BD Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit

Instruction Manual

Catalog No. 551811

Regulatory information

BD flow cytometers are class I (1) laser products.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
23-11112-00 Rev. A	1/2010	Initial document
23-11112-01 Rev. 01	10/2010	Setup and acquisition instructions removed

Contents

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About this kit

This section covers the following topics:

Purpose of the kit

Use of the kit

The BD™ CBA Human Inflammatory Cytokines Kit can be used to quantitatively measure interleukin-8 (IL-8), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor (TNF), and interleukin-12p70 (IL-12p70) protein levels in a single sample. The kit performance has been optimized for analysis of specific proteins in tissue culture supernatants, EDTA-treated plasma, and serum samples using one of two protocols, depending on the sample source. The kit provides sufficient reagents for 80 tests.

Principle of CBA assays

BD CBA assays provide a method of capturing a soluble analyte or set of analytes with beads of known size and fluorescence, making it possible to detect analytes using flow cytometry.

Each capture bead in the kit has been conjugated with a specific antibody. The detection reagent provided in the kit is a mixture of phycoerythrin (PE)–conjugated antibodies, which provides a fluorescent signal in proportion to the amount of bound analyte.

When the capture beads and detector reagent are incubated with an unknown sample containing recognized analytes, sandwich complexes (capture bead + analyte + detection reagent) are formed. These complexes can be measured using flow cytometry to identify particles with fluorescence characteristics of both the bead and the detector.

Principle of this assay

Six bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for IL-8, IL-1β, IL-6, IL-10, TNF, and IL-12p70 proteins. The six bead populations are mixed together to form the bead array that is resolved in a red channel (FL3 or FL4) of a flow cytometer (see Figure 1).

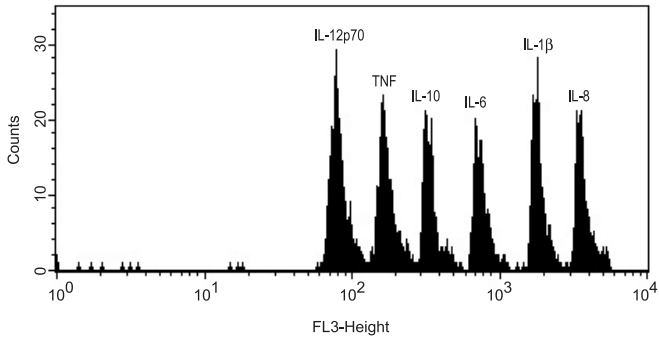

Figure 1

During the assay procedure, you will mix the inflammatory cytokine capture beads with the recombinant standards or unknown samples and incubate them with the PE-conjugated detection antibodies to form sandwich complexes. The intensity of PE fluorescence of each sandwich complex reveals the concentration of that cytokine. After acquiring samples on a flow cytometer, use FCAP Array™ software to generate results in graphical and tabular format.

Advantages over ELISA

The broad dynamic range of fluorescent detection via flow cytometry and the efficient capturing of analytes via suspended particles enable BD CBA assays to measure the concentration of an unknown in substantially less time and using fewer sample dilutions compared to conventional ELISA methodology.

- The required sample volume is approximately one-sixth the quantity necessary for conventional ELISA assays due to the detection of six analytes in a single sample.
- A single set of diluted standards is used to generate a standard curve for each analyte.
- A BD CBA experiment takes less time than a single ELISA and provides results that would normally require six conventional ELISAs.

Limitations

Assay limitations

This kit is designed to be used as an integral unit. Do not mix components from different batches or kits.

The theoretical limit of detection of the BD CBA Human Inflammatory Cytokines Kit is comparable to conventional ELISA, but due to the complexity and kinetics of this multi-analyte assay, the actual limit of detection in a given experiment may vary slightly. See Theoretical limit of detection (page 34) and Precision (page 40).

The BD CBA Kit is not recommended for use on stream-in-air instruments for which signal intensities may be reduced, adversely affecting assay sensitivity. Stream-in-air instruments include the BD FACStar™ Plus, BD FACSVantage™, and BD Influx™ flow cytometers (BD Biosciences).

Serum spike recoveries for IL-1β, TNF, and IL-12p70 are lower than for the other proteins in this assay. This variation is due to assay conditions and serum proteins and may affect quantitation of these proteins in serum samples.

The sensitivity for the detection of IL-1β in this assay is less than for the other proteins measured. It is possible that, due to operator variation, instrument settings, and instrument performance, the 20 pg/mL standard curve point for IL-1β may not have signal intensity above the 0 pg/mL background level.

