Bio-Plex Signal Transduction (Phosphoprotein) 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 quantitate 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 the Signal Transduction (Phosphoprotein) Assays

Bio-Plex phosphoprotein assays report the level of a protein that is phosphorylated at a specific site or sites, while total target assays report the level of the protein regardless of its phosphorylation state. Signal Transduction assays are available in singleplex and multiplex formats. Assays are available for the detection of signal transduction proteins (phosphoproteins) involved in a range of processes; including Akt signaling, MAP kinase signaling, and immunology/inflammation. Please visit the Bio-Rad website for a complete list. Pre-mixed assay panels (phospho 3-plex, phospho 5-plex and total target 5-plex) as well as custom premixed assays (x-plex) assays are available. We will order “off-the-shelf” multiplex kits for you, however, custom kits should be ordered by the customer.

Published studies involving Bio-Plex phosphoprotein assays:

Bouali S, *et al*. **P53 and PTEN expression contribute to the inhibition of EGFR downstream signaling pathway by cetuximab.** Cancer Gene Ther 2009, 16(6):498

Chergui F, *et al*. **Validation of a Phosphoprotein Array Assay for Characterization of Human Tyrosine Kinase Receptor Downstream Signaling in Breast Cancer.** Clin Chem 2009, 55:1327

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Sample Preparation Guidelines

Samples should be prepared using the Bio-Plex Cell Lysis Kit (catalogue #171-304011, 171-304012). For dilutions, the appropriate species-specific diluent kit should be used.

Type of Sample	Lysate Preparation
Adherent cells	Aspirate culture medium and quickly rinse cells with ice-cold cell wash buffer (same volume as culture medium). Keep cells on ice. Prepare 500 mM PMSF (store as aliquots at -20ºC; aliquots can be frozen and thawed up to five times). Prepare adequate volume of lysing solution (see instruction manual). Add lysing solution to cells. Agitate cells by placing plate on ice and pipet contents up and down five times; for adherent cells, scrape cells with a cell scraper. Agitate plate on a microplate shaker at 300 rpm for 20 min at 4ºC (otherwise, transfer cell lysate to centrifuge tube and rotate for 20 min at 4ºC. Determine lysate protein concentration (which should be 200 – 900 µg/mL). Add an equal volume of assay buffer to the lysate. Store samples at -20ºC
Cells grown in suspension	Add ice-cold wash buffer (twice the volume of the culture medium), centrifuge cells at 1,000 rpm for 5 min at 4ºC. Aspirate supernatant. Prepare 500 mM PMSF (store as aliquots at -20ºC; aliquots can be frozen and thawed up to five times). Prepare adequate volume of lysing solution (see instruction manual). Add lysing solution to cells. Agitate cells by placing plate on ice and pipet contents up and down five times; for adherent cells, scrape cells with a cell scraper. Agitate plate on a microplate shaker at 300 rpm for 20 min at 4ºC (otherwise, transfer cell lysate to centrifuge tube and rotate for 20 min at 4ºC. Determine lysate protein concentration (which should be 200 – 900 µg/mL). Add an equal volume of assay buffer to the lysate. Store samples at -20ºC
Tissue samples	Rinse tissue sample with cell wash buffer once. Cut the tissue into 3 x 3 mm pieces and transfer them to a 2 mL tissue grinder. Prepare 500 mM PMSF (store as aliquots at -20ºC; aliquots can be frozen and thawed up to five times). Prepare adequate volume of lysing solution (see instruction manual). Add 500 µL lysing solution to tissue grinder and grind the tissue sample on ice using about 20 strokes. Transfer ground tissue to clean microfuge tube and freeze sample at -70ºC. Thaw samples and sonicate. Centrifuge the samples at 4,500 x g for 4 min. Collect supernatant without disturbing pellet. Determine lysate protein concentration (which should be 200 – 900 µg/mL). Add an equal volume of assay buffer to the lysate. Store samples at -20ºC

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

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If you are running the Histone H3 assay, please contact us for special instructions on lysate preparation.

Note: It is highly recommended that you run dilutions of each sample. Also, highly lipemic, hemolysed, and viscous samples may not be suitable for Bio-Plex cytokine assays and can lead to aberrant results.

Volume of Sample required:
The assay requires at least 25 µL of undiluted cell lysate (50 µL cell lysate + assay buffer). Additional dilutions are optional, but recommended.

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 -20ºC.

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

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Sample submission form

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 Information

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

Type of samples (cell culture lysate or tissue lysate):

Number of unique samples:

Run serial dilution of each sample? Yes No

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Dilution Instructions: For the phosphoprotein assays, each 96-well plate can accommodate 95 unique samples (or 47 unique samples run in duplicate), if no samples require dilutions. We recommend doing at least one dilution (1 in 10), although several serial dilutions may be best (1 in 10, 1 in 100, 1 in 1000), depending on the nature of your experiments. Dilutions of each unique sample are performed to be certain that at least one of these sample concentrations are within the correct concentration for the assay.

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**	**Estimated Protein Concentration**	**Total volume sent (µL)**
M14	*Adherent cell culture*	*A1*	*250 µg/mL*	*50 µL (in assay buffer/diluent)*
M14	*Adherent cell culture*	*A2*	*25 µg/mL (1:10 dilution)*	*50 µL (in assay buffer/diluent)*
M14	*Adherent cell culture*	*A3*	*2.5 µg/mL (1:100 dilution)*	*50 µL (in assay buffer/diluent)*

Note that each assay plate can handle 95 samples (1 well is run as a blank).

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Shipping Address/Contact information:
Please ship/deliver all samples at -70 ºC.
Please **DO NOT SEND** infectious, or potentially infectious, human samples.

Attention:
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