

# MultiCyt QBeads PlexScreen Human QBeads Inflammation Panel Kit

## Product Information

Catalog No. 97097 1x 384-well  
Catalog No. 97098 5x 384-well

## Background

The Human QBeads Inflammation Panel Kit allows the measurement of seven human cytokines and chemokines from either serum or *in vitro* samples. The cytokines/chemokines included are implicated in inflammatory responses to disease states including autoimmune diseases, chronic inflammation, and infections, for example, viral infections such as COVID-19.

Analytes offered in the Human QBeads Inflammation Panel Kit include: Human Interferon gamma (IFN $\gamma$ ), Interleukin-2 (IL-2), Interleukin-6 (IL-6), CCL2 (MCP-1), CCL3 (MIP-1 $\alpha$ ), CXCL9 (MIG), and CXCL10 (IP-10).

## Components

Lyophilized Cytokine Standards – 7 vials  
Cytokine Capture Beads (50x) – 7 vials  
Cytokine Detection Reagent (ready to use) – 1 bottle  
Human Capture Bead Buffer – 1 bottle

Human Capture Bead Buffer for Serum Samples – 1 bottle  
Human Assay Buffer – 1 bottle  
Human Wash Buffer – 1 bottle  
USB Flash Drive Containing Analysis Template

## Storage

Store at 2–8°C

## Use

QBeads function on the same principle as a sandwich immunoassay. Capture beads are directly combined with the sample. Samples can be from cell culture supernatant or serum. Once the analytes are bound by the capture beads, fluorescent detection antibodies are added to the reaction which bind the analytes forming a “sandwich.” The fluorescence signal is now

associated with the bead complex, with the intensity of fluorescence directly correlating to the quantity of bound analyte. Quantitative readouts from this assay can be measured as fluorescence intensity, or interpolated to a concentration (pg/mL) in solution via the use of a standard curve.

