

iQue Qbeads[®] Plexscreen iQue Qbeads[®] Human Inflammation Panel Kit

Background

The iQue Qbeads[®] Human 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 iQue Qbeads[®] Human Inflammation Panel Kit include: Human Interferon gamma (IFN γ), Interleukin-2 (IL-2), Interleukin-6 (IL-6), CCL2 (MCP-1), CCL3 (MIP-1 α), CXCL9 (MIG), and CXCL10 (IP-10).

Product Information

Catalog Numbers

Catalog No. 97097 1x 384-well

Catalog No. 97098 5x 384-well

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

Recommended Use

iQue 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

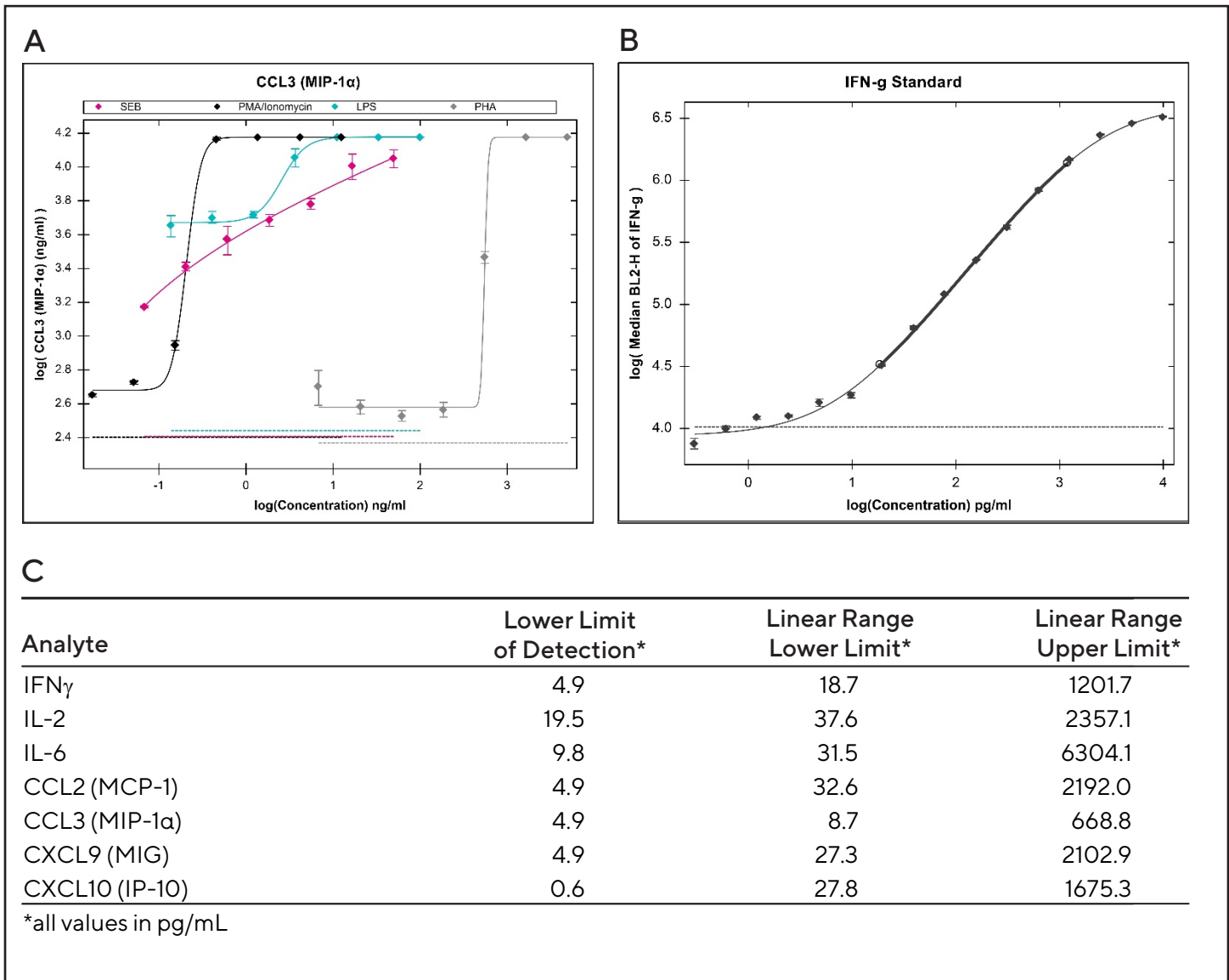


Figure 1: (A) Concentration-dependent increase in cytokine expression after 24 hours stimulus with various chemicals. Human peripheral blood mononuclear cells (PBMCs) were treated in culture with either Staphylococcal Enterotoxin type B (SEB), Phorbol 12-Myristate-13-Acetate | Ionomycin (PMA/Ionomycin), Lipopolysaccharide (LPS), or Phytohaemagglutinin (PHA) and cytokine secretion was measured with the iQue Qbeads[®] Human Inflammation Panel Kit. Example data for CCL3 (MIP-1 α) shown. The dashed lines represent the fluorescent background when the treatment concentration is zero. (B) Representative standard curve for IFN γ . 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 iQue ForeCyt[®] software using 4 parameter logistic (4PL) curve fit with 1/Y² weighting.