Quantitative results or protein levels for the same sample or recombinant protein run in ELISA and BD CBA assays may differ. A spike recovery assay can be performed using an ELISA standard followed by BD CBA analysis to assess possible differences in quantitation.

The BD CBA Human Inflammatory Cytokines Kit assay has been shown to detect IL-8, IL-6, and TNF produced by the activation of cells from the non-human primate rhesus and cynomolgus models. Direct quantitation of proteins from the rhesus and cynomolgus models has not been validated using this kit, and results may vary.

Kit contents

Contents

This kit contains the following components sufficient for 80 tests.

Vial label	Reagent	Quantity
A1	Human IL-8 Capture Beads	1 vial, 0.8 mL
A2	Human IL-1β Capture Beads	1 vial, 0.8 mL
A3	Human IL-6 Capture Beads	1 vial, 0.8 mL
A4	Human IL-10 Capture Beads	1 vial, 0.8 mL
A5	Human TNF Capture Beads	1 vial, 0.8 mL
A6	Human IL-12p70 Capture Beads	1 vial, 0.8 mL

Vial label	Reagent	Quantity
B	Human Inflammatory Cytokine PE Detection Reagent	1 vial, 4 mL
C	Human Inflammatory Cytokine Standards	2 vials, 0.2 mL lyophilized
D	Cytometer Setup Beads	1 vial, 1.5 mL
E1	PE Positive Control Detector	1 vial, 0.5 mL
E2	FITC Positive Control Detector	1 vial, 0.5 mL
F	Wash Buffer	1 bottle, 260 mL
G	Assay Diluent	1 bottle, 30 mL
H	Serum Enhancement Buffer	1 bottle, 10 mL

Bead reagents

Human Inflammatory Cytokine Capture Beads (A1–A6): An 80-test vial of each specific capture bead (A1–A6). The specific capture beads, having discrete fluorescence intensity characteristics, are distributed from brightest (A1) to dimmest (A6).

Cytometer Setup Beads (D): A 30-test vial of setup beads for setting the initial instrument PMT voltages and compensation settings is sufficient for 10 instrument setup procedures. The Cytometer Setup Beads are formulated for use at 50 µL per test.

Antibody and standard reagents

Human Inflammatory Cytokine PE Detection Reagent (B): An 80-test vial of PE-conjugated anti-human IL-8, IL-1ß, IL-6, IL-10, TNF, and IL-12p70 antibodies, formulated for use at 50 µL per test.

Human Inflammatory Cytokine Standards (C): Two vials containing lyophilized recombinant human proteins. Each vial should be reconstituted in 2.0 mL of Assay Diluent to prepare the top standard.

PE Positive Control Detector (E1): A 10-test vial of PE-conjugated antibody control formulated for use at 50 µL per test. This reagent is used with the Cytometer Setup Beads to set the initial instrument compensation settings.

FITC Positive Control Detector (E2): A 10-test vial of FITC-conjugated antibody control formulated for use at 50 µL per test. This reagent is used with the Cytometer Setup Beads to set the initial instrument compensation settings.

Buffer reagents

Wash Buffer (F): A 260-mL bottle of phosphate buffered saline (PBS) solution (1X), containing protein and detergent, used for wash steps and to resuspend the washed beads for analysis.

Assay Diluent (G): A 30-mL bottle of a buffered protein solution (1X) used to reconstitute and dilute the Human Inflammatory Cytokine Standards and to dilute unknown samples.

Serum Enhancement Buffer (H): A 10-mL bottle of a buffered protein solution (1X) used to dilute mixed Capture Beads when testing serum or plasma samples.

Note: Source of all serum proteins is from USDA-inspected abattoirs located in the United States.

Storage and handling

Storage	Store all kit components at 2 to 8°C. Do not freeze.
Warning	Components A1–A6, B, D, E1–E2, F, G, and H contain sodium azide. Sodium azide yields a highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discharging to avoid accumulation of potentially explosive deposits in plumbing.

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Before you begin

This section covers the following topics:

Workflow overview

Workflow

The overall workflow consists of the following steps.

Step	Description
1	Preparing Human Inflammatory Cytokines Standards (page 18)
2	Mixing Human Inflammatory Cytokine Capture Beads (page 20)
3	Diluting samples (page 22)
4	Performing instrument setup with Cytometer Setup Beads (instructions can be found at bdbiosciences.com/cbasetup) **Note:** Can be performed during the incubation in step 5.
5	Performing the Human Inflammatory Cytokine Assay (page 24)
6	Acquiring samples (instructions can be found at bdbiosciences.com/cbasetup)
7	Data analysis (page 30)

Incubation times

To help you plan your work, the incubation times are listed in the following table.