## Example Data

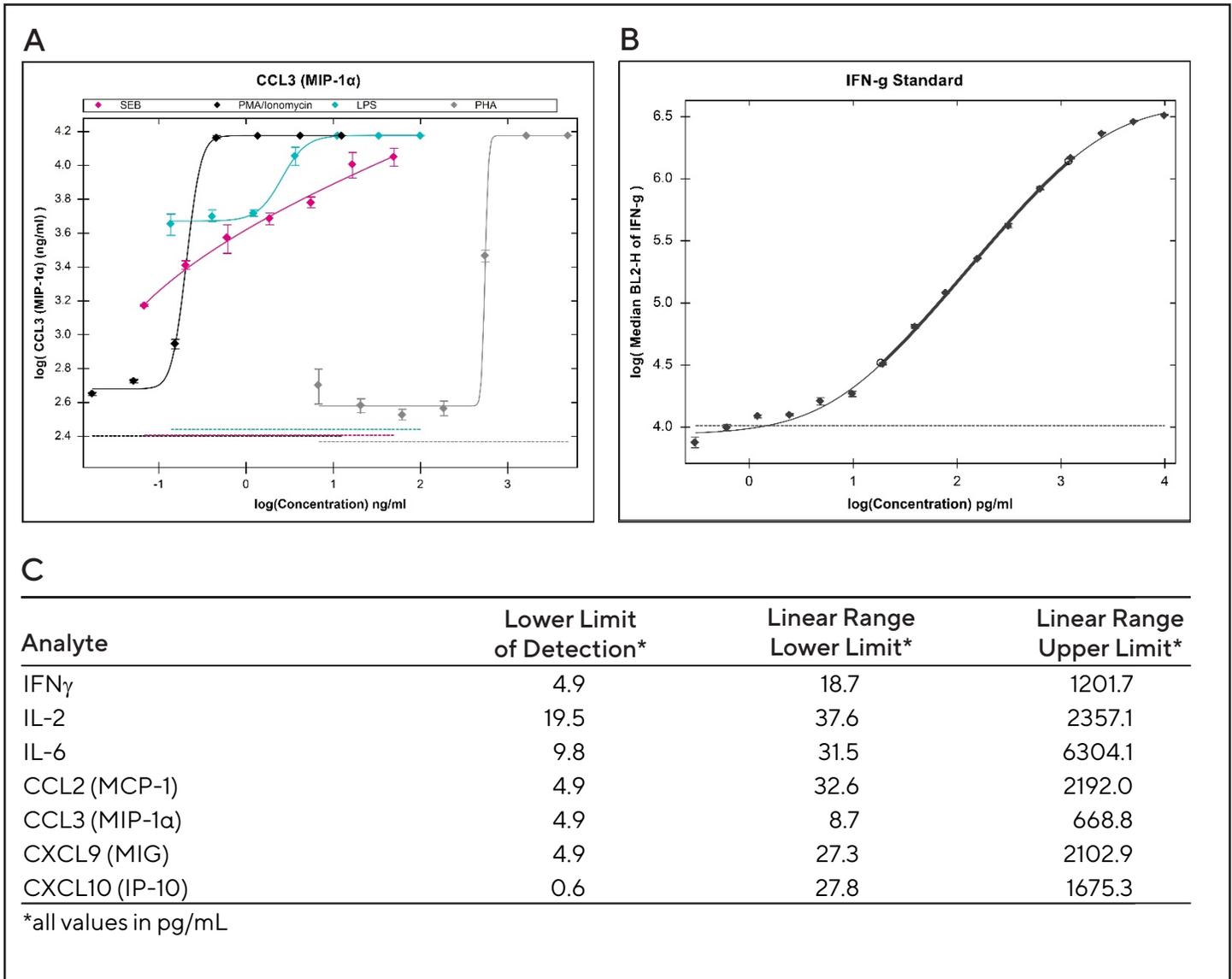


Figure 1: (A) Concentration-dependent increase in cytokine expression after 24 hours stimulus with various chemicals. Human peripheral blood mononuclear cells (PBMCs) were treated in culture with either Staphylococcal Enterotoxin type B (SEB), Phorbol 12-Myristate-13-Acetate/Ionomycin (PMA/Ionomycin), Lipopolysaccharide (LPS), or Phytohaemagglutinin (PHA) and cytokine secretion was measured with the Intellicyt<sup>®</sup> Human QBeads Inflammation Panel. Example data for CCL3 (MIP-1 $\alpha$ ) shown. The dashed lines represent the fluorescent background when the treatment concentration is zero. (B) Representative standard curve for IFN $\gamma$ . The bold line indicates the linear range, with the detection range wider than the linear range. The dashed line represents the fluorescent background when the standard concentration is zero. (C) Lower Limit of Detection (LLOD), Linear Range Lower Limit, and Linear Range Upper Limit for each analyte. LLOD was calculated by adding 3 standard deviations to the mean of samples without the analyte (n=12). Linear ranges were determined by ForeCyt<sup>®</sup> software using 4 parameter logistic (4PL) curve fit with 1/Y<sup>2</sup> weighting.

# Work Flow

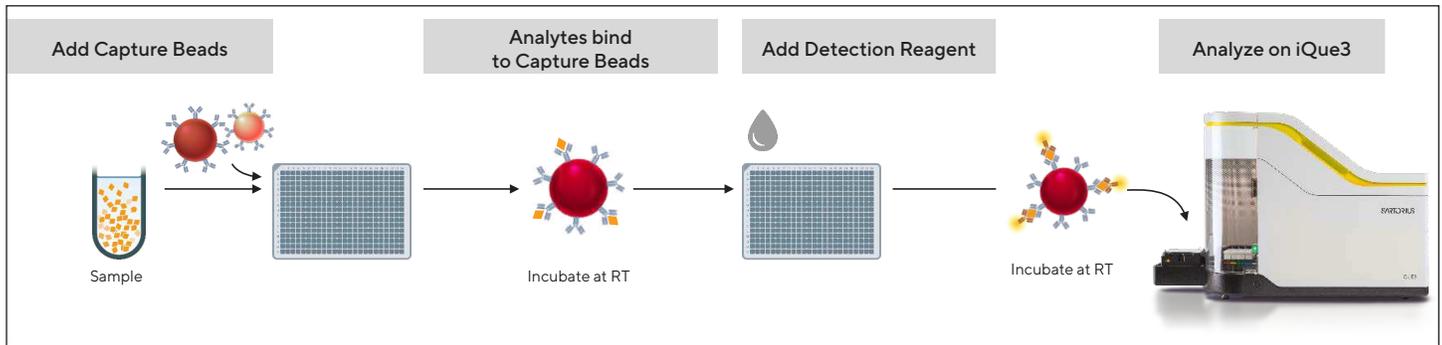


Figure 2: Human QBeads Inflammation Panel Kit cytokine work flow.

## Protocol and Procedure for 1 x 384-well plate:

### 1. Prepare the Human QBeads Inflammation Panel Kit Cytokine Standards:

- 1.1 From the provided glass vials, combine the seven Cytokine Standard spheres from the kit into a 1.5 mL microfuge tube or 15 mL conical tube. Use only 1 glass vial of each cytokine for the standard preparation.
- 1.2 Slowly add 1 mL of Human Assay Buffer to the tube. DO NOT MIX. Mixing at this step causes the reagent to foam.
- 1.3 Let stand for 15 minutes at room temperature to fully reconstitute.
- 1.4 Once dissolved, gently mix by pipetting up and down; do not vortex.
- 1.5 Prepare a 1:2 serial dilution of the reconstituted Cytokine Standards with Human Assay Buffer (Figure 3).