Work Flow

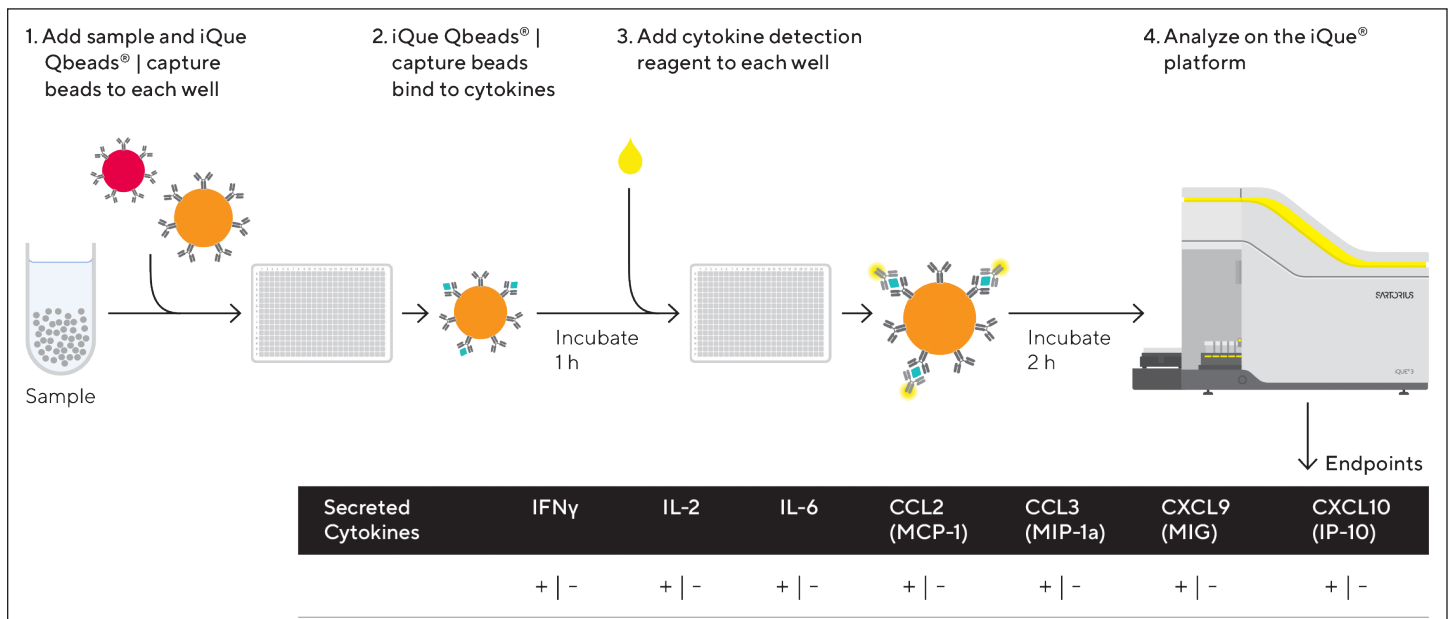


Figure 2: iQue Qbeads[®] Human Inflammation Panel Kit work flow.

Protocol and Procedure for 1 x 384-well plate:

1. Prepare the iQue Qbeads[®] Human 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**).

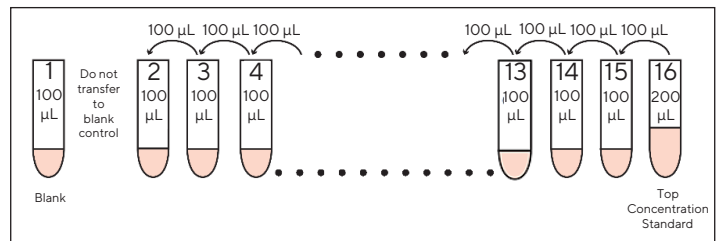


Figure 3: Standard Curve dilutions.

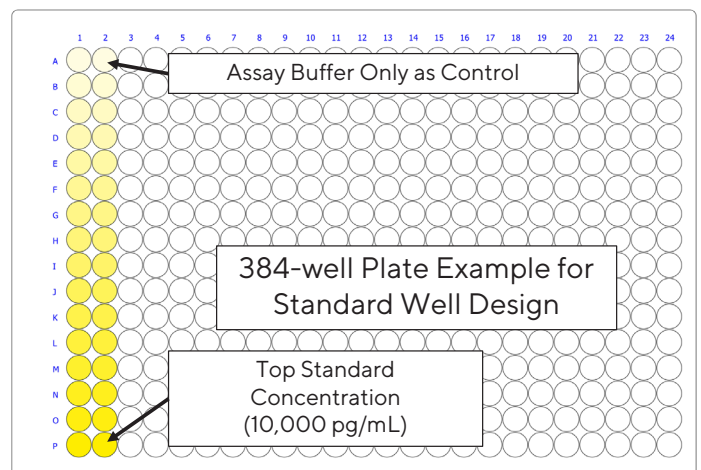


Figure 4: Suggested Standard Curve plate design.

