Institut für HIV Forschung
SOP# 22-00 **(April 2015)**

Luminex

- Allow all reagents to reach room temperature before beginning
- Add 250uL deionized H2O to cytokine/chemokine standard vial, allow to sit at RT for 10 minutes. DO NOT INVERT
- Centrifuge samples at 10,000rpm for 10 minutes
- Following 10 min of incubation for standard, invert vial 5 times and allow to sit at RT for another 5 minutes
- Prepare a serial dilution of standards to be used in Assay Buffer (provided with kit). Generally this is a 1:5 dilution scheme where you add 50uL from the previous vial into 200uL Assay buffer. Use Assay Buffer as blank control.
- Sterilize samples and standards by adding 1 part 0.5% Tween in PBS to 9 parts standards or samples. This is generally done in a 96 well plate for ease of multi-channel pipetting afterwards. Usually I plate 135uL standard/sample and add 15uL of the pre-made Tween solution to each well.
- Incubate at RT for 15min
- While sterilization is taking place, take individual vials of beads or pre-mixed bead vial and sonicate for 30s, then vortex for 30s
- Reconstitute beads in assay buffer to a total volume of 6-6.5mL. For pre-mixed bead vials I usually add 3mL Assay Buffer and vortex for 30s. For the kits where you add individual bead vials, I account for 100uL volume from each vial, so 10 beads at 100uL = 1mL volume from beads, so I would add these to 5mL Assay Buffer
- Plate samples and beads with appropriate buffers in luminex plate (just a regular 96-well culture plate) : In wells which will contain standards, add 25uL R10 or Serum Matrix (provided with kit). In wells which will contain sample, add 25uL Assay Buffer
- Plate standards and samples by adding 25uL of them to appropriate wells. I always do my standards in duplicate. For cell culture supernatant I plate samples in duplicate, for serum I plate in triplicate
- Add 25uL prepared bead solution to each well.
- Seal with plate sealer, cover with aluminium foil, and incubate rotating on a shaker at ~400rpm. For serum samples I incubate O/N at 4C, for cell culture supernatant I incubate at RT for 2h. The longer incubation time overnight increases assay sensitivity for low-level cytokines.
- Wash wells 2x with 175uL Wash Buffer (provided with kit, be sure to dilute 1:10 in deionized H2O). For using the magnet, make sure to leave the plate on the magnet for at least 90s before dumping out solution. Once solution is dumped out, you can remove the plate from the magnet, add the wash, and then immediately place the plate back on the magnet.
- Prepare detection antibody solution by adding 3mL Assay Buffer to provided antibody

solution vial.

- Add 25uL prepared antibody solution to each well.
- Cover with plate sealer, cover with foil, incubate at RT on shaker for 1h
- WITHOUT WASHING, add 25uL L-SAPE (provided with kit, different number L-SAPE depending on which kit you're using) to each well.
- Cover with plate sealer, cover with foil, and incubate at RT on shaker for 30min
- Wash 2x with 175uL Wash Buffer and resuspend each well with 150uL Wash Buffer. Plate must now be read within the next 12 hours.