Procedure	Incubation time
Preparing standards	15 minutes
Preparing mixed capture beads (when analyzing serum or plasma samples only)	30 minutes
Preparing Cytometer Setup Beads	30 minutes
Performing the assay	3 hours

Required materials

Materials required but not provided

In addition to the reagents provided in the BD CBA Human Inflammatory Cytokines Kit, the following items are also required:

- A dual-laser flow cytometer equipped with a 488-nm or 532-nm and a 633-nm or 635-nm laser capable of distinguishing 576-nm, 660-nm, and >680-nm fluorescence. The following table lists examples of compatible instrument platforms.

Flow cytometer	Reporter channel	Bead channels
BD FACSArray™	Yellow	Red
BD FACSCanto™ platform BD™ LSR platform BD FACSAria™ platform	PE	APC
BD FACSCalibur™ (single laser) BD FACSCalibur (dual laser)	FL2	FL3 FL4
Note: Visit bdbiosciences.com/cbasetup for setup protocols.		

- BD Falcon™ 12 × 75-mm sample acquisition tubes for a flow cytometer (Catalog No. 352008)
- 15-mL conical, polypropylene tubes (BD Falcon, Catalog No. 352097), or equivalent
- FCAP Array software (Catalog No. 641488 [PC] or 645447 [Mac®])

Materials required for plate loader-equipped flow cytometers

- Millipore MultiScreen$_{HTS}$-BV 1.2 µm Clear non-sterile filter plates [Catalog No. MSBVN1210 (10 pack) or MSBVN1250 (50 pack)]
- Millipore MultiScreen$_{HTS}$ Vacuum Manifold, (Catalog No. MSVMHTS00)
- MTS 2/4 Digital Stirrer, IKA Works, VWR (Catalog No. 82006-096)
- Vacuum source
- Vacuum gauge and regulator (if not using the recommended manifold)

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Assay preparation

This section covers the following topics:

Preparing Human Inflammatory Cytokines Standards

Purpose of this procedure

The Human Inflammatory Cytokines Standards are lyophilized and should be reconstituted and serially diluted immediately before mixing with the Capture Beads and the PE Detection Reagent.

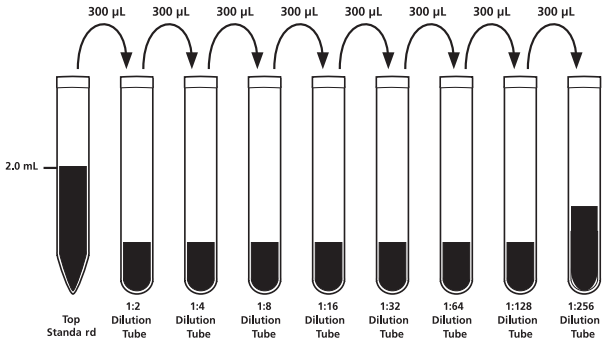
You must prepare fresh standards to run with each experiment. Do not store or reuse reconstituted or diluted standards.

Procedure

To reconstitute and serially dilute the standards:

1. Open one vial of lyophilized Human Inflammatory Cytokine Standards. Transfer the standard spheres to a 15-mL polypropylene tube. Label the tube "Top Standard."
2. Reconstitute the standards with 2 mL of Assay Diluent.
    a. Allow the reconstituted standard to equilibrate for at least 15 minutes at room temperature.
    b. Gently mix the reconstituted protein by pipette only. Do not vortex or mix vigorously.
3. Label eight 12 × 75-mm tubes and arrange them in the following order: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256.
4. Pipette 300 µL of Assay Diluent in each of the 12 × 75-mm tubes.
5. Perform a serial dilution:
    a. Transfer 300 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly by pipette only. Do not vortex.

b. Continue making serial dilutions by transferring 300 µL from the 1:2 tube to the 1:4 tube and so on to the 1:256 tube.



6. Prepare one 12 × 75-mm tube containing only Assay Diluent to serve as the 0 pg/mL negative control.

Concentration of standards

See the Performing the Human Inflammatory Cytokine Assay (page 24) for a listing of the concentrations (pg/mL of all six recombinant proteins in each standard.

Next step

Proceed to Mixing Human Inflammatory Cytokine Capture Beads (page 20).

Mixing Human Inflammatory Cytokine Capture Beads

Purpose of this procedure

The Capture Beads are bottled individually (A1–A6). You must pool all six bead reagents immediately before using them in the assay.

Mixing the beads

To mix the Capture Beads:

1. Determine the number of assay tubes (including standards and controls) that are required for the experiment (for example, 8 unknowns + 9 standard dilutions + 1 negative control = 18 assay tubes).
2. Vigorously vortex each Capture Bead suspension for 3 to 5 seconds before mixing.

