Luminex – Preparing Luminex to Run an Assay

NOTE: Before you can use this instrument, you need proper training and/or authorization from Dr. Harrison.

Required Kit Protocol Changes:

- Centrifuge serum/plasma samples at 1,400 xg for 5 min prior to running assay.
- Creating the well map: Run all samples, standards etc. in duplicate and set them up so the duplicates run vertically. Start with the blank samples followed by the standards, beginning with the lowest concentration and going to the high concentration, and then the QC (see example at end of protocol).
- Fill in ALL blanks on the well map sheet; batch should match file name for saved results.
- During final step in protocol, resuspend samples in wash buffer instead of sheath fluid.
- During the run, include alcohol flushes after every column with samples; this will help prevent clogging.

Remember to schedule the use of this instrument on the HIP laboratory calendar.

LUMINEX START-UP:

1. Turn on the instrument; two switches on the back lower right-hand side of the Luminex (Bio-Plex 200) and an additional switch on the back lower right-hand side of the Bio-Plex-HTF.
2. Power on the computer and open the program "Luminex xPONENT" from the desktop.
3. Select 'Log-in' without entering a username.
4. Gently rock the sheath fluid in the cardboard box beneath the Luminex to ensure contents are mixed. Verify fluid is not expired; expiration date should be on box.
5. After a 30 min warm up, an alert will appear within the application indicating it is ready for use.
6. Take out the Calibration and Verification beads from the refrigerator. They need at least 30 minutes to come to room temperature before use.

CLEAN PROBE:

NOTE: Before cleaning or removing the probe, watch the "Luminex® 200™ Clean and Set Probe Height" video in the LX200 folder on the desktop.

7. Remove the plastic cover on the instrument that covers the probe.
8. Remove the probe housing unit at the top of the Luminex machine; if you apply firm but gentle pressure it will 'pop' out. It will remain attached so carefully lie it on top of the instrument.
9. Unscrew the Cheminert fitting from the top end of the probe.
10. Unscrew the knob on the front of the probe arm, do *not* touch the knob on top of the probe arm.
11. Pull the probe out through the top, removing it completely.
12. Remove syringe with tubing attached (in drawer beneath the Luminex) and fill with DI water. Then, attach the hose to the narrow end of the probe and flush the probe with until a stream appears. If you have problems getting a constant stream you can try using 70% ethanol.
13. Fill the sonicator with DI water and place the narrow end of the probe in the water with the sonicator running. Hold the probe in the water until drops of water come out of the other end of the probe, count at least 30 drops. This is tricky so you will need to adjust the positioning of the probe in the sonicator until you see drops form.
14. Cleaning is complete, so replace both the probe and the probe housing unit (it will 'pop' back in with firm, gentle pressure). Be careful not to crimp the lines that carry fluid to the probe. Proceed with adjusting the probe height before replacing the plastic cover.

ADJUST THE PROBE HEIGHT:

15. Select the Maintenance tab at the top of the screen.

16. Select Probe and Heating on the left side of the screen.
17. Take the plate labeled '96-well plate for adjusting probe height' from the drawer beneath the Luminex.
18. Add "alignment discs" to well A1 according to table below. (Note: maintenance plates and typical 96-well plates will require two 5.08 mm disks, as highlighted below). Alignment tools are in the purple-top tubes in drawer beneath Luminex.

LX200 (xPONENT 3.1)	
Plate Type	Alignment disk/sphere
Standard flat-bottom Plate	two 5.08 mm disks
Round-bottom Plate	two 3.35 mm disks
Filter Plate	three 5.08 mm disks
Half-volume with flat-bottom Plate	two 5.08 mm disks
V-bottom Plate	1 sphere

19. Select "Eject" to open instrument door and insert plate. Select "Retract".
20. Confirm on the screen that the pin is placed in A1, so the probe will descend into the correct well.
21. Unscrew the knob on the front of the probe arm (*not* the one on top of the probe arm) one-third to one-half turn. Raise the probe to its highest position and then tighten the knob. On the computer, select "lower probe."
22. Loosen the knob on the front of the probe arm and lower the probe until it rests on the alignment discs. Then, tighten the knob and select "raise probe."
23. Use the red tape on the door of the machine to open the door and watch while you lower and raise the probe a few times. Verify the probe is not pressing too firmly on the plate, you will know if it causes the plate to depress when the probe makes contact.
24. Reattach plastic cover and put away the plate.

PREPARE LUMINEX:

NOTE: Before running an assay, calibration must have been completed within the past 7 days. Verification, initialization, and prime must be performed on the day the assay is being run.

25. Make sure the calibration and verification beads have been at room temp for 30 min.
26. From the Home menu, select "System Initialization."
27. Remove the white plastic auto-maintenance plate from the Luminex machine.

28. Ensure that the Luminex calibration and verification lot numbers on the screen match the lot numbers on the kits you are about to use.
    - If lot numbers are not listed, insert the CD from the kit into the computer. Click "Lot Management on the left side of the screen, click "Import Kit," and select the CD.

29. Insert plastic wells (located inside cal/ver boxes) into the maintenance plate as shown on the computer screen.
30. Vortex each vial for 15 seconds and then place 5 drops of each reagent into the designated well, be careful not to get bubbles. Verify color-coded bottles match the plate map on the screen so beads go in correct wells.
31. Fill the designated reservoirs with DI H_2O and 70% ethanol.
32. Select "Eject" to open instrument door then place the plate in the tray. Select "Retract," then "Run."
33. If either calibration and/or verification fails, consult the troubleshooting instructions on page 60 – 63 before the LX200 End-User tab of the white Luminex Learning binder (in the drawer below the computer).
34. When finished, add a tick mark to each of the cal/ver boxes (these indicate a use of the beads) and then return the cal/ver kits to the refrigerator.
35. Luminex is ready to run an assay.

PLATE WELL MAP EXAMPLE:

Batch: 02.02.2021_RECYTMAG_PRACTICE Cat: RECYTMAG-65K

Analytes: IL-4, IL-10 Lot #: 3384235

Date: 02.02.2021 Exp: 12-31-2020

	1	2	3	4	5	6	7	8	9	10	11	12
A	blk	S4	blk2	S4d	S8d	QC1d	OD-1053 S6	OD-1054- 60	OH-2139 24	OD-1053- S2d	OD-1054 S2d	OH-2140 40d
B												
C	S1	S5	S1d	S5d	S9d	QC2d	OD-1054 24	OH-2140 16	OH-2139 40	OD-1053 S6d	OD-1054 60d	OH-2139 24d
D												
E	S2	S6	S2d	S6d	QC1	OD-1053 28	OD-1054 28	OH-2140 36	OH-2139 44	OD-1054 24d	OH-2140 16d	OH-2139 40d
F												
G	S3	S7	S3d	S7d	QC2	OD-1053 S2	OD-1054 S2	OH-2140 40	OD-1053 28d	OD-1054 28d	OH-2140 36d	OH-2139 44d
H												

Sample Type: rat serum Plate #: 1

Dilution Factor: QCd: 1:3 dilution. Notes: S4 - one standard is off.

Sd: S5 diluted. $\frac{1}{2}$ dilutions