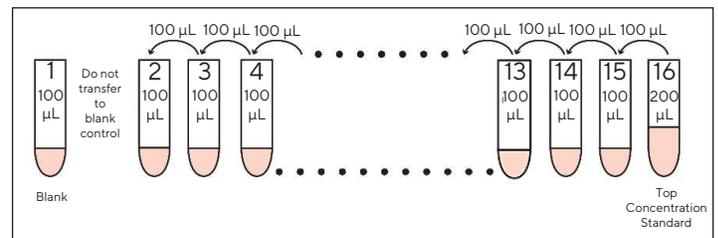


Figure 3: Standard Curve dilutions.

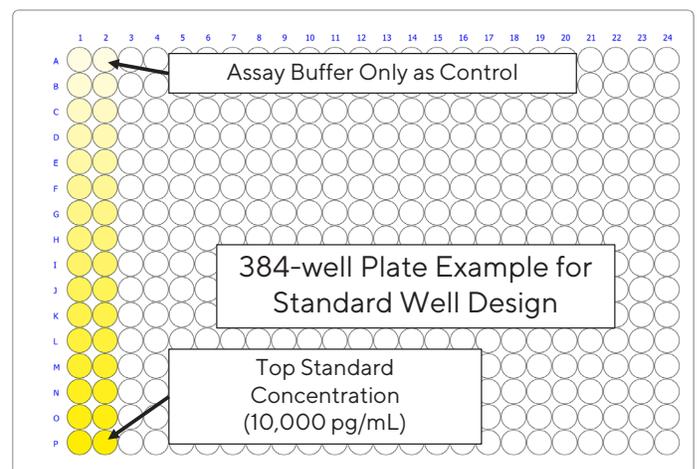


Figure 4: Suggested Standard Curve plate design.

## 2. Dilute the Cytokine Capture Beads:

- 2.1 Vortex the Human QBeads Inflammation Panel Kit Cytokine Capture Beads vials for at least 15 seconds.
- 2.2 In a 15 mL conical tube, combine 90  $\mu\text{L}$  of capture beads for each analyte. Add 3.87 mL of Human Capture Bead Buffer to bring the total volume to 4.5 mL. (If using serum samples, use Human Capture Bead Buffer for Serum Samples)

## 3. Perform the Assay:

**Note: During liquid transfers, change pipette tips to avoid cross-well contamination**

- 3.1 Transfer **10  $\mu\text{L}$**  of sample to each well of the assay plate designated as Sample during the plate set up on the ForeCyt<sup>®</sup> Design section.
- 3.2 Transfer **10  $\mu\text{L}$**  of cytokine standards prepared earlier to each well of the assay plate designated as Standards in the ForeCyt<sup>®</sup> Design section. Give the assay plate a quick spin (300 xg, 5 seconds) to ensure that all samples are at the well bottom.
- 3.3 Transfer **10  $\mu\text{L}$  of Diluted Capture Beads** to each well. Agitate the reagent occasionally to prevent bead settling. Give the assay plate a quick spin (300 xg, 5 seconds) and a brief shake (2,000 RPM, 20 seconds).
- 3.4 Cover the plate to prevent evaporation and protect from light. Incubate the plate at room temperature for 1 hour.
- 3.5 After the incubation: Add **10  $\mu\text{L}$  Cytokine Detection Cocktail** per well to the assay plate. Give the assay plate a quick spin (300 xg, 5 seconds) and a brief shake (2,000 RPM, 20 seconds).
- 3.6 Cover the plate to prevent evaporation and protect from light. Incubate the plate at room temperature for 2 hours.
- 3.7 After incubation, add **50  $\mu\text{L}$  Human Wash Buffer**.
- 3.8 Spin the assay plate (300 xg, 5 minutes).
- 3.9 Aspirate the supernatant.

- 3.10 Add **10  $\mu\text{L}$  Human Wash Buffer** per well. An additional quick spin (300 xg, 5 seconds) and brief shake (2000 RPM, 20 seconds) should be performed to ensure that all samples are at the well bottom.

## 4. Plate Acquisition and Data Analysis

- 4.1 Launch ForeCyt<sup>®</sup> Software
- 4.2 Import the provided experiment template (included on USB key in the kit package). Create a New Experiment using the provided template.
- 4.3 In the Design section, assign wells to sample. In the Standards subsection, Edit the standard set if necessary to ensure proper plate layout. (See Figure 4)
- 4.4 In the Protocol section: Adjust sip times if desired.
- 4.5 Click "Run" on the Controller to acquire the plate. If a gate does not completely encompass a bead population, click the gate label to shift the position of the gate. (See Figure 5)

