The Flow Cytometry Facility

University of Glasgow

STANDARD OPERATING PROCEDURE FOR THE LUMINEX

All users must follow the SOP & fill in the logbook.
If you are unsure about anything please do not hesitate to ask for help.

Before running samples you should complete the Luminex safety form, have it signed off by your PI, and submit it to the Facility manager for approval.

If you are running potentially biohazardous samples jump to the end of the SOP for details of how to alter the working procedure.

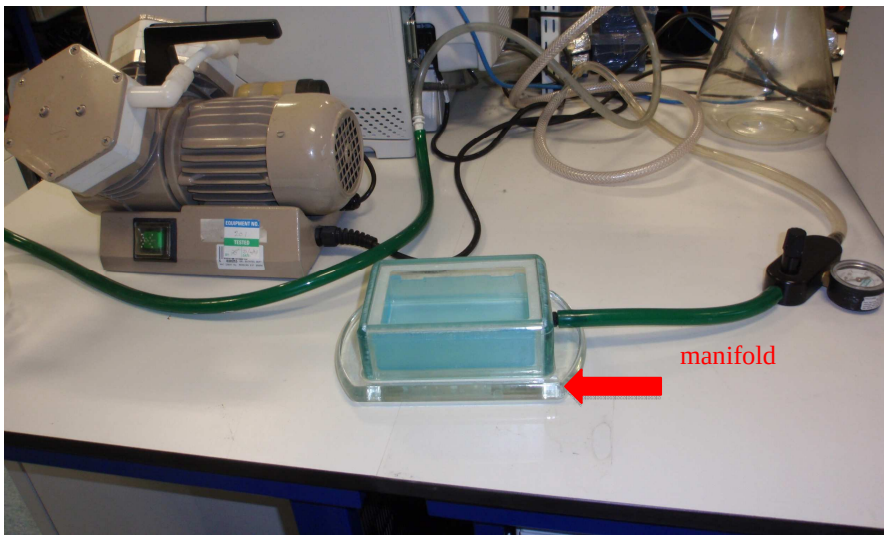
Sample Prep Guidelines

Serum, plasma & tissue culture supernatants are suitable samples for analysis.
Serum is preferred for cytokine assays as it generally yields better cytokine levels.
If plasma is used then heparin should be avoided as this interferes with the Ab:Ag interaction. EDTA and citrate are better.
When whole blood is collected it should be kept at 4°C until processed to avoid haemolysis (rupturing of the red blood cells). Don't agitate whole blood as this will increase the rate of haemolysis.
For serum collection whole blood should be allowed to clot for a minimum of 30 minutes (maximum of 2 hours) at room temperature before centrifugation to separate the serum from the red blood cells (10 mins at 1000g).
After centrifugation aliquot the serum into 50-100ul aliquots and freeze these at -70°C.
Avoid multiple freeze thaw cycles as cytokines degrade significantly after 2-3 freeze/thaws.

Clarify all samples by ultracentrifugation 1000 x g for 10 minutes before analysis to prevent aggregates clogging the filter plates.

Setup Procedure

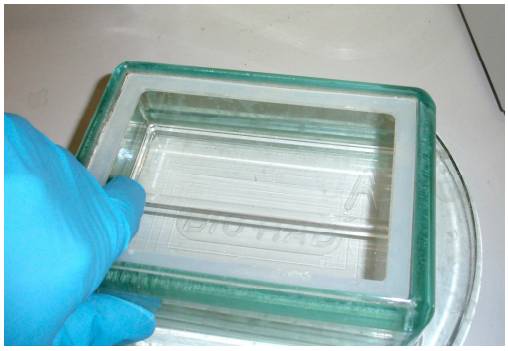
1. Check that there is enough sheath fluid and that the waste line is feeding into the designated sink.
2. The machine takes up a lot of space, so working room on the bench is limited. Keep the bench clean and tidy. Dispose of pipette tips in the sweetie jar provided. If you remove the vacuum manifold, or plate shaker to another bench be sure to return them. Empty the manifold when required.



It is essential to set the manifold vacuum pressure correctly before washing your plates or you may burst the membrane on the bottom of the plate

Remove the blue trough from inside the manifold and switch on the vacuum pump.

Put you finger over the tubing hole that supplies the vacuum.



