BioPlex Training, ProdMed Lab

Luminex xMAP technology allows for the simultaneous analysis of multiple analytes in samples- including cytokines, total proteins, and phosphoproteins. The principle of the assay is similar to a capture sandwich immunoassay- an antibody to the target protein is covalently coupled to internally dyed beads. When the beads are incubated with sample, the protein of interest is captured and then a biotinylated antibody for a different epitope is added to the reaction, which is then detected with streptavidin-phycoerythrin. Using a dual-laser flow-based reader, beads are analyzed for the detection antibody and the internal bead signature, identifying both the protein analyzed and the level bound to the bead.

The ProdMed Lab has a Luminex 200 with BioPlex software. The general policies for this are outlined in this document and must be followed. This is an expensive piece of equipment with expensive reagents. Failure to follow the rules can result in loss of priveleges to use the instrument.

Who can use the BioPlex?

- Researchers and students associated with or collaborating with ProdMed employees at the discretion of Janczak.
- Regardless of above status, you **MUST** be trained to use the equipment.

Reagents for the BioPlex

Reagents consist of kits for specific analytes, buffers, cell lysis buffers, calibration kits, and filter plates.

- Individual kits are your responsibility to buy. Be aware of overlap of your kit needs with others in the lab- there may be price breaks for buying bulk packs that you can split.
- Buffers are bought in bulk to get a price break. These are ordered by Janczak and are found in the refrigerator with glass doors.
- Cell Lysis Buffer is bought in bulk to get a price break. This is ordered by Janczak and are found in the refrigerator with glass doors.
- Calibration Kits/Sheath Fluid are shared by all users and bought by Janczak. The calibration kit can be found in the refrigerator with glass doors.
- Sheath fluid is in the lab near the BioPlex instrument.
- Filter Plates may be company specific. For BioRad kits, there are plates in the buffer kits and additional plates are ordered from Millipore (cat # MSBVN1250). They will be ordered by Janczak and are found in the cabinet near the BioPlex.

For all common reagents – help everyone out by letting Janczak know when things are running low or if you are going to need a lot of something in the future. Don't expect instant replacements.

Instrument Startup/Use/Shutdown

General

1) Sign up for use of instrument in the ProdMed Lab Outlook calendar – estimated time is 1 hour for startup/shutdown procedures and 30 minutes per plate to read.
2) In addition, fill in instrument log next to the BioPlex
3) Each user has there own folder for protocols and result files- these should be stored in MyDocuments\BioPlex Users\last name. It is your responsibility to back up your data and configuration files on a different storage device as files will be removed from the BioPlex PC without prior notice.
4) There is an area set up with the plate shaker to do assays across from the BioPlex reader. Vortexers and plate shakers must not be placed on the same counter top as the BioPlex as the vibrations mess up the BioPlex laser settings. Please keep the work space clean. Be sure to cover your plate during all incubations to prevent photobleaching. The magnetic plate washer is stored on the shelf above the BioPlex.

Start Up Routine – (40 minutes)

1) Turn on instrument using the two power switches at the back of the instrument.
2) Turn on computer if not already on, and log in.
3) Open BioPlex Manager 4.1
4) On Quick Guide menu, click on 'Start Up'
5) Follow instructions using MCV plate which is stored under the left front panel of the BioPlex.
6) Laser takes 30 minutes to warm up before you can proceed. To check time remaining select 'Instrument' on top tool bar, then 'Instrument Info' and look in Device Status. Can also look at status bar at bottom of the screen.
7) Once the laser is ready, on Quick Guide menu, click on 'Calibrate'
8) Follow instructions using Calibration Kit (in refrigerator) and MCV plate.
    a. Enter your name
    b. Match calibration values to the control number on each tube. New kits will be added by Janczak to the list.
    c. Always do CAL 1 – this calibrates the bead classification.
    d. CAL 2 – calibrates reporter fluorescence. For phosphoprotein assays select PMT HIGH; for cytokine generally select PMT LOW for CAL 2.
    e. Be sure to vortex each calibration vial for 30s before use.
    f. Calibration is OK for changes of +/- 2^{o}C or until shut down. The software will tell you if calibration needs to be redone.
    g. If you run a plate at one PMT setting and your next is at the other PMT setting, be sure to redo the CAL 2 calibration.

Reading plates

1) Set up your protocol by selecting 'New Protocol' in Quick Guide or 'Open Protocol' if previously used.

2) Details can be found in the BioPlex Manager 4.1 manual, but here is a quick outline:
    a. Describe/format plate: indicate number & direction of replicates, mark wells as Blank, Standard, Unknown (X), or Controls
    b. Copy and paste your unknown descriptions from a previously made column in Excel
    c. Enter standards information if doing cytokines (kit should provide concentrations of the standard curve you made) or controls if doing phosphoprotein assays.
3) Run Protocol
    a. Select bead region map (usually 100)
    b. Set number of beads (recommended is **25 for phospho** assays, **100 for cytokine** assays)
    c. In Advanced Settings, check that DD Gates match the kit instructions and any other kit specific changes. Normally want 'pause if errors' unselected, and 'autosave' selected.
    d. Click Start- give your results a unique file a name and be sure it is placed in correct directory under My Documents.
    e. Data is also saved in the protocol file- allowing reanalysis if you don't properly set up the plate. Therefore, if you use a previously set up protocol, do 'Save As' and give the protocol a new name to prevent overwriting raw data file.

    NOTE: most parts of the protocol set up can be changed after the plate is run and reanalyzed. You absolutely MUST correctly tell the number of wells to assay and pick the correct analytes however.

If reading multiple plates, or another user is planning to use the instrument in less than 2 hours…

1) On Quick Guide menu, click on 'Wash between plates'
2) Follow instructions using MCV plate.

Shut Down

If done and no other user is signed up for next 2 hours, you must shut down the instrument. THIS IS CRITICAL FOR KEEPING THE BIO-PLEX RUNNING. Most problems are a result of clogging of the fluidics resulting from a failure to flush and disinfect before idle periods.

1) On Quick Guide menu, click on 'Shut Down'
2) Follow instructions using MCV plate- takes 10 minutes.
3) Rinse MCV plate with MilliQ water and invert to dry on KimWipe.
4) Shut down BioPlex Manager software
5) Shut down instrument using power strip.

Troubleshooting

1) In protocol run advanced settings you can opt to pause run for various errors. If errors occur you will then be prompted to do “Remove Air Bubbles” or “Unclog” functions on the top bar. Follow the instructions. The assay can then be continued in “Rerun/Recovery” mode in the protocol. You select the checkboxes of wells that need to be rerun.
2) If a needle can not be unclogged by these functions, it needs to be changed out and thoroughly cleaned. This must be done by either the Janczak or another user who is authorized for Troubleshooting.
3) Monthly validations of the optics and fluidics will be done by Janczak. If you notice a lot of problems especially with bead classification, please let him know and the validation can be done as a check.