Intelliplate
4 Hampton Screen	5 Hampton Screen	6 Protein Chill Block
7 Wash Station	8 Reservoir	9 Reservoir

Location Range: A1 to H12

Fill All Plate

**Well Selection:**

Drop Volume ( $\mu$ l) 0.5

Liquid Class Protein 10% Glycerol

Percent Speed 50

Delay Length (mm) 0

Use Custom Delay

Use Linear Pattern

**Callouts:**

- Select plate
- Select wells
- Select well
- Set volume
- Set liquid class based on sample viscosity
- Recommended speed: 20-50%

## Screen 6: Prime Dispense

Select plate and well. Set the volume to same volume to be dispensed in protein well.  
Recommended dwell time: 2 sec.

**Select plate**

**Select well**

Set volume to same as to be dispensed in protein well

Rec. dwell time: 2 sec

## Screen 7: Dispense Screen Into Protein Well

Select plate and well. Set the volume. Needles should be set to near bottom of plate. Recommended dwell time: 2 sec.

The screenshot displays the Phoenix 1.9 software interface for configuring a dispensing protocol. The main window is titled "Phoenix 1.9 - New Protocol". The interface is divided into several sections:

- Protocol Start:** A list of steps for the protocol, including "Loop 3 loops", "Aspirate 80  $\mu$ l from Tray 5", "Dispense 60  $\mu$ l in Tray 2 in location A,1", "Nano Aspirate 50  $\mu$ l from Tray 6", "Nano Dispense 0.5  $\mu$ l drop in Tray 2", "Dispense 0.5  $\mu$ l in Tray 2 in location A,1", "Empty in Tray 2 in location A,1", "Replace plate in tray 2", "Replace plate in tray 5", "Wash 30 times 90  $\mu$ l in Tray 7", and "Wash Nano Dispenser 3 times".
- Plate Selection:** A grid of 9 plates is shown, including "Intelliplate", "Hampton Screen", "Protein Chill Block", "Wash Station", and "Reservoir". A callout "Select plate" points to this grid.
- Well Selection:** A diagram of a 96-well plate is shown with a callout "Select well" pointing to it.
- Dispensing Controls:**
  - Volume ( $\mu$ l):** Set to 0.5. A callout "Set volume" points to this field.
  - Distance Above Bottom (mm):** Set to 0.1. A callout "Needles should be set near plate bottom" points to this field.
  - Tip Touch:** A dropdown menu with "ALL" and "NONE" options.
  - Liquid Class:** Set to "Screen DSP-2". A callout "Rec. dwell time: 2 sec" points to this field.

## Screen 8: Final Screen Dispense

Select plate and well. Empty the remaining screen into a reservoir.

Phoenix 1.9 - New Protocol

File Configuration

Open Protocol Save Run Protocol Stop Protocol Info Undo Redo Delete Connect

Protocol Start

Loop: 3 loops

- Aspirate 80 µl from Tray 5
- Dispense 60 µl in Tray 2 in location A,1
- Nano Aspirate 50 µl from Tray 6
- Nano Dispense 0.5 µl drop in Tray 2
- Dispense 0.5 µl in Tray 2 in location A,1
- Dispense 0.5 µl in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 30 times 90 µl in Tray 7
- Wash Nano Dispenser 3 times

1 Intelliplate 2 Intelliplate 3 Intelliplate

4 Hampton Screen 5 Hampton Screen 6 Protein Chill Block

7 Wash Station 8 Reservoir 9 Reservoir

1

A

Volume (µl) 0 Empty

Distance Above Bottom (mm) 1

Tip Touch

ALL NONE

Liquid Class Screen DSP-2

Select plate

Select well

Empty remaining screen into reservoir

start Phoenix 1.9 - New Pr... Document1 - Microsof... 4:19 PM



## Screen 9: Exchange Protein Plate

Replace the protein plate.

Protocol Start

Loop: 3 loops

- Aspirate 80 µl from Tray 5
- Dispense 60 µl in Tray 2 in location A,1
- Nano Aspirate 50 µl from Tray 6
- Nano Dispense 0.5 µl drop in Tray 2
- Dispense 0.5 µl in Tray 2 in location A,1
- Dispense 0.5 µl in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 30 times 90 µl in Tray 7
- Wash Nano Dispenser 3 times

Replace protein plate

Remove plate

Replace plate with selected plate below:

Name	Maker	Type
Viel 0.6 ml		96 Wells
96 Flat Bottom	Greiner	96 Wells
384	Greiner	384 Wells
96 Lid - offset		96 Wells
LLNL Screen Block	Nunc	96 Wells
djwPCRholder	djw	96 Wells
96 Block/Clear	Greiner	96 Wells
96 Block 1/2 Height	Greiner	96 Wells
384 - Polystyrene - Sq.	Greiner	384 Wells
Intelliplate96	Jaime	96 Wells
Emerald Screen		96 Wells
1536 - Clear - Square	Greiner	1536 Wells
Wash Station	ARI	96 Wells



## Screen 10: Exchange Screen Plate

Replace the screen plate.