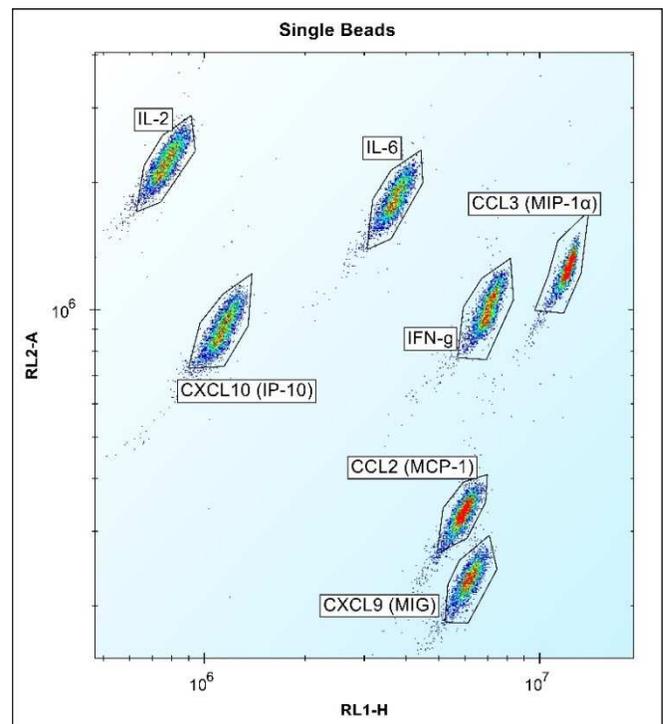


Figure 5: Human QBeads Inflammation Panel Kit gating.

**NOTE:** Each Human QBeads Inflammation Panel Kit contains the reagent volumes needed to run a 1 x 384-well or 5 x 384-well format of the kit.

Other analytes are available; for a custom QBeads kit, contact your local salesperson or visit the QBeads Assay Builder website: <https://intellicyt.com/qbeads-assay-builder/> to create your own multiplexed bead-based assay.

## Quick Guide

### 1. Reagent preparation

|  |                            |                          |
|--|----------------------------|--------------------------|
| Combine seven different lyophilized Cytokine Standards into one vial:<br><input type="checkbox"/> IFN $\gamma$ <input type="checkbox"/> IL-2 <input type="checkbox"/> IL-6 <input type="checkbox"/> CCL2(MCP-1)<br><input type="checkbox"/> CCL3(MIP-1 $\alpha$ ) <input type="checkbox"/> CXCL9(MIG) <input type="checkbox"/> CXCL10(IP-10)<br><b>Add 1 mL Human Assay Buffer to solubilize.</b>  | Incubate RT,<br>15 minutes | <input type="checkbox"/> |
| Start time _____ ↓ Stop time _____   |                            |                          |
| Prepare 1:2 serial dilution of Cytokine Standards with Human Assay Buffer.   |                            | <input type="checkbox"/> |
| Dilute Cytokine Capture Beads into Capture Bead Buffer. Combine 90 $\mu$ L of each Bead:<br><input type="checkbox"/> IFN $\gamma$ <input type="checkbox"/> IL-2 <input type="checkbox"/> IL-6 <input type="checkbox"/> CCL2(MCP-1)<br><input type="checkbox"/> CCL3(MIP-1 $\alpha$ ) <input type="checkbox"/> CXCL9(MIG) <input type="checkbox"/> CXCL10(IP-10)<br><b>Add 3.87 mL Human Capture Bead Buffer</b><br>(If using serum samples, use Human Capture Bead Buffer for Serum Samples) |                            | <input type="checkbox"/> |

### 2. Assay protocol

|  |                               |                          |
|--|-------------------------------|--------------------------|
| Add 10 $\mu$ L/well samples and standards to the assay plate.<br>Quick spin   Brief shake* |                               | <input type="checkbox"/> |
| ↓  |                               |                          |
| Add 10 $\mu$ L/well diluted Cytokine Capture Beads Cocktail.<br>Quick spin   Brief shake*  | Incubate RT,<br>1 hour, Dark  | <input type="checkbox"/> |
| Start time _____ ↓ Stop time _____   |                               |                          |
| Add 10 $\mu$ L/well Cytokine Detection Cocktail.<br>Quick spin   Brief shake*              | Incubate RT,<br>2 hours, Dark | <input type="checkbox"/> |
| Start time _____ ↓ Stop time _____   |                               |                          |
| Add 50 $\mu$ L/well Wash Buffer.<br>Spin at 300 xg for 5 minutes. Aspirate supernatant.    |                               | <input type="checkbox"/> |
| ↓  |                               |                          |
| Add 10 $\mu$ L/well Wash Buffer. Quick spin   Brief shake*<br>Acquire data.                |                               | <input type="checkbox"/> |

#### Notes

\* Quick spin: 300 xg, 5 seconds | Brief shake: 2000 RPM, 20 seconds

## Sales and Service Contacts

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Publication No. #17109 Rev.A.

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