Using the black knob adjust the pressure until the gauge reads 3.0. You may now wash your plates.

When shaking your plates use the designated shaker on the side bench near the Luminex. Shake at a speed of 500 ± 50 rpm unless your protocol says otherwise.

Failure to agitate the plate sufficiently can cause aggregation of your samples.

3. Take the Calibration kit provided, from the fridge under the HTF, and leave it on the bench to equilibrate for 30 minutes.

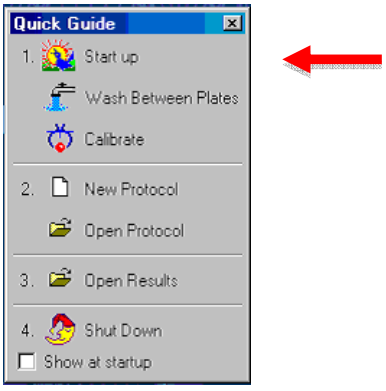
4.Turn on the power supply 1) vacuum pump, 2) HTF, 3) array reader and 4) microplate platform at the power switch on the trunking for each, working from left to right. Turn on the P.C. and monitor last

N.B do not try to turn the machine switches off at the back as you are likely to move the reader in relation to the platform which may misalign the lasers, and also lead to bending of the needle !

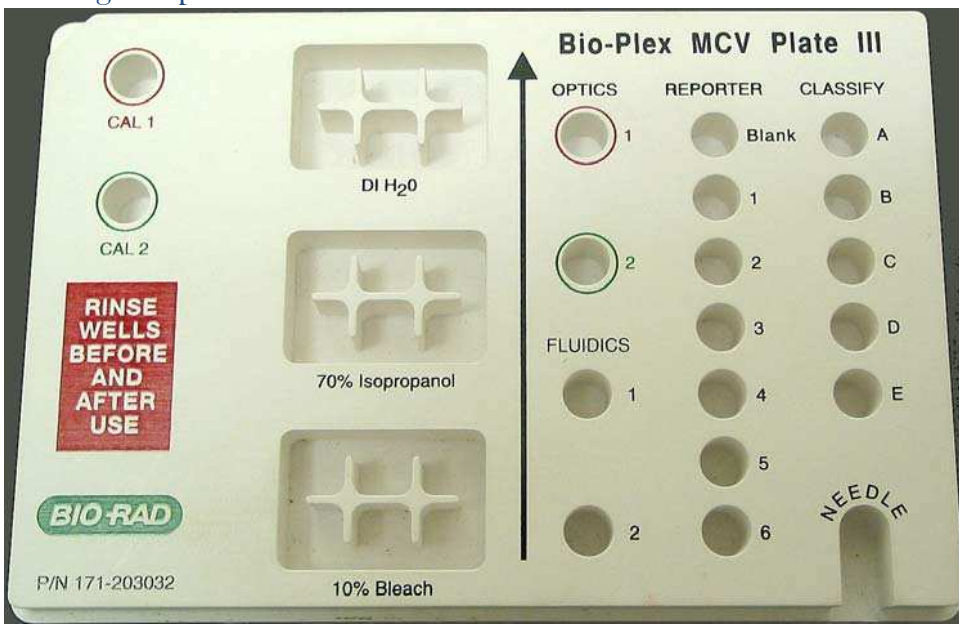
5.The password to log on to the desktop is BDIS.

6.Open Bioplex Manager 4.1 by clicking on the icon on the desktop:

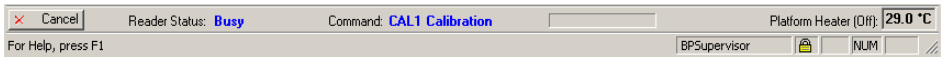
7.On the *Quick Guide* Menu click START UP and follow the on screen prompts to prepare the MCV plate (start up takes approx 10 minutes):



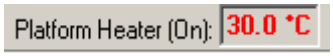
8.Use the MCV Plate provided on top of the machine to validate, calibrate and wash the machine. On arrival, give it a clean by filling all the wells with distilled water, then emptying this into the sink, and blotting the plate on blue roll.



