

Bio-Plex Cytokine Assays

Sample Preparation Guidelines & Sample Submission Form

About the Bio-Plex System

The Bio-Plex system (Bio-Rad) is a multiplex analysis system that can simultaneously analyse up to 100 different biomolecules (proteins, peptides, or nucleic acids). This suspension array system, which is based Luminex's xMAP technology, incorporates several technologies including fluorescently dyed microspheres (beads), flow cytometry, lasers and associated optics to measure the biochemical reactions that occur on bead surfaces, and a digital signal processor to manage the data. This fast and reproducible multiplex system has small sample requirements and offers greater flexibility than planar arrays.

How it works: The system uses a liquid suspension array with up to 100 uniquely colour-coded bead sets. Each of the 100 beads is internally labelled with a specific ratio of two fluorophores to assign it a unique spectral address. The beads are then conjugated with different biomolecules, allowing the capture of specific analytes from the sample. A fluorescently-labelled reporter molecule is then added to the sample in order to detect and quantify each captured analyte. The beads are drawn through a flow cell where two lasers excite each bead. Fluorescent signals are recorded, translating the signals into data for each bead-based assay.

About Bio-Plex Cytokine Assays

Cytokines are important cell signaling proteins that mediate a range of physiological responses, including immunity, inflammation, and hematopoiesis. Cytokines are also associated with a number of diseases, including cancer and infection. Bio-Plex cytokine assays are multiplex bead-based arrays designed to quantify multiple cytokines in diverse matrices including serum samples, plasma samples, and tissue culture supernatants. For optimal recovery and sensitivity, it is important to properly prepare samples.

Serum or plasma samples require 15 μ l of sample and have a detection range of ~ 0.2-3200 pg/mL. Cell and tissue cultures require 55 μ L of sample and have a detection range of 2-32000 pg/mL. For the cytokine assays, each 96-well plate can accommodate 78 unique samples (or 39 unique samples run in duplicate). 16 wells are required for the cytokine standards and two wells are run as blanks (media only).

Publications using Bio-Plex cytokine assays:

Labidi SI, *et al.* **Serum cytokines in follicular lymphoma. Correlation of TGF- β and VEGF with survival.** Ann Hematol 2009, Jul 7 [[Epub ahead of print](#)]

Lee KS, *et al.* **Simultaneous measurement of 23 plasma cytokines in late-life depression.** Neurol Sci 2009, 30(5):435

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Revised: November 2009

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Sample Preparation Guidelines

Type of Sample	Sample Preparation	Additional Notes
Serum and Plasma	EDTA and citrate plasma are acceptable but heparin plasma may absorb cytokines. Also avoid using hemolysed samples. Allow blood to clot and collect serum. For plasma, centrifuge immediately after collection in tubes containing anticoagulant. Freeze at -70°C and avoid freeze-thawing.	Once we receive the samples, one volume of the serum sample will be diluted with three volumes of the appropriate Bio-Plex sample diluent prior to running the assay.
Tissue Culture Supernatant	If necessary, dilute with culture medium. For serum-free culture medium, add a carrier protein (ie. BSA) to samples at a concentration of at least 0.5%. Centrifuge samples at 13,200 rpm for 10min at 4 °C and store at -70°C. Avoid freeze-thawing.	Please provide 5 mL (per plate of samples) of the <i>exact same</i> medium along with your samples as cytokine standards must be diluted in the same medium or matrix in which the cells were prepared. Please send medium from the same lot. If the medium contained FBS, the sample sent to us should contain FBS at the same concentration.
Other Biological Fluid samples	Keep all samples on ice until ready for use. Dilute with buffer as similar to sample as possible if required. Add a carrier protein (ie BSA) to samples at a concentration of at least 0.5%.	Please provide 5 mL (per plate of samples) of the <i>exact same</i> medium along with your samples as cytokine standards must be diluted in the same medium or matrix in which the cells were prepared.

Please note that we are unable to process infectious, or potentially infectious, human samples

Volume of Sample Required:

For serum and plasma samples, a volume of 15 µL is required; thus we request 32 µL of each sample (if sample is to be run in duplicate). For cell culture samples, we require 55 µL (115 µL for duplicates). It is highly recommended that you run your samples in duplicate.

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Tubes:

Please send your samples in either a) 96-well plate(s) (conical bottom preferred) or b) 0.5 mL 8-tube strip tubes. For 1-8 samples, samples can be sent in 1.5mL eppendorf tubes. Please do not send your samples in mini (PCR) tubes or collection tubes.

Tube Labelling:

Please label the tubes clearly using a permanent marker. Please keep the sample IDs to a maximum of five characters. Easy codes (e.g. numbers #1-100) are preferred.

Sample Shipping:

All samples must be non-infectious and non-hazardous. Please ship/deliver all samples frozen at -70°C.

Cytokine Assay Standards:

Cytokine Standards (included with each assay kit) are used to generate the standard curve. In order to select the best standard dilutions for your samples, please let us know the expected range of cytokine concentrations in your samples. The cytokine standards can be diluted in one of two ways; one will provide a standard curve suitable for samples with cytokine concentrations between 1.95 and 32,000 pg/mL and the other for cytokine concentrations between 0.2 and 3,200 pg/mL. The former dilution set, which covers a wider concentration range, will be used if the expected concentrations of the samples are unknown. The latter set of dilutions will provide greater sensitivity for lower cytokine concentrations between 0.2 and 3,200 pg/mL.

Note: Highly lipemic, hemolysed, and viscous samples may not be suitable for Bio-Plex cytokine assays and can lead to aberrant results.

For a complete quote, please provide us with the following information: the assay kit required and the number of samples. The service price includes the assay kit/reagents, instrument calibration and validation reagents, all consumables, assay set-up, and Bio-Plex data acquisition.

Contact

geneservice@microarrays.ca

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Sample submission form

Please submit one form for each assay

Customer Information

Name: _____

Phone number: _____

Email address: _____

PI: _____

Name of Organisation/Company: _____

(Please provide the name, phone number, and email address of the person responsible for the microarray experiment in case we have any questions)

Billing Address: (if billing directly to Purchasing Department, please provide a purchase order number).

Assay/Sample Information

Type of assay (name of assay, vendor, catalogue number): _____

Number of unique samples: _____

Run each sample in duplicate? Yes No

Type of samples (plasma, cell culture supernatant, etc): _____

Type of medium: _____
(please ship 5mL of medium with cell culture samples)

Sample species: _____

Expected concentration of unknown : (circle one)
1.95 – 32,000 pg/mL OR 0.2-3,200 pg/mL OR unknown

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For samples submitted in a 96-well plate:

Please ensure that the plates are well sealed to avoid contamination.

List of Samples: Please include a list (Excel spreadsheet or chart below) with detailed information about the nature of each sample, well location, and expected concentration.

Sample ID	Sample Type	Well location on 96-well plate	Expected Concentration	Total volume sent (µL)
<i>M14</i>	<i>Cell culture</i>	<i>C1</i>	<i>3 ng/mL</i>	<i>120</i>

Note that each assay plate can handle 78 samples (16 wells reserved for cytokine standards and two wells for blanks).

Shipping Address/Contact information:

Please ship/deliver all samples at -70 °C.

Please **DO NOT SEND** infectious, or potentially infectious, human samples.

Attention:
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