

## Luminex – Preparing xPONENT to Run an Assay

**NOTE:** Before you can use this instrument, you need proper training and/or authorization from Dr. Harrison. Remember to schedule the use of this instrument on the HIP laboratory calendar.

### CREATE A PROTOCOL:

**NOTE:** You must create a protocol if it is the first time you are running a specific kit or if you are running a kit that you have run before but you now have different analytes included in the kit. If this is not the case then start with Create a Batch.

1. Open the program “Luminex xPONENT” from the desktop.
2. Select the “Protocol” menu, then select the option “Create new Protocol”.
3. Under the Settings tab: name the Protocol after the kit name. The Version needs to be a unique number; start with 1 if it is the first time to set up a given kit. The manufacturer, bead type, DD gating, volume, timeout, and reporter gain can be found in the protocol included with the kit under ‘Equipment Settings.’
4. Set analysis type to quantitative (if your kit includes a standard curve) and include the number of standards from the kit. Be sure to leave the number of controls at 0.
5. Under the Analytes tab: click a region to move it to the right side. Name the regions for each analyte; be sure to include units and change the count to match the events according to the kit protocol (it is usually 50).
6. Under Plate Layout: Select the number of replicates from the drop-down menu and highlight a region according to the plate map by clicking and dragging with the mouse. Be sure to check the box that replicates should be read vertically. Only include blanks and standards here; unknowns and controls will be entered later when you set up the batch.
7. Save protocol when finished.

### PAIR STANDARD CURVE WITH PROTOCOL:

8. Make sure your protocol is highlighted and select “New Std/Ctl” from the bottom row of options to link a standard curve to your protocol. Do not select the tab on the left labeled ‘Std & Ctrls.’
9. Std/Ctrl name must be unique. Name the standard curve after the kit and lot # [i.e., RADPKMAG-33081]. Name must be entered for every standard (S1, S2, S3... will suffice). Lot #, expiration, and manufacturer will be the same for every standard, so they can be entered on the first row, then select the down arrow next to “Apply Values” to auto-copy for all standards.
10. Concentrations for standards are found in the kit protocol. Manually enter the concentrations from the lowest to highest for each analyte. Using the dilution factor drop-down box and ‘Apply Dilutions’ button will only work if samples are entered from high to low, so it should not be used. Once again, do not enter control info here.
11. Save the Std/Ctrl when finished.

### CREATE A BATCH:

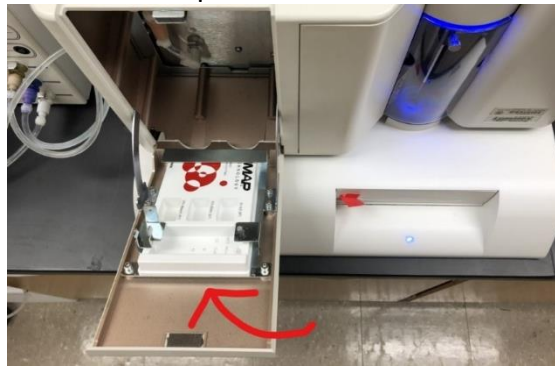
**Note:** A new batch must be made for every assay you run.

12. Select the “Batch” menu, then the option “Create a new Batch from an Existing Protocol.”
13. Select the protocol previously created for this assay.
14. Under the Stds & Ctrls tab, select next and this will auto-populate with the previously created standard curve from your protocol.
15. Under the “Plate Layout tab:” Select the number of replicates from the drop down and highlight the region of the plate that has samples by clicking and dragging across the plate image. The background and standards will auto-populate from the protocol. Enter samples and controls as unknowns.

16. Enter sample names for unknowns; double click the name under the ID section and you will be able to type in sample names. Enter QC1 and QC2 for your controls.
17. Add a dilution factor to the wells if necessary.
18. Highlight each column that has only samples (not the standards) and enter a command for an alcohol flush after these wells to prevent clogging.
19. Batch can be saved and run when the plate is ready, or run immediately.
  - If the batch is saved for later use, navigate to the 'Batch' tab, and find your saved batch in the table below. It will be listed as "pending" if it is saved but not yet run.
20. Once the batch is finished, then save the batch results. Create a folder within you PI's folder on the desktop, name the folder with the date and kit that was run ('YYYY.MM.DD\_KitName').
21. In the "Results" menu, highlight the completed batch, then select the 'export' icon to save a .csv file of the results with the same name format as the folder. This file can then be opened with Belysa software for data analysis.

#### SHUTDOWN THE INSTRUMENT:

22. Select "Shutdown" from the home screen.
23. Remove the white plastic auto-maintenance plate from the Luminex machine.



24. Enter the appropriate solutions in their respective wells as per diagram on the screen.
  - Wells with "Wash" can be filled with DI water.
  - Wells with "Drain" can be left empty.
  - Wells with "Alcohol Flush" can be filled with 70% ethanol.
  - Wells with "Sanitize" can be filled with 10% bleach solution. This needs to have been made fresh within the last 7 days, if not make up a fresh solution.
25. Insert the maintenance plate into the instrument and run the shutdown protocol.
26. When protocol is complete, plate can be ejected, rinsed, and replaced in its slot.
27. Exit the software, and turn off all three switches on the Luminex.
28. Power down the computer and monitor.