9.The status bar shows the current state of the instrument

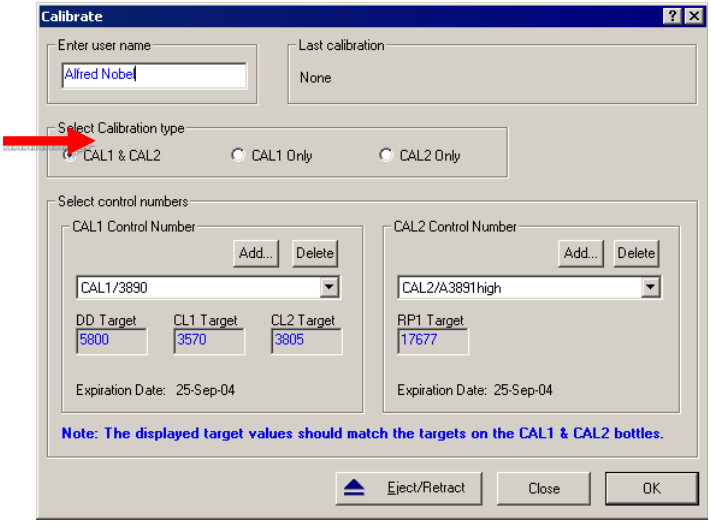


The optics automatically begins to warm up when you first turn on the array reader.

Whilst the platform heater is warming up or cooling down the temperature is shown in red in the status bar 

10.The machine needs to be calibrated every time you use it. If you run several plates on one day and the platform temperature changes significantly the software will prompt you to recalibrate. To calibrate select the Calibrate icon from the Quick Guide menu: 

In the pop up calibration window *always* select the CAL1& CAL2 option.



Be sure to check that the control number in the calibration window is the same as that on the bottle. Enter the correct target values for each tube when prompted.
11. Remember to vortex the calibration beads for 30 seconds before loading 5 drops into the appropriate well of the MCV plate. Follow the on screen prompts to calibrate the machine.

NOTE - Please inform the Facilities Manager when the beads are running low so more can be ordered *before* they run out!

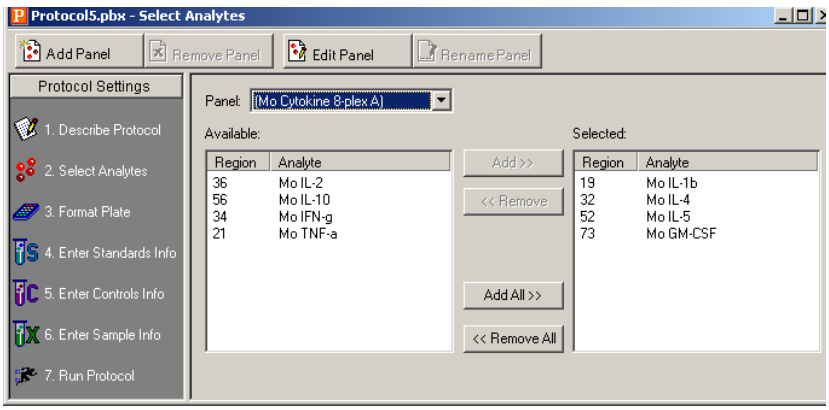
Return the box of Calibration beads to the fridge when you have finished. Once the machine has been calibrated successfully you are ready to read your plates.

Reading Your Plates

Check you kit instructions to see whether the platform needs to be at a specific temperature throughout the assay. Click on the platform heater icon on the main toolbar to bring up the dialog box to set the desired temperature.

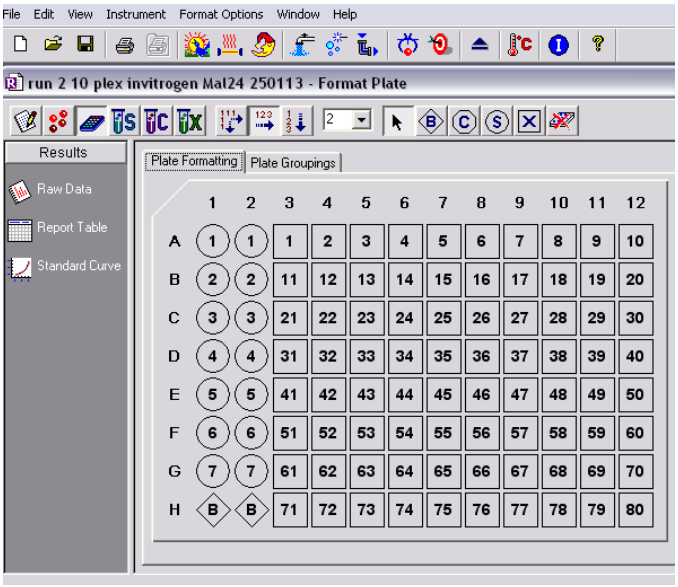
To set up your protocol in the Quick Guide menu either click the New Protocol icon or Open Protocol if you already have a saved protocol.
Full details of how to run your plate can be found in the software manual. The software is user friendly & will guide you through the following steps:

