

Immune Monitoring

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Cytokine Analysis FAQ

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What is cytokine analysis service?

We perform quantitative immunoassays on your biological samples of serum, plasma or cell culture supernatants to give you concentrations of various cytokines and other analytes.

Which cytokine assays are available?

As of January 2021, we have assays for these *human* analytes:

| IL1 ra | IL10 | CCL14 | FGF basic | MIP 1 alpha | TIMP1 |
|------------|----------------|--------------|---------------------|----------------|---------------|
| IL1 alpha | IL12p70 | CCL17/TARC | GCSF | MIP 1 beta | TIMP2 |
| IL1 beta | IL15 | CXCL9/MIG | GMCSF | PDL1 | TNFR1/TNFRp55 |
| IL2 | IL17 | CXCL4** | Granzyme B | RANTES** | TNFR2/TNFRp75 |
| IL2R alpha | IL18 | CD59 | gp130 | Resistin | TNF alpha |
| IL3 | IL21 | CD137 | HGF | Reg3A | TSLP |
| IL4 | IL22 | CRP** | IP-10 | ROR1 | VCAM 1** |
| IL5 | IL27 | Elafin | Interferon alpha 2a | SCF | VEGF |
| IL6 | IL32 | Endoglin | Interferon gamma | Sema4A | |
| IL6R alpha | IL33 | Eotaxin | Leptin** | ST2 | |
| IL7 | Adiponectin** | E-Selectin | MCSF | Thrombopoietin | |
| IL8 | Angiopoietin 2 | FAS | MCP1 | TIM1 | |
| IL9 | BAFF | Flt 3 ligand | | TIM3 | |

**For serum/plasma samples, these analytes must be assayed and charged separately.

And for these *mouse* analytes:

| IL2 | IFN gamma | IL12 |
|-----|-----------|-------|
| IL6 | TNF alpha | GMCSF |
| | | IL15 |

How much does it cost?

To see prices, you must log into the Fred Hutch iLab page, click on Immune Monitoring and then Cytokine Analysis. The price chart will appear on the cytokine assay request form that pops up.

How much sample volume do you need?

Please provide us with at least 75-100 microliters of sample. We will refreeze leftover sample volume to return to you.

What should I put the samples in?

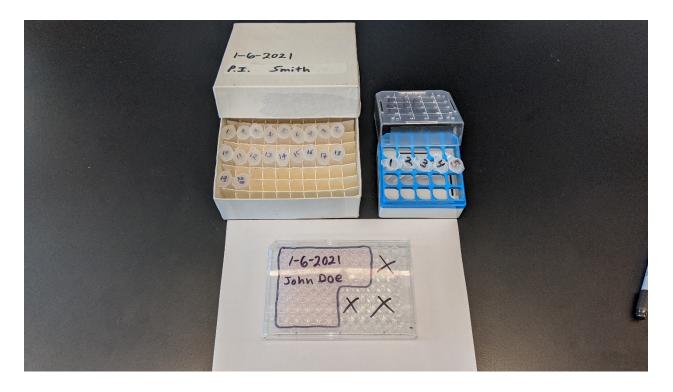
Please put serum and plasma samples into plastic screwcap cryotubes or micro-centrifuge tubes and then into a freezer box labeled with your name, principal investigator's name and date (see picture below).

Cell culture supernatants should be cell free and put into the same type of tubes, or else transferred into a clean 96-well plate with lid and frozen. If using a 96-well plate, DO NOT OVERFILL the wells. This can cause cross-contamination between wells. Use no more than 150 microliters per well for 96-well plates.

Please clearly label your cryo-tubes or microfuge tubes on the caps, using a simple 1, 2, 3, 4... numbering system.

NO GLASS sample tubes please.

NO WHOLE BLOOD. Blood must be processed into serum or plasma and then aliquoted and frozen.



Should I bring frozen or thawed samples?

Please freeze your samples and then keep them frozen on dry ice when you bring them to us.

I'll be drawing whole blood. Should I use serum or plasma?

That is your choice. Serum is used a little more often than plasma. Most cytokines and other analytes will be about the same whether the blood is processed into serum or plasma, though there are a few analytes (such as the chemokine CXCL4) that are drastically different between the two. If you do choose plasma, please use citrate or EDTA as anticoagulant and NOT HEPARIN, which binds to many proteins and might interfere with the assay.

How many samples can I bring at once? What about batch size?

You can bring anywhere from a few samples up to a few hundred all at once. It is usually cheaper to wait and group your samples into one larger batch rather than several smaller ones. For example, if you will be creating a total of 40 samples over time, it would be cheaper to submit all 40 samples together than to submit two separate batches of 20.

How do I get my samples assayed?

The first step is to create a cytokine assay request by logging into the Fred Hutch iLab web page. Once logged in, click on *Immune Monitoring* and then *Cytokine Analysis*. Fill out the assay request form that pops up, including your budget information and sample information, and select the cytokines you want from the box next to either *human Luminex assays available* or *mouse Luminex assays available*. Click *Save* and then *Submit*.

The second step is to bring your samples to the Immune Monitoring shared resource. We are on the 5th floor of the historic Steam Plant Building about one block away from the main Fred Hutch campus on South Lake Union in Seattle. You can email Rick Lawler (<u>rlawler@fredhutch.org</u>) to arrange sample drop off.

The address is: 1201 Eastlake Ave. E. Room S5-101 Seattle, WA 98102

When do I get my data? And in what format?

We will email your results to you as an Excel file once we have finished running the assay(s). How long this takes depends mostly on the size of your sample batch and the lab's workload. For batches of 90 samples or less, we can usually get data to you within about two weeks of samples being dropped off.

Can I buy a Luminex assay kit and have you run it for me?

Sorry, but we generally do NOT run Luminex assay kits (or ELISA kits) for investigators. You can buy such a kit, run it yourself in your own lab and then bring the finished assay plate to read on our Luminex reader, but you must schedule this several business days in advance. Please email Rick Lawler (<u>rlawler@fredhutch.org</u>) to schedule Luminex usage.