2. Dilute the Cytokine Capture Beads

- 2.1 Vortex the iQue Qbeads® Human Inflammation Panel Kit Cytokine Capture Beads vials for at least 15 seconds.
- 2.2 In a 15 mL conical tube, combine 90 µL 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 µL** of sample to each well of the assay plate designated as Sample during the plate set up on the iQue Forecyt® Design section.
- 3.2 Transfer **10 µL** of cytokine standards prepared earlier to each well of the assay plate designated as Standards in the iQue Forecyt® Design section. Give the assay plate a quick spin (300 x g, 5 seconds) to ensure that all samples are at the well bottom.
- 3.3 Transfer **10 µL of Diluted Capture Beads** to each well. Agitate the reagent occasionally to prevent bead settling. Give the assay plate a quick spin (300 x g, 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 µL Cytokine Detection Cocktail** per well to the assay plate. Give the assay plate a quick spin (300 x g, 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 µL Human Wash Buffer**.
- 3.8 Spin the assay plate (300 x g, 5 minutes).
- 3.9 Aspirate the supernatant.
- 3.10 Add 10 µL Human Wash Buffer per well. An additional quick spin (300 x g, 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 iQue Forecyt® 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. (**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. (**Figure 5**)

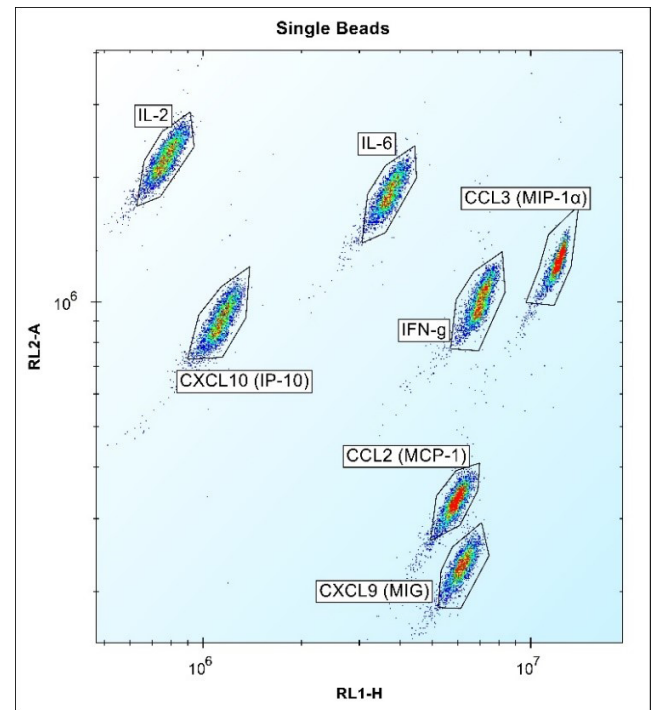


Figure 5: iQue Qbeads® Human Inflammation Panel Kit gating

Note: Each iQue Qbeads® Human 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 iQue Qbeads® kit, contact your local salesperson or visit the iQue Qbeads® Assay Builder website: <https://intellicyt.com/qbeads-assay-builder/> to create your own multiplexed bead-based assay.

Quick Guide

1. Reagent preparation

Combine 7 different Lyophilized Cytokine Standards into the same tube.

INF γ IL-2 IL-6 CCL2 (MCP-1) CCL3 (MIP-1 α) CXCL9 (MIG) CXCL10 (IP-10)

Add 1 mL Human Assay Buffer to solubilize.

Incubate RT
15 min.

Start time _____ ↓ Stop Time _____

Make 1:2 serial dilution of Combined Cytokine Standards with Human Assay Buffer.



Dilute the Cytokine Capture Beads into Capture Bead Buffer. Combine 90 μ L of each bead.

INF γ IL-2 IL-6 CCL2 (MCP-1) CCL3 (MIP-1 α) CXCL9 (MIG) CXCL10 (IP-10)

Add 3.87 mL Human Capture Bead Buffer. (If using serum samples, use Human Capture Bead Buffer for Serum Samples.)

2. Assay Protocol

Add 10 μ L/well Standards or Samples to appropriate wells. Quick Spin*.



Add 10 μ L/well diluted Cytokine Capture Beads Cocktail.
Quick Spin | Brief Shake*

Incubate RT
1 hour, Dark

Start time _____ ↓ Stop Time _____

Add 10 μ L/well Cytokine Detection Cocktail.

Quick Spin | Brief Shake*

Incubate RT
1 hour, Dark

Start time _____ ↓ Stop Time _____

Add 50 μ L/well Wash Buffer. Long Spin [300 x g, 5 min.] Aspirate supernatant.



Add 10 μ L/well Wash Buffer. Quick Spin | Brief Shake*. Acquire data.

*Quick Spin=300 x g, 5 sec. | Brief Shake=2000 RPM, 20 sec.

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For further contacts, visit
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