# Ranked abundance of 115 proteins following simultaneous measurement by multiplex immunoassay in serum, plasma, and cell culture supernatant samples

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## Introduction

The MILLIPLEX® PLEXpedition panel, a customizable multiplex immunoassay kit, was developed to simultaneously screen up to 115 targets including cytokines, chemokines, growth factors, matrix metalloproteinases, and biomarkers of bone health, metabolism, and cardiovascular disease. Two Luminex® instruments, the xMAP® INTELLIFLEX® and the FLEXMAP 3D® systems, can accommodate all 115 bead regions with comparable sensitivity and sample correlation observed between them. The analyte selection incorporates many biomarkers critical to the field of immunology, using assays developed for two MILLIPLEX® 48-plex human cytokine, chemokine, and growth factor kits (Cat. No. HCYTA-60K and Cat. No. HCYTB-60K). Sample correlation in serum, plasma, and cell culture supernatants demonstrated that this discovery biomarker panel with a matrix-free format can be used to transition seamlessly to our highly qualified panels, MILLIPLEX® Human Cytokine Panels A and B, which have optimized serum matrix and lot-to-lot verification for absolute quantification of protein concentration. To probe the relative abundance of each analyte in typical sample matrices, the 115-plex assay was run with a set of cell culture supernatants and healthy and disease serum and plasma samples. Additionally, analytes were ranked by their response to stimulation by LPS or ConA in PBMCs. Our results highlight the utility of the novel MILLIPLEX® PLEXpedition screening kit for evaluating relative abundance in biological samples.

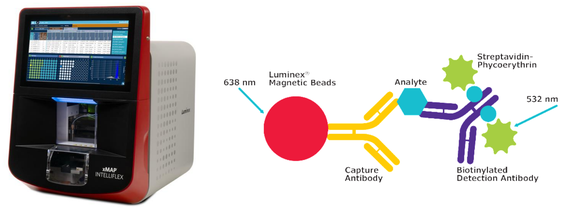


**Figure 1: MILLIPLEX® immunoassay format.**
MILLIPLEX® assays use magnetic microspheres (beads) conjugated to capture antibodies. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set, allowing researchers to simultaneously measure the analytes targeted by the capture antibodies. Native protein is analyzed by means of a "sandwich" immunoassay, pairing the capture beads with a biotinylated detection antibody.

## Methods

**Immunoassays and Data Analysis**: The MILLIPLEX® PLEXpedition Screening Panel (Cat. No. HPLX1-115SP) was used to evaluate all samples according to the kit protocol. Human Cytokine/Chemokine/ Growth Factor Panel A (Cat. No. HCYTA-60K) multiplex immunoassay was run according to kit protocol, with serum and plasma concentrations obtained from a standard curve run in serum matrix and the concentrations of all other samples obtained from a standard curve run in assay buffer. Each kit was run on the xMAP® INTELLIFLEX® instrument (pictured above) and data was acquired via xPONENT® v. 4.3 software. Data analysis was performed for all immunoassays using the Belysa® Immunoassay Curve Fitting Software (Cat. No. 40-122). Figures were prepared in GraphPad Prism and Microsoft Excel.

**Samples:** All human serum and plasma samples tested were obtained from commercial vendors. The following samples were tested: Matched serum and plasma from healthy donors; serum and plasma from individuals with cardiovascular disease; serum and plasma from individuals with autoimmune disease; serum and plasma collected from individuals under fasting conditions and post-feeding; cerebrospinal fluid (CSF), Bronchoalveolar Lavage (BAL), synovial fluid, pooled urine, and pooled milk.

Cell culture supernatants were collected from PBMC's with or without stimulation by 1 µg/mL lipopolysaccharide (LPS) or Concanavalin A (ConA) for 48 hours.

## Results

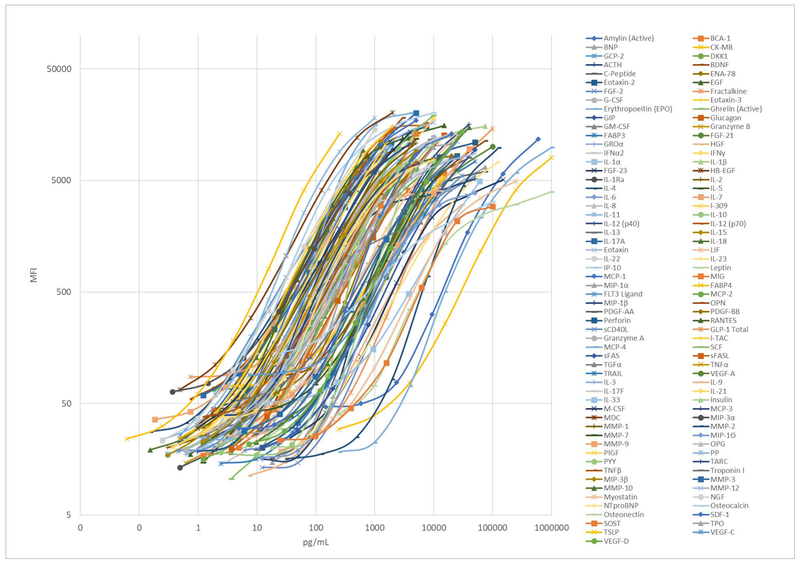


**Figure 2: MILLIPLEX® PLEXpedition Screening Panel standard curves.** The standard curves for all 115 MILLIPLEX® PLEXpedition analytes run in multiplex format are displayed above with concentration (pg/mL) on the x-axis and Mean Fluorescence Intensity (MFI) on the y-axis.