The screenshot shows the Phoenix 1.9 software interface. The top menu bar includes File, Configuration, and a toolbar with icons for Open Protocol, Save, Run Protocol, Stop, Protocol Info, Undo, Redo, Delete, and Connect. The left sidebar contains icons for Loop, Pause, Aspirate, Dispense, Mix, Exchange, NanoAspirate, NanoDispense, Wash, and NanoWash. The main area displays a protocol list with the following steps:

- Loop: 3 loops
- Aspirate 80  $\mu$ l from Tray 5
- Dispense 60  $\mu$ l in Tray 2 in location A,1
- Nano Aspirate 50  $\mu$ l from Tray 6
- Nano Dispense 0.5  $\mu$ l drop in Tray 2
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 30 times 90  $\mu$ l in Tray 7
- Wash Nano Dispenser 3 times

A callout box labeled "Replace screen plate" points to the "Replace plate in tray 5" step in the protocol list and the "Intelliplate" option in the plate selection table. The plate selection table is as follows:

Name	Maker	Type
Viel 0.6 ml		96 Wells
96 Flat Bottom	Greiner	96 Wells
384	Greiner	384 Wells
96 Lid - offset		96 Wells
LLNL Screen Block	Nunc	96 Wells
djwPCRholder	djw	96 Wells
96 Block/Clear	Greiner	96 Wells
96 Block 1/2 Height	Greiner	96 Wells
384 - Polystyrene - Sq.	Greiner	384 Wells
Intelliplate96	Jaime	96 Wells
Emerald Screen		96 Wells
1536 - Clear - Square	Greiner	1536 Wells
Wash Station	ARI	96 Wells

## Screen 11: Wash 96 Syringe Head

You can use the faster plunger speed. The Empty Time should be greater than the Fill Time.

Phoenix 1.9 - New Protocol

File Configuration

Open Protocol Save Run Protocol Stop Protocol Info Undo Redo Delete Connect

Protocol Start

Loop 3 loops

- Aspirate 80 µl from Tray 5
- Dispense 60 µl in Tray 2 in location A,1
- Nano Aspirate 50 µl from Tray 6
- Nano Dispense 0.5 µl drop in Tray 2
- Dispense 0.5 µl in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 3 times 90 µl in Tray 7
- Wash Nano Dispenser 3 times

Can use faster plunger speed

Empty time should be greater than fill time

1 Intelliplate 2 Intelliplate 3 Intelliplate

4 Hampton Screen 5 Hampton Screen 6 Protein Chill Block

7 Wash Station 8 Reservoir 9 Reservoir

Number of Cycles 3

Volume (µl) 90

Distance Above Bottom (mm) 1

Liquid Class Water(asp)

Fill Time (s) 10

Empty Time (s) 15

start Phoenix 1.9 - New Pr... Document1 - Microsof...

4:20 PM

## Screen 12: Nano Wash

The screenshot displays the Phoenix 1.9 software interface for setting up a Nano Wash protocol. The window title is "Phoenix 1.9 - New Protocol". The interface includes a menu bar with "File" and "Configuration", and a toolbar with buttons for "Open Protocol", "Save", "Run Protocol", "Stop", "Protocol Info", "Undo", "Redo", "Delete", and "Connect".

On the left side, there is a vertical toolbar with icons for "Loop", "Pause", "Aspirate", "Dispense", "Mix", "Exchange", "NanoAspirate", "NanoDispense", "Wash", and "NanoWash".

The main area is titled "Protocol Start" and contains a list of steps for a "Loop: 3 loops":

- Aspirate 80  $\mu$ l from Tray 5
- Dispense 60  $\mu$ l in Tray 2 in location A,1
- Nano Aspirate 50  $\mu$ l from Tray 6
- Nano Dispense 0.5  $\mu$ l drop in Tray 2
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Dispense 0.5  $\mu$ l in Tray 2 in location A,1
- Empty in Tray 2 in location A,1
- Replace plate in tray 2
- Replace plate in tray 5
- Wash 3 times 90  $\mu$ l in Tray 7
- Wash Nano Dispenser 3 times

On the right side, there is a grid of component selection buttons:

1 Intelliplate	2 Intelliplate	3 Intelliplate
4 Hampton Screen	5 Hampton Screen	6 Protein Chill Block
7 Wash Station	8 Reservoir	9 Reservoir

Below the grid, there are input fields for "Number of Cycles" (set to 3) and "Volume ( $\mu$ l)" (set to 100).

The Windows taskbar at the bottom shows the "start" button, open applications including "Phoenix 1.9 - New Pr...", and the system clock showing "4:20 PM".

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