1) Select your Analytes Plexes are saved by other users and can be used if you have the same kit. Click on the *Panel* pull down menu to see saved plexes. Check the bead region is the same.

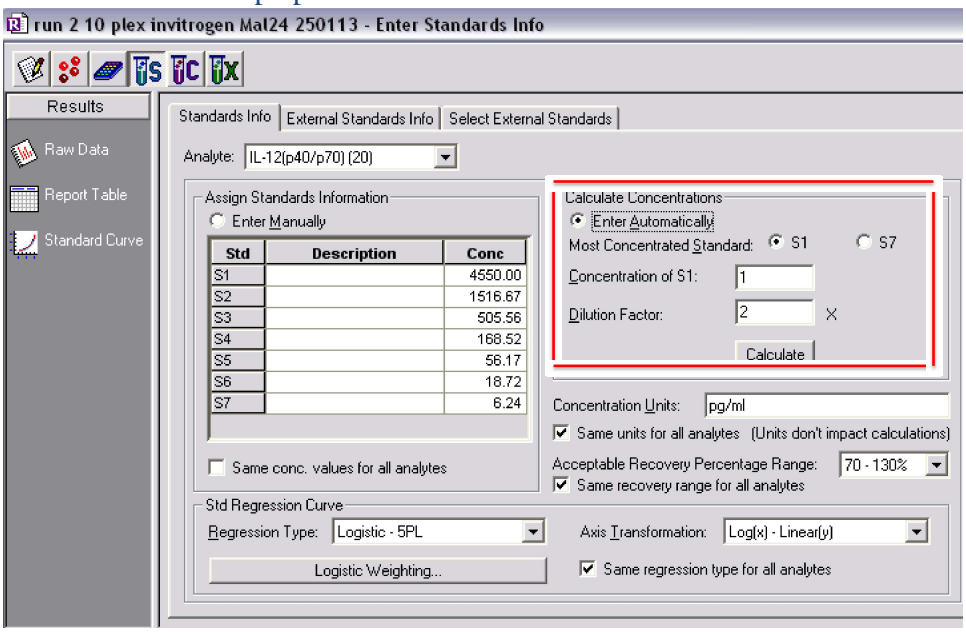


2) Format the plate:  number of replicates, which wells are blank, standard, controls or test samples.

 Only the wells which have been formatted will be read so don't miss anything out!