   Note: The antibody-conjugated beads will settle out of suspension over time. Vortex the vial immediately before taking a bead-suspension aliquot.
3. Add a 10-µL aliquot of each Capture Bead, for each assay tube to be analyzed, into a single tube labeled "Mixed Capture Beads" (eg, 10 µL of IL-8 Capture Beads × 18 assay tubes = 180 µL of IL-8 Capture Beads required).
4. Vortex the bead mixture thoroughly.

Resuspending the beads

If you are using serum or plasma samples, you must perform this procedure to reduce the chances of false-positive results due to serum or plasma proteins. This procedure is optional for all other sample types.

To resuspend the Capture Beads in Serum Enhancement Buffer:

1. Centrifuge the mixed Capture Beads at 200*g* for 5 minutes.
2. Carefully aspirate and discard the supernatant.

3. Resuspend the mixed Capture Beads pellet in Serum Enhancement Buffer (equal to the volume removed in step 2) and vortex thoroughly.
4. Incubate the mixed Capture Beads for 30 minutes at room temperature, protected from light.

Next step

The mixed Capture Beads are now ready to be transferred to the assay tubes. Discard excess mixed Capture Beads. Do not store after mixing.

To begin the assay, proceed to Performing the Human Inflammatory Cytokine Assay (page 24). If you need to dilute samples having a high-protein concentration, proceed to Diluting samples (page 22).

Diluting samples

Purpose of this procedure

The standard curve for each protein covers a defined set of concentrations from 20 to 5,000 pg/mL. It might be necessary to dilute test samples to ensure that their mean fluorescence values fall within the range of the generated standard curve. For best results, dilute samples that are known or assumed to contain high levels of a given protein. This procedure is not required for all samples.

Procedure

To dilute samples with known high-cytokine concentration:

1. Dilute the sample by the desired dilution factor (for example, 1:2, 1:10, or 1:100) using the appropriate volume of Assay Diluent.

   Optimal recovery from serum samples typically requires a 1:4 dilution.

2. Mix sample dilutions thoroughly.

Next step

Perform instrument setup using the Cytometer Setup Beads. For details, go to bdbiosciences.com/cbasetup and select the appropriate flow cytometer under CBA Kits: Instrument Setup.

Or, if you wish to begin staining your samples for the assay, proceed to Performing the Human Inflammatory Cytokine Assay (page 24), and you can perform instrument setup during the 3-hour staining incubation.

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Assay procedure

This section covers the following topics:

Performing the Human Inflammatory Cytokine Assay

Before you begin

1. Prepare the standards as described in Preparing Human Inflammatory Cytokines Standards (page 18).
2. Mix the Capture Beads as described in Mixing Human Inflammatory Cytokine Capture Beads (page 20).
3. If necessary, dilute the unknown samples. See Diluting samples (page 22).

Procedure for supernatant samples

To perform the assay:

1. Vortex the mixed Capture Beads and add 50 µL to all assay tubes.
2. Add 50 µL of the Human Inflammatory Cytokine Standard dilutions to the control tubes as listed in the following table.

Tube label	Concentration (pg/mL)	Standard dilution
1	0 (negative control)	no standard dilution (Assay Diluent only)
2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8
8	1,250	1:4
9	2,500	1:2
10	5,000	Top Standard

3. Add 50 µL of each unknown sample to the appropriately labeled sample tubes.

4. Add 50 µL of the Human Inflammatory Cytokine PE Detection Reagent to all assay tubes.
5. Incubate the assay tubes for 3 hours at room temperature, protected from light.

   Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation.
6. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.
7. Carefully aspirate and discard the supernatant from each assay tube.
8. Add 300 µL of Wash Buffer to each assay tube to resuspend the bead pellet.

Procedure for serum/plasma samples

To perform the assay:

1. Vortex the mixed Capture Beads and add 50 µL to all assay tubes.
2. Add 50 µL of the Human Inflammatory Cytokine Standard dilutions to the control tubes as listed in the following table.

Tube label	Concentration (pg/mL)	Standard dilution
1	0 (negative control)	no standard dilution (Assay Diluent only)
2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8

Tube label	Concentration (pg/mL)	Standard dilution
8	1,250	1:4
9	2,500	1:2
10	5,000	Top Standard

3. Add 50 µL of each unknown sample to the appropriately labeled sample tubes.
4. Incubate the assay tubes for 1.5 hours at room temperature, protected from light.

   Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation, or during the incubation in step 8.
5. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.
6. Carefully and consistently aspirate and discard the supernatant, leaving approximately 100 µL of liquid in each assay tube.
7. Add 50 µL of the Human Inflammatory Cytokine PE Detection Reagent to all assay tubes. Gently agitate the tubes to resuspend the pellet.
8. Incubate the assay tubes for 1.5 hours at room temperature, protected from light.
9. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.
10. Carefully aspirate and discard the supernatant from each assay tube.
11. Add 300 µL of Wash Buffer to each assay tube to resuspend the bead pellet.

Procedure for filter plates for supernatant samples

To perform the assay:

1. Wet the plate by adding 100 µL of wash buffer to each well.
2. Place the plate on the vacuum manifold.
3. Aspirate for 2 to 10 seconds until the wells are drained.
4. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.
5. Add 50 µL of each of the following to the wells in the filter plate:
   - Capture Beads (vortex before adding)
   - Standard or sample (add standards from the lowest concentration to the highest, followed by samples)
   - Human Inflammatory Cytokine PE Detection Reagent
6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
7. Incubate the plate for 3 hours at room temperature on a non-absorbent, dry surface.

   Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.
8. Remove the cover from the plate and apply the plate to the vacuum manifold.
9. Vacuum aspirate for 2 to 10 seconds until the wells are drained.
10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.

11. Add 120 µL of wash buffer to each well to resuspend the beads.
12. Cover the plate and shake it for 2 minutes at 1,100 rpm before you begin sample acquisition.

Procedure for filter plates for serum/plasma samples

To perform the assay:

1. Wet the plate by adding 100 µL of wash buffer to each well.
2. Place the plate on the vacuum manifold.
3. Aspirate for 2 to 10 seconds until the wells are drained.
4. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.
5. Add 50 µL of each of the following to the wells in the filter plate:
   - Capture Beads (vortex before adding)
   - Standard or sample (add standards from the lowest concentration to the highest, followed by samples)
6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
7. Incubate the plate for 1.5 hours at room temperature on a non-absorbent, dry surface.

   Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.
8. Remove the cover from the plate and apply the plate to the vacuum manifold.
9. Vacuum aspirate for 2 to 10 seconds until the wells are drained.

10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.
11. Add 200 µL of wash buffer to each well. Cover the plate and shake for 2 minutes at 1,100 rpm.
12. Repeat step 8 through step 10.
13. Add 100 µL of assay diluent to each well.
14. Add 50 µL of Human Inflammatory Cytokine PE Detection Reagent to each well.
15. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
16. Incubate the plate for 1.5 hours at room temperature on a non-absorbent, dry surface.

    Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.
17. Repeat step 8 through step 10.
18. Add 120 µL of wash buffer to each well to resuspend the beads.
19. Shake the plate for 2 minutes at 1,100 rpm before you begin sample acquisition.

Next step

Acquire the samples on the flow cytometer. For details, go to bdbiosciences.com/cbasetup and select the appropriate flow cytometer under CBA Kits: Instrument Setup.

CBA samples must be acquired on the same day they are prepared. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

To facilitate the analysis of samples using FCAP Array software, we recommend the following guidelines:

- Acquire standards from lowest (0 pg/mL) to highest (Top Standard) concentration, followed by the test samples.
- If running sample dilutions, acquire sequentially starting with the most concentrated sample.
- Store all FCS files (standards and samples) in a single folder.

When you are finished acquiring samples, proceed to Data analysis (page 30).

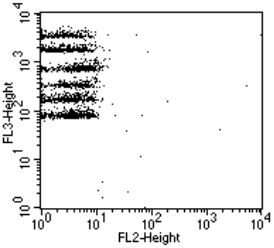
Data analysis

How to analyze

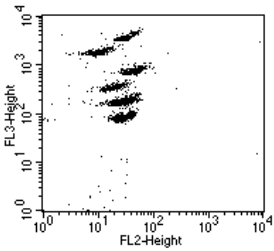
Analyze BD CBA Human Inflammatory Cytokines Kit data using FCAP Array software. For instructions on analysis, go to bdbiosciences.com/cbasetup see the *Guide to Analyzing Data from BD CBA Kits Using FCAP Array Software*.

Typical data

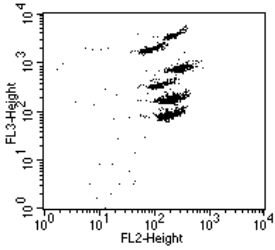
The following data, acquired using BD CellQuest software, shows standards and detectors alone.