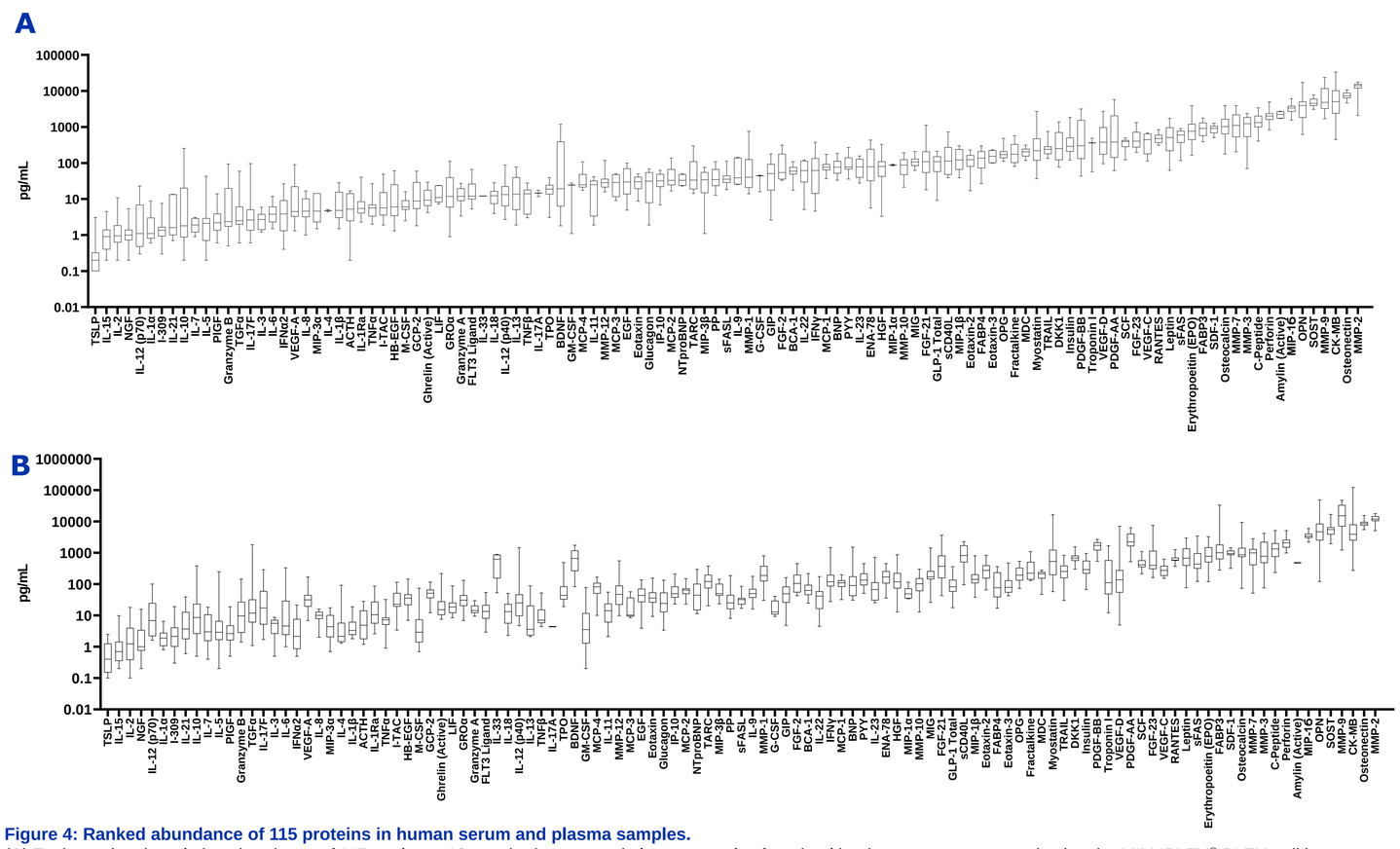


**Figure 4: Ranked abundance of 115 proteins in human serum and plasma samples.**
(**A**) To determine the relative abundance of 115 analytes, 10 matched serum and plasma samples from healthy donors were measured using the MILLIPLEX® PLEXpedition Screening Panel. Analytes were ranked according to median concentration (pg/mL). Boxes represent 25%-75% range, with error bars extending to the minimum and maximum values. (**B**) A set of diseased samples consisting of autoimmune disease (n=16) and cardiovascular disease (n=6) were also evaluated using the MILLIPLEX® PLEXpedition Screening Panel. Data is displayed as described above.