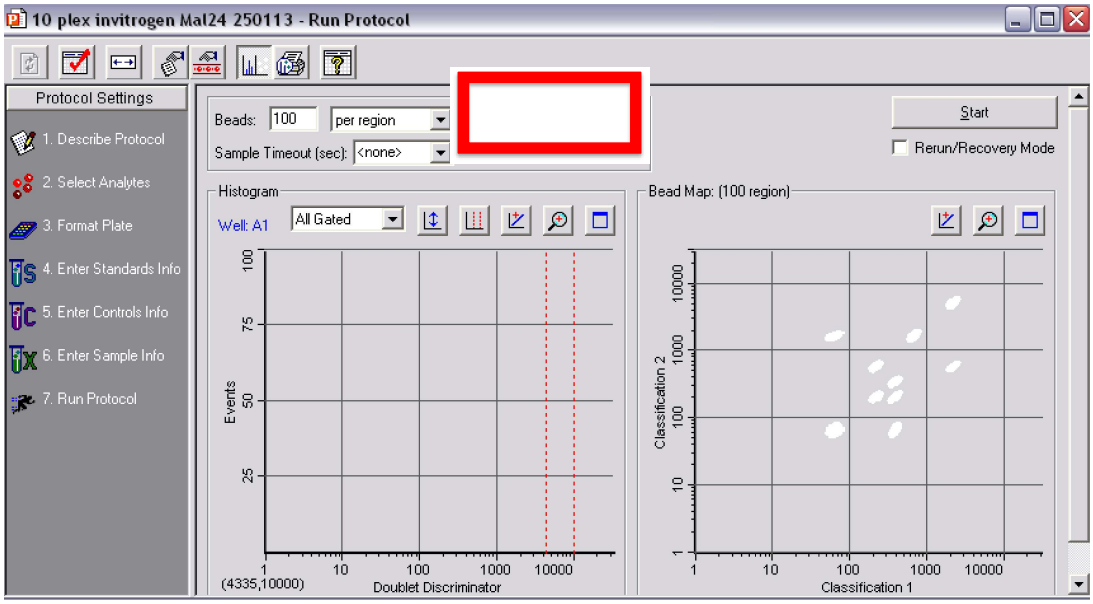
3) Enter standards information from your kit: 
Check the "enter automatically" box, select S1 or S7 – whichever is the most concentrated standard. Look on the data sheet supplied with your kit and enter concentration of S1 e.g. 4550pg/ml. Enter the dilution factor and the machine calculates the value of all the standard and populates the table S1-S7



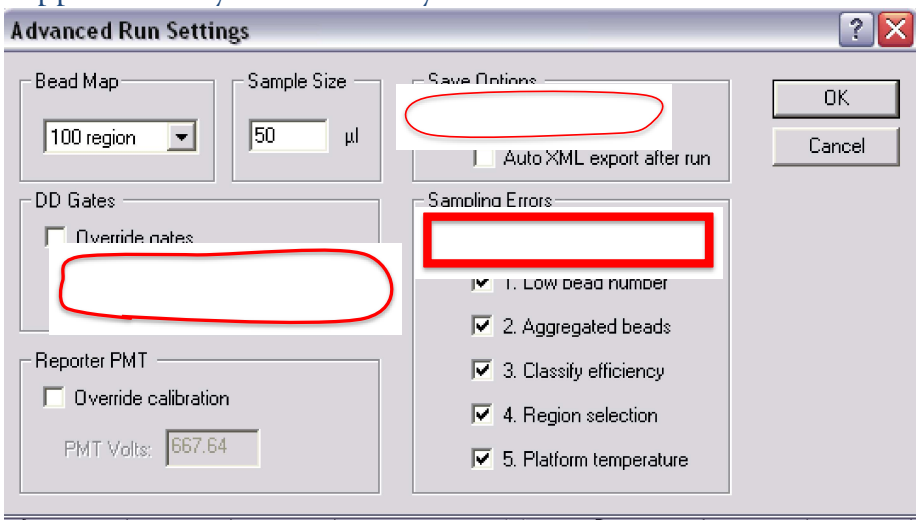
4) OPTIONAL :Enter Controls information from your kit:  These are controls spiked with known concentrations of your analytes to double check the accuracy of the assay.

5) Enter Sample information if needed: e.g you may need to enter dilution factor details.

6) Run Protocol Window: This allows you to display your raw data or raw data and histogram/Bead map. Select the number of beads to be read. The default is 100 beads *per region* for most Cytokine assays and 25 beads *per region* for Phosphoprotein assays. Check your kit.



Click on Advanced Settings:
Set your doublet discrimination gates according to the values supplied with your kit. Always tick the *autosave* after run box.



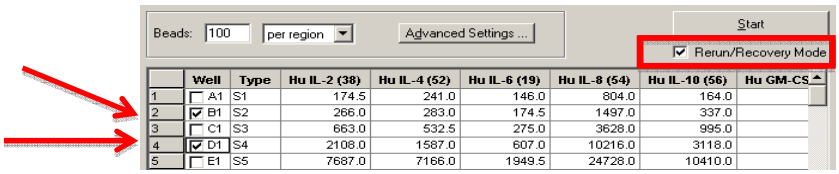
Check the Pause Run if error occurs box.The machine will automatically stop reading the plate in the event of any of the five problems listed.

When you click *Start* the software asks you to specify where to save the data, and everything is automatically saved there.