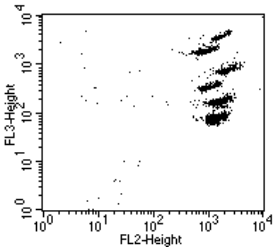
Negative control: 0 pg/mL



Standard: 20 pg/mL



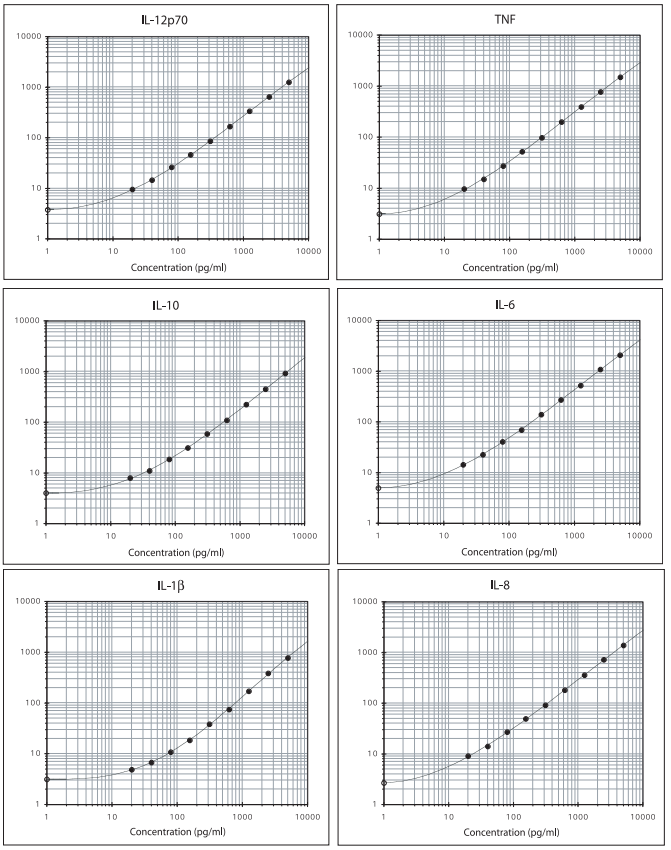
Standard: 156 pg/mL



Standard: 1,250 pg/mL

Standard curve examples

The following graphs represent standard curves from the BD CBA Human Inflammatory Cytokine Standards.



5

Performance

This section covers the following topics:

Theoretical limit of detection

Experiment details

The individual standard curve range for a given protein defines the minimum and maximum quantifiable levels (for example, 20 pg/mL and 5,000 pg/mL) using the BD CBA Human Inflammatory Cytokines Kit. By applying the four-parameter curve fit option, it is possible to extrapolate values for sample intensities not falling within the limits of the standard curve. It is up to the researcher to decide the best method for calculating values for unknown samples using this assay. The theoretical limit of detection for each protein using the BD CBA Human Inflammatory Cytokines Kit is defined as the corresponding concentration at two standard deviations above the median fluorescence of 20 replicates of the negative control (0 pg/mL).

Limit of detection data

Cytokine	Median fluorescence	Standard deviation	Limit of detection (pg/mL)
IL-8	3.4	0.4	3.6
IL-1β	3.7	0.3	7.2
IL-6	4.7	0.4	2.5
IL-10	4.1	0.4	3.3
TNF	3.9	0.3	3.7
IL-12p70	4.0	0.3	1.9

Recovery

Experiment details

Individual proteins were spiked into various matrices at three different levels within the linear assay range. The matrices used in these experiments were not diluted before addition of the protein. The plasma samples in these experiments were EDTA treated. Results are compared with the same concentrations of the proteins spiked in the Assay Diluent.

Recovery data

Protein	Matrix	Standard spike conc. (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
IL-8	Pooled donor sera (n=5)	2,500 625 80	2,145 548 66	86% 88% 82%
	Pooled donor plasmas (n=5)	2,500 625 80	2,051 473 53	82% 76% 67%
	Cell culture supernatant	2,500 625 80	2,709 661 91	108% 106% 113%
IL-1β	Pooled donor sera (n=5)	2,500 625 80	1,656 424 42	66% 68% 52%
	Pooled donor plasmas (n=5)	2,500 625 80	1,776 403 47	71% 65% 58%
	Cell culture supernatant	2,500 625 80	2,813 623 81	113% 100% 101%

