Luminex Requisition Form

Lab PI _____ Phone #_____ Lab Phone #_____

Individual Requesting Luminex _____

Today's Date _____ Scheduled Date of Assay_____

Account string to be charged_____

Cytokine Kits (Circle Which)

Human 41-plex (Millipore) Human Cardiovascular (Millipore) Custom __-Plex

Samples

Type:
_____ Cell culture supernatant
_____ Serum
_____ Plasma
_____ Tissue homogenate
_____ Lavage fluid
_____ Other (describe) _____

Delivered:
_____ in U-well plate

Sample medium (except serum and plasma samples):
What medium are your samples in?

___ RPMI ___ AIM V ___ PBS Other (describe) _____

Does your medium contain FBS, FCS, HS, or BSA? _____NO _____YES

Which? _____ How much? _____ %

Cytokine concentrations:
The range of the standard curve for each cytokine varies greatly. Please ask is you're unsure whether your sample needs to be diluted.

Number of samples:
_____ singles

_____ duplicates

_____ triplicates

*_____ **Total # of wells**

Dilution factor:
_____ undiluted

_____?-fold

_____ pre-diluted in your lab

_____ request Dart Lab to dilute

Are any or all of your samples HIV/AIDS positive? _____
Hep B positive? _____ **Hep C positive?** _____

If so, on the attached plate map, please indicate which samples are positive and for which disease.

Sample Amount: Please deliver in U-well plate according to provided plate map

Millipore kit: 40ul supernatant (see kit protocol for plasma or serum dilution) samples

Sample Identification: Please send an Excel file to ryan.andreozzi@gmail.com with your sample identification information (i.e., Pt 456 Mo. 2) in one Excel cell/sample.

Please note that Bio-Rad Kits do not have Quality Control Wells

Points to consider BEFORE harvesting samples:

- We need to dilute the cytokine standards in the identical medium your samples are in. So freeze 10 ml culture medium (e.g. RPMI/10% FBS) at experiment set-up.
- For whole blood, collect plasma rather than serum. The clotting process causes platelet degranulation. Platelet granules are a source of many cytokines.
- Harvest your samples then centrifuge them 14,000 rpm for 3 minutes in a cold microcentrifuge (to pellet particulates). Remove supernatants, avoiding any lipid layer, and aliquot.
- **Do not freeze and thaw samples.** Cytokines deteriorate with freezing and thawing. Aliquot and freeze small volumes (~180 ul for triplicates) at harvest.
- If serum-free culture medium is used, add 0.5% BSA (5 mg/ml) as carrier protein before freezing.

Points to consider when preparing samples to be given to Dart Lab:
Warning: Hemolyzed samples are not suitable for Bio-Plex cytokine assays and will not be accepted (the instrument gets clogged).
Thaw samples the morning of the assay.
Keep all thawed samples on ice until ready for use.
We add 25 ul sample per well, so provide us with **at least 40 uL.**
Add your samples to a U-bottom 96-well plate as shown in the Luminex template.

Serum Isolation
Allow the whole blood samples to clot for 1–2 hr at 37°C. Alternatively, use a serum separator tube and allow the blood samples to clot for 30 min at room temperature. Centrifuge at 1,000 x g at 4°C. Collect the serum, avoiding any lipid layer, and assay immediately or freeze at –20°C in small aliquots.

Plasma Isolation
EDTA tubes are preferred. If using Sodium Heparin, be certain to have no more than 10 IU/mL of blood. Sodium Heparin is known to interfere with the detection of certain cytokines. Centrifuge at 1,000 x g at 4°C for 10 min. Collect the supernatant and either filter through a sterile 0.22 µm filter or centrifuge 14,000 rpm for 3 minutes in a refrigerated microfuge. Collect the plasma, avoiding any lipid layer, and assay immediately or freeze at –20°C.