If something goes wrong - Rurun /Recovery mode

After plate reading has stopped part way through due to an error, or the user choosing to stop the plate -the software allows you to switch on the Rerun/Recovery Mode. This enables you to rerun the same plate, or just part of it:

Tick the boxes of the samples you wish to rerun



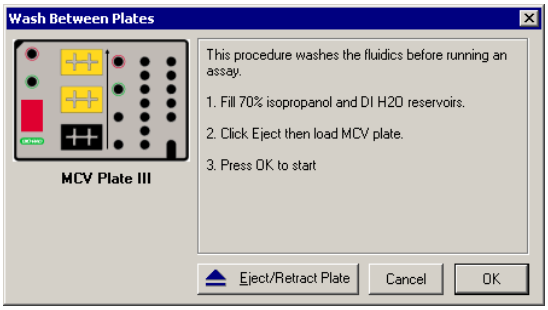
When you have selected the samples simply click on start and the machine will resume.

Wash Between Plates

This is mandatory for all users. After reading any plate you must **always** run *Wash between plates* to ensure the fluidics lines are properly flushed through, otherwise the machine will block.
Even if you will run subsequent plates, or there is another user booked after you must still always run *Wash Between Plates*

1) On Quick Guide menu, click on the Wash between plates icon:

2) Use the MCV plate and follow the on screen prompts.



If no one is booked to use the machine within 2 hours – or you are the last booked user of the day follow the shut down procedure.

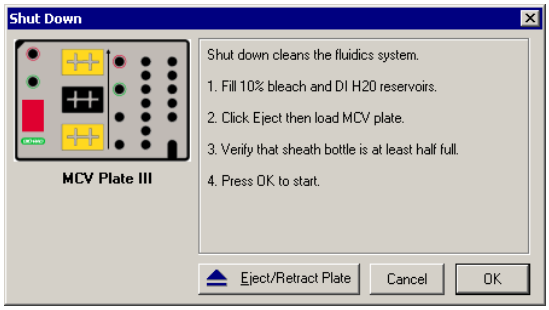
The last booked user is completely responsible for instrument shutdown. If you need help please ask.

N.B even if your booking is not the last one of the day, it may become so if other users cancel. Check the online diary before leaving, to ensure have not become the last user of the day.

Instrument Shut Down

1) On Quick Guide menu, click on 'Shut Down'

2) Follow instructions using MCV plate- takes 10 minutes.



3) Rinse the MCV plate with distilled water blot, and leave to dry on blue roll on top of the reader.

4) Close BioPlex Manager software and shut down the PC.

5) Switch off the power to the PC, Array reader, platform, HTF and Vacuum pump at the plugs on the electrical trunking.

6) Remove all your rubbish and dispose of it according to the Facility safety rules. Clean the bench with 70% EtOH. Empty & rinse out the blue manifold trough.

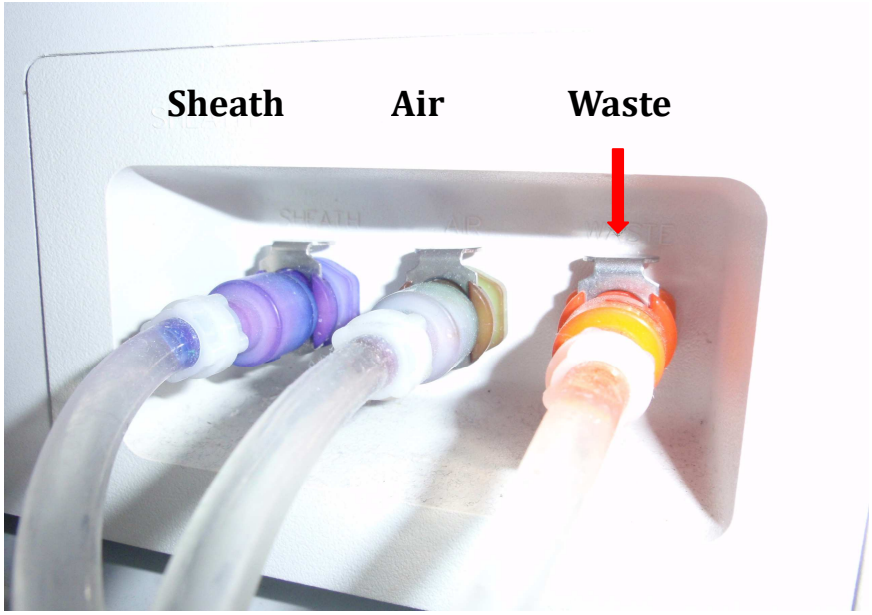
The machine will be validated once a month, unless there are any issues with the machine.

