Luminex FAQ

- **When do you run Luminex assays?**
  - Luminex assays are run three times a month, with one week dedicated to human Luminex assays, one week for mouse, and one week for intracellular assays.
  - The schedule for upcoming runs, as well as offered panels and prices, can be found on the UVa Flow Cytometry Core website
  - Request forms for Luminex runs must be submitted 14 days in advance to ensure we have the reagents available.
- **How much sample should I bring?**
  - For plasma, serum, and cell lysate we generally run samples in duplicates of 25uL each. To ensure there is enough volume, we request that you provide a minimum of 60uL per sample per kit.
  - In the case of cell cultured supernatant, lavage, or any other samples with lower protein concentration due to dilution factors, we try to run 50uL of each replicate, so we request a minimum of 120uL per sample per kit.
  - **Example**: If you are requesting a Mouse Pro-inflammatory and a Mouse Th17 luminex on the same samples of mouse serum, we would need 60uL for the pro-inflammatory kit (25uL + 25uL + 10uL of extra buffer) AND an additional 60uL for the Th17 kit for a total of 120uL per sample.
- **How much protein should my samples have?**
  - The assays recommend 1-25ug of total protein per well. Since we usually add 25uL of sample per well, total protein concentration of your sample is recommended to be between 0.04 ug/uL and 1 ug/uL.
  - Lysates at 5 ug/uL concentration may not have lysed properly and should be avoided, as not all of the proteins may have been solubilized equally.
- **Should I bring media?**
  - For all samples that are not plasma or serum source, you will need to provide roughly 2mL of background material per kit. This will be used to determine the background level of protein in the sample. If your plasma or serum samples have been diluted with any material, bring the material used to dilute the samples and be sure to mention the dilution factor in your Luminex submission form.
    - Cell culture supernatant: The media the cells were cultured in, eg. RPMI+5% FBS
    - Lavage: The media used to wash out the body cavity, eg. DPBS
    - Cell lysate: Cell lysis buffer used.
- **What cell lysis buffer should I use?**
  - Millipore Sigma recommends their own lysis buffer which has been tested in their assays and shown not to interfere with results: Cat. #43-040 https://www.emdmillipore.com/US/en/product/MILLIPLEX-MAP-Lysis-buffer-for-Multiplexing,MM_NF-43-040 . Some of this buffer is available at the core for users to try out, but it is recommended that you provide your own.

        - **Note**: Cat# 43-040 does NOT include protease/phosphatase inhibitors, as these must be added fresh. For users looking at pathway signaling, you will need to provide your own. Example products include Millipore Cat. No. 20-201 and Sigma AEBSF Cat. No. 101500
    - Non-ionic detergents (NP40, Tergitol, IPEGAL) are recommended in lysis buffers for solubilizing cytoplasmic proteins.
    - Partially ionic detergents (Triton® X-100) are recommended in lysis buffers for cytoplasmic or membrane-bound proteins.
    - Ionic detergents (sodium dodecyl sulfate, SDS) are recommended in lysis buffers for membrane-bound, nuclear or mitochondrial proteins. If using SDS in the lysis buffer (i.e., Radioimmunoprecipitation assay (RIPA) buffer), then cell lysate must be diluted to less than 0.05% SDS for assays to detect intracellular proteins, such as cell signaling proteins.
- **How long until I get the data?**
    - Luminex assays are run over a period of two days. If we are running the assay on Tuesday, you can generally expect the data to be available by the end of the following day, in this case Wednesday. We occasionally receive many orders at the same time that can push this timeline back.
- **How do I access the data?**
    - When data analysis is complete, you will receive an email that the data has been finished and moved to your PI's folder on the flow cytometry core server. Due to billing and ownership of data, the data will be saved in the folder of the PI who is associated with the PTAO that was used for the booking. If you are collaborating with someone, a member of the lab that provided the payment must give you the data.
- **How do I interpret the data?**
    - Generally, users will receive two excel documents: a **Detailed report** and a **Summary report**.
        - The **summary report** will have analyte concentrations and values based off of the average of the replicate samples. It also has images of the standard curves and the fit values used to determine analyte concentration based off of the MFI values. The summary report is usually the one users are most interested in.
        - The **detailed report** includes all of the data in the summary report, but also goes into more detail for each analyte, including MFI values, values of individual replicates, etc. This report is very large and is used for troubleshooting, such as looking at samples with high CVs between the two replicates.
    - If users are looking at phosphorylated data, such as the STAT kits or the 9-Plex Multipathway panel, only a single excel sheet is provided. Samples are measured qualitatively compared to known cell line lysates that are provided with the kits. It is not possible to get quantitative values, so samples will need to include some sort of baseline to compare against, such as a time zero timepoint, an untreated/unstimulated sample, or genotype.

- **When can I bring samples to the core?**
    - Once you've submitted a Luminex request and it has been approved, you can bring the samples by at any time (even days in advance). We will store samples in our -80°C freezer until the day of the assay. We ask that you bring samples to the core no later than noon the day of the run.
- **How are the samples stored?**
    - Samples are stored in a -80°C freezer with remote temperature monitoring. Staff of the core receive text and email notifications of any changes in temperature beyond the set ranges 24/7.
- **Can I get unused sample back?**
    - If you would like to recover unused sample, eg. You provide 200 uL and we only used 50uL, you can come back the day after the Luminex assay is started to pick up your remaining samples. We keep all submitted samples stored at -80°C for a period of 6 months, during which time users can request their leftover sample.
- **Can I run X species/analyte?**
    - If you're interested in running panels with species or analyte combinations we don't have listed, please contact Lesa Campbell (lc4ae@virginia.edu) to discuss the specialty panel design and receive a quote. Please have an idea of the specific analytes you'd like to include, the species, the sample type (cell lysis, serum, etc), and a rough estimate of the number of samples.
- **Can I be trained to run the MagPix?**
    - Users can be trained to run the Luminex instrument themselves. In iLab, users can schedule time on the MagPix (instrument used to run Luminex assays) by going to schedule equipment > Benchtop Analyzer > MagPix. Users will need to confirm that a flow core staff member is also available by looking at Staff Calendars 1,2, or 3. Training takes roughly 30 minutes, but additional time should be booked if you plan on running a plate as well. Generally, a full 96 well plate takes about 45 minutes to run, but some kits and manufacturers may vary.
    - After completing a first training session with a staff member, the next booking will be run with a staff member shadowing your work, eventually either approving unassisted use or deciding you need more training.
- **Can I use other manufacturers kits?**
    - Users who have been trained on the MagPix can use their own kits from any manufacturer supported by the instrument. Panels run by the core are from kits provided by Millipore Sigma (Milliplex brand).
- **I've never performed a Luminex assay and I'm not entirely sure what I'm looking for. What's a good place to start?**
    - If you're beginning a new project and looking for any changes in analytes, we offer large panel kits for mouse (32 analytes) and human (47 analytes). These kits can be costly if you plan on running many samples, so you may want to select a few samples with a variety of conditions, run them on the large panel, find out what analytes seem to show changes with your conditions, and then determine if a smaller, more cost-effective kit may be a better option for a large batch run. Additionally, you may always email either

Alex Wendling (ajw4sr@virginia.edu) or Mike Solga (mds4z@virginia.edu) to go over any additional questions you may have.

- **Do you have any other resources I can check out?**
  - **Milliplex FAQ**
  - **Milliplex Tips and Tricks Booklet - 2019 edition**