Protein	Matrix	Standard spike conc. (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
IL-6	Pooled donor sera (n=5)	2,500 625 80	1,713 484 55	69% 78% 69%
	Pooled donor plasmas (n=5)	2,500 625 80	2,254 573 73	90% 92% 92%
	Cell culture supernatant	2,500 625 80	2,646 592 81	106% 95% 101%
IL-10	Pooled donor sera (n=5)	2,500 625 80	2,016 556 67	81% 89% 84%
	Pooled donor plasmas (n=5)	2,500 625 80	2,308 589 70	92% 94% 88%
	Cell culture supernatant	2,500 625 80	2,449 600 79	98% 96% 99%
TNF	Pooled donor sera (n=5)	2,500 625 80	1,507 419 45	60% 67% 56%
	Pooled donor plasmas (n=5)	2,500 625 80	1,851 457 55	74% 73% 69%
	Cell culture supernatant	2,500 625 80	2,864 665 88	115% 107% 110%

Protein	Matrix	Standard spike conc. (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
IL-12p70	Pooled donor sera (n=5)	2,500 625 80	1,366 368 46	55% 59% 57%
	Pooled donor plasmas (n=5)	2,500 625 80	1,752 409 53	70% 65% 66%
	Cell culture supernatant	2,500 625 80	2,540 600 81	102% 96% 101%

Linearity

Experiment details

In two experiments, the following matrices were spiked with IL-8, IL-1β, IL-6, IL-10, TNF, and IL-12p70 and were then serially diluted with Assay Diluent.

Linearity data

Matrix	Dilution	Observed					
		IL-8 (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TNF (pg/mL)	IL-12p70 (pg/mL)
Cell culture media	Neat	5,200	5,800	5,200	5,100	6,000	6,000
	1:2	2,520	2,665	2,590	2,494	2,597	2,560
	1:4	1,260	1,291	1,280	1,302	1,241	1,283
	1:8	602	596	635	643	606	618
	1:16	296	312	310	329	320	302
	1:32	154	143	150	156	157	154
	1:64	74	74	75	74	78	72
	1:128	39	39	41	40	45	42
	1:256	19	17	20	19	23	20
	Slope	1.01	1.04	1.01	1.01	0.99	1.02
Pooled human sera (n=5)	Neat	4,468	2,884	3,410	4,662	3,213	2,962
	1:2	2,583	1,815	2,016	2,452	2,038	1,757
	1:4	1,363	900	1,017	1,204	1,041	963
	1:8	618	458	486	559	519	470
	1:16	322	248	278	304	302	252
	1:32	129	102	99	129	124	109
	1:64	76	61	69	79	83	67
	1:128	39	46	32	36	35	30
	1:256	19	26	14	18	17	15
	Slope	1.00	0.88	1.00	1.01	0.95	0.97
Pooled human plasma (n=5)	Neat	4,493	3,692	4,734	5,408	4,284	4,391
	1:2	2,582	2,049	2,654	4,284	2,270	2,359
	1:4	1,405	1,130	1,301	2,270	1,223	1,218
	1:8	697	572	651	1,223	648	643
	1:16	347	275	315	648	294	309
	1:32	169	138	160	294	166	165
	1:64	80	72	81	166	84	83
	1:128	43	37	43	84	44	42
	1:256	22	23	19	44	20	21
	Slope	0.98	0.95	1.00	0.91	0.96	0.97

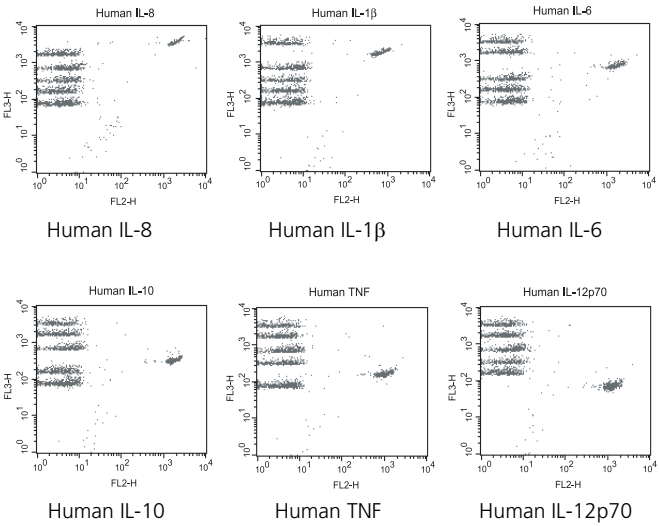
Specificity

Experiment details

The antibody pairs used in the BD CBA Human Inflammatory Cytokines Kit assay have been screened for specific reactivity with their corresponding specific proteins. Analysis of samples containing only a single recombinant protein found no cross-reactivity or background detection of protein in other Capture Bead populations using this assay.

Specificity data

Data for the detection of individual proteins was analyzed using BD CellQuest software.



Precision

Intra-assay precision

Ten replicates of each of three different levels of IL-8, IL-1β, IL-6, IL-10, TNF, and IL-12p70 were tested.