If you have any problems when running the machine, please report this to the Facilities Manager.

Fill in the log to indicate you have cleaned and shutdown the machine and tidied up after yourself.

Additional steps required if running potentially biohazardous samples

Wear gloves and a lab coat.
If you spill any supernatant on the bench immediately clean with 70% ethanol or Virkon.
Remove the waste line (which feeds into the sink) from the side of the array reader.



Put bleach or virkon (10% of total volume) in the designated waste bottle and connect this to the waste port valve on the side of the machine. Bleach tablets or virkon powder are available free from the FCF.

During preparation of your sample plate, be sure to put either 10% bleach or virkon in the blue trough inside the vacuum manifold before each plate-washing step.

Keep an eye on the waste bottle. Do not allow it to overflow!

When you have finished running your plate clean and decontaminate the machine as follows:

Perform a wash between plates

1) On the *Instrument menu* Click > Additional Function > Sanitise – bleach is run through the fluidics during this extended clean.
2) Now initiate the Instrument Shut down

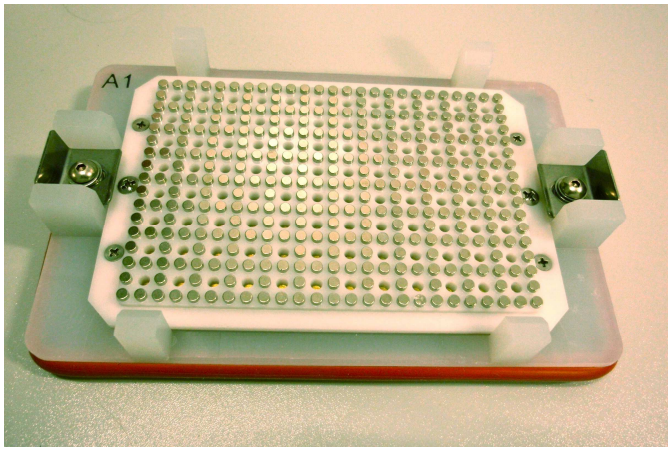
Remove all your rubbish.
Dispose of all waste appropriately in accordance with the Facility safety rules i.e.

Take the waste bottle and empty the decontaminated waste down the designated sink in the Flow Cytometry Facility.

All pipette tips should be placed in the metal tin for autoclaving.
Gloves and plates should be placed in the autoclave waste bin.
Wipe down the bench with Virkon or 70% ethanol.

HAND HELD MAGNETIC SEPARATION BLOCK

Some companies are now offer kits on Polystyrene beads, and/or on Magnetic beads. Our software is able to run both kinds of assays.
We now have a hand held magnetic separation block, which replaces the vacuum pump/manifold trough for washing plates if you have a magnetic kit. This is kept in the Flow lab & available on request.



Procedure

Basically this is like the washing step in a conventional ELISA assay.

1. Put assay plate containing the magnetic beads on the hand held separator block. Critical step - secure the plate using the adjustable clip.

2. Allow the plate to sit on the block for 60 seconds to allow the beads to be bound by the magnetic separator.

3. Next firmly hold the block, invert it, to dump the liquid into an appropriate waste receptacle. If your samples are potentially bio hazardous dump the liquid into a trough containing Virkon, otherwise you may use the designated sink.

4. Gently tap the plate three to four times on a wad of absorbent tissue to remove any residual liquid.

5. Now open the adjustable clips and remove the plate away from the block.

6. Use a multi channel pipette to load 200ul of wash buffer to each well.

7. Use a plate sealer to seal the plate and place on a plate shaker. Shake the plate on setting 6 (500-600rpm) for 30 seconds.

8. Remove the plate sealer and secure the plate to the magnetic separation block

as in step 1. Repeat step 2- 5 inclusive to discard the wash buffer. Repeat as necessary for the number of wash cycles required by your protocol.