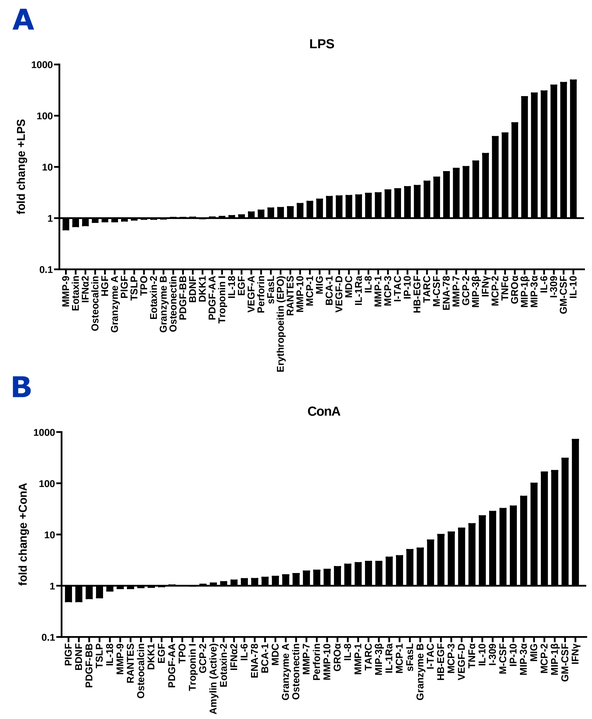


**Figure 3: Relative responses of analytes to stimuli in PBMC supernatant.**
The concentrations of all analytes in the 115-plex were analyzed in unstimulated PBMC supernatant and compared to PBMC supernatant stimulated by (**A**) LPS or (**B**) ConA. The fold change of the concentration for each detectable analyte was determined and analytes were plotted in in ranked order of fold change, from low to high. The analytes most responsive to LPS and ConA stimulation were IL-10 and IFNγ, respectively, which each showed >100-fold changes in concentration.

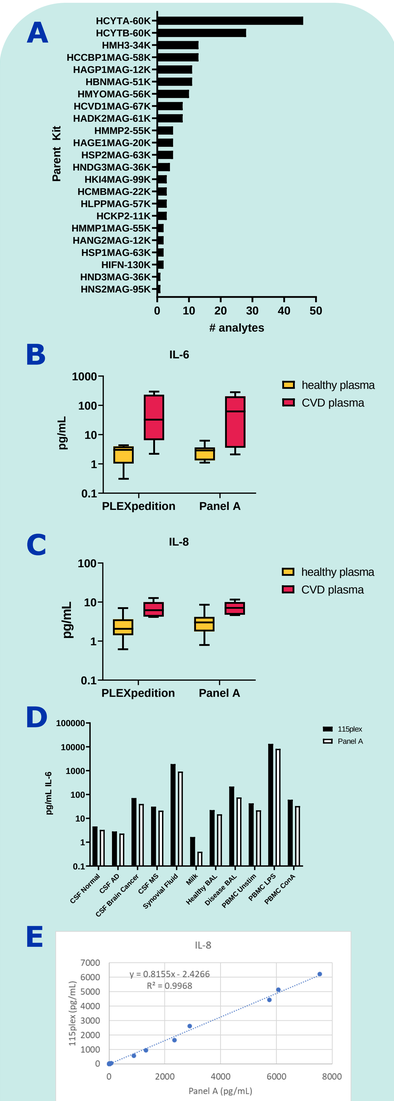


**Figure 5: Seamless transition from MILLIPLEX® PLEXpedition Screening kit to MILLIPLEX® Qualified assay format.**
(**A**) Analyte contribution to MILLIPLEX® PLEXpedition from 23 parent kits. (**B, C**) Discrimination of healthy (n=10) and cardiovascular disease (n=5) plasma samples for (**B**) IL-6 and (**C**) IL-8 using MILLIPLEX® PLEXpedition and Panel A. (**D**) IL-6 concentrations obtained from different sample types (CSF, BAL, synovial fluid, milk, PMBC supernatants) using MILLIPLEX® PLEXpedition and Panel A, respectively. (**E**) Correlation of IL-8 in 64 samples measured with MILLIPLEX® PLEXpedition and Panel A.

## Summary

MILLIPLEX® PLEXpedition Screening Panel (Cat. No. HPLX1-115SP) is a configurable multiplex immunoassay kit to facilitate measurement of up to 115 protein biomarkers. Sample detection was demonstrated in a variety of biofluids including serum, plasma, CSF, BAL, synovial fluid, milk, urine, and cell culture supernatants. This kit was used to evaluate fold changes in PBMC supernatants in response to immunostimulatory challenges and to determine the relative abundance of each analyte in a set of healthy, matched serum and plasma samples. The data generated from the MILLIPLEX® PLEXpedition Screening Panel can be used by researchers to identify markers of interest and subsequently transition to our Qualified MILLIPLEX® panels, each of which has an optimized serum matrix and lot-to-lot verification for reproducible quantification of protein concentration.



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