IL-8	80 pg/mL	625 pg/mL	2,500 pg/mL
Actual mean conc. (pg/mL)	74	578	2,221
SD	3	12	104
%CV	4%	2%	5%

IL-1β	80 pg/mL	625 pg/mL	2,500 pg/mL
Actual mean conc. (pg/mL)	69	540	2,220
SD	5	19	129
%CV	7%	4%	6%

IL-6	80 pg/mL	625 pg/mL	2,500 pg/mL
Actual mean conc. (pg/mL)	77	557	2,171
SD	5	25	163
%CV	6%	5%	8%

IL-10	80 pg/mL	625 pg/mL	2,500 pg/mL
Actual mean conc. (pg/mL)	76	551	2,116
SD	5	25	121
%CV	6%	5%	6%

TNF	80 pg/mL	625 pg/mL	2,500 pg/mL
Actual mean conc. (pg/mL)	70	527	1,971
SD	6	34	207
%CV	9%	6%	10%

IL-12p70	80 pg/mL	625 pg/mL	2,500 pg/mL
Actual mean conc. (pg/mL)	74	562	2,094
SD	3	19	132
%CV	4%	3%	6%

Inter-assay precision

Three different levels of IL-8, IL-1β, IL-6, IL-10, TNF, and IL-12p70 (80, 625, and 2,500 pg/mL) were tested in four experiments conducted by different operators.

Note: The number of replicates refers to the total number of assay tubes tested at a given concentration of protein.

IL-8	80 pg/mL	625 pg/mL	2,500 pg/mL
Number of replicates	8	8	8
Actual mean conc. (pg/mL)	74	626	2,618
SD	3	24	172
%CV	4%	4%	7%

IL-1β	80 pg/mL	625 pg/mL	2,500 pg/mL
Number of replicates	8	8	8
Actual mean conc. (pg/mL)	69	589	2,594
SD	9	55	196
%CV	13%	9%	11%

IL-6	80 pg/mL	625 pg/mL	2,500 pg/mL
Number of replicates	8	8	8
Actual mean conc. (pg/mL)	78	630	2,669
SD	6	55	271
%CV	8%	9%	10%

IL-10	80 pg/mL	625 pg/mL	2,500 pg/mL
Number of replicates	8	8	8
Actual mean conc. (pg/mL)	77	644	2,669
SD	6	57	306
%CV	8%	9%	11%

TNF	80 pg/mL	625 pg/mL	2,500 pg/mL
Number of replicates	8	8	8
Actual mean conc. (pg/mL)	74	631	2,607
SD	6	94	351
%CV	8%	15%	13%

IL-12p70	80 pg/mL	625 pg/mL	2,500 pg/mL
Number of replicates	8	8	8
Actual mean conc. (pg/mL)	77	654	2,563
SD	5	49	223
%CV	6%	7%	9%

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Reference

This section covers the following topics:

Troubleshooting

Recommended actions

These are the actions we recommend taking if you encounter the following problems.

For best performance, vortex samples immediately before analyzing on a flow cytometer.

Problem	Recommended action
Variation between duplicate samples	Vortex the Capture Beads before pipetting. Beads can aggregate.
Low bead number in samples	• Avoid aspiration of beads during the wash step. • Do not wash or resuspend beads in volumes higher than the recommended volumes.
High background	• Test various sample dilutions, the sample may be too concentrated. • Remove excess Human Inflammatory Cyokines PE Detection Reagent by increasing the number of wash steps, as the background may be due to non-specific binding.
Little or no detection of protein in sample	Sample may be too dilute. Try various sample dilutions.
Less than six bead populations are observed during analysis or distribution is unequal	• Ensure that equal volumes of beads were added to each assay tube. • Vortex Capture Bead vials before taking aliquots. Once Capture Beads are mixed, vortex to ensure that the beads are distributed evenly throughout the solution.

Problem	Recommended action
Debris (FSC/SSC) during sample acquisition	• Increase the FSC threshold or further dilute the samples. • Increase the number of wash steps, if necessary. • Make a tighter FSC/SSC gate around the bead population. • Centrifuge or filter samples to reduce debris before staining samples with the BD CBA Human Inflammatory Cytokines Kit.
Overlap of bead population fluorescence (FL3) during acquisition	This may occur in samples with very high protein concentration. Ensure that instrument settings have been optimized using the Cytometer Setup Beads.
Standards assay tubes show low fluorescence or a poor standard curve	• Verify that all components are properly prepared and stored. • Use a new vial of standards with each experiment, and once reconstituted, do not use after 12 hours. • Ensure that incubