

Vapor Diffusion Screening

Douglas Instruments

Step-by-Step Instructions

Hardware Preparation

1. Place CrystalClear™ strips with plastic frame or other VD plate on the left side of the table of the Plate Loader.
2. Place the Protein load plate to the right of the VD plate.
3. Place the black plastic rinse block to the right of the Protein load plate as in Fig 1. Place a glass vial in each well of the rinse block. Fill the glass vials with water as follows:
Vials 1, 2 and 3: 95% full. Vial 4: empty. Vial 5 may contain pure silicone oil if you wish to select the option to dispense oil.

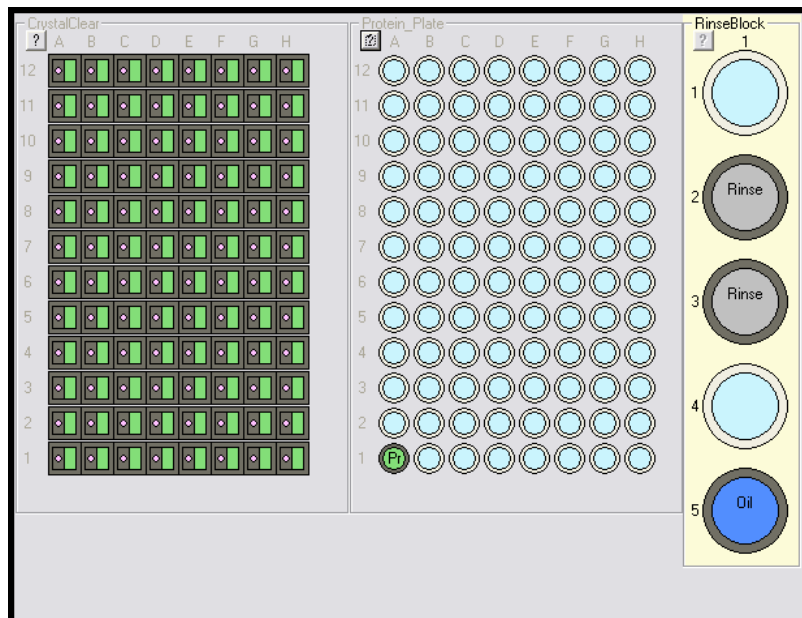
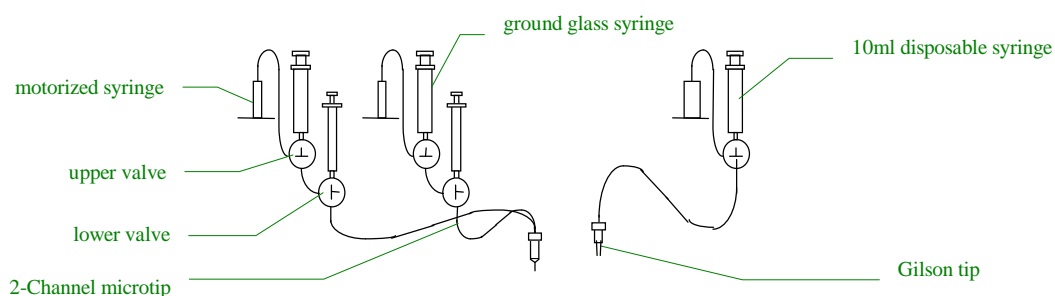


Fig 1.


4. Connect a 2-channel Microtip to the first two channels (red and green). Place the tip in the 2-channel "collet" and mount on the left arm (Z) of the Plate Loader. Connect a blue Gilson Disposable Tip to the sixth channel (black), and mount on the right arm (V) of the Plate Loader. Flush the Gilson tip with air to empty it.
5. Turn the lower valves of channels 1 and 2 to the flush position (\downarrow). Place small glass vials under the microtip to collect waste. Use the disposable syringes to fill both bores of the microtip with pure degassed water. Now turn the lower valves to the dispense position (\uparrow).



Software Preparation

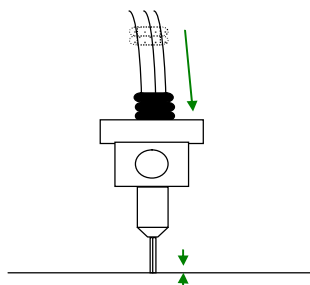
6. Switch on the computer and the MCC control unit. Launch the HTML Screens application by clicking on the "Screens" shortcut.



7. Navigate to Vapor Diffusion and then click on "Single protein up to 3 drops" or the image.
8. The program Wasprun will load. If desired, change the location of the block on the Destination Plate. You can also change the drop volume, concentration and dispensing conditions.
 - a. If using < 3 drop plate Protein proportions for additional drop positions will be ignored.
 - b. Set Protein Proportion to 0.00 for drops that should not be dispensed on multi-well plates
9. When your experiment is ready, Select "Dispense".
10. You will now move to a separate program walled Wasp. To the left of the start button is a button with an icon
 
 showing the dispensing chassis. If you wish to debubble the system or install a new microtip, click on this button, which will in turn load the program Front Panel, which is used for controlling the robotics.

Debubbling and Setting the Height of the Microtip

11. At the beginning of each day the system will require debubbling. Place a small bottle or vial under the Microtip in the Z-arm, select **Syringes | Debubble**, and follow instructions. Any air bubbles that were present at the top of the motorized syringes should have passed out into the connecting tubing.
12. Now follow instructions and remove the air bubbles from the connecting tubing as follows:
 - Remove the PTFE tubing from the needles of debubbled motorized syringes.
 - Expel water and air bubbles from the tubing using the ground glass syringe.
 - Reconnect the tubing carefully, ensuring no air bubbles re-enter.
13. Occasionally there may be bubbles between the upper and lower valves. Turn the top valves to the **flush position** (⊥) and flush the bubbles out through the microtip with the ground glass syringes.
14. If you are installing a new 2-channel Microtip, remove the destination plate, select **Plate Loader | Install Tip**. The arm(s) will move to its lowest position. Follow the instructions on the screen:
 - Move one o-ring towards the tip and the other two away from the tip
 - Adjust the height of the Microtip until it is just touching the table by pushing through the lower o-ring
 - Mark the height by moving the top two o-rings down to the top of the collet.



- Do not continue to the 'testing' phase of this routine.

Running the Experiment

15. Press **Alt/Tab** to return to Wasp. Click on the large **Start** button.
16. The system will now check the positions of the motorized syringes. If necessary, you will be instructed to turn valves and the syringes will be moved in order to carry out the experiment.
17. You will now have the opportunity to adjust the tip position. Use the arrow keys and the 'Raise' / 'Lower' button to center the tip in the well for optimum drop location. Lower the tip until a slight movement can be detected between the collet and arm when in the lowered position. Save the configuration while in the 'Lowered' position.
18. A page of configuration information and instructions will now appear. Note that all air bubbles must be removed from all tubes of the first two channels including the microtip. Remember to remove any droplet on the end of the microtip to prevent this from being sucked in later on.
19. Press any key to start the experiment, which will now be carried out automatically. When the program pauses, put tape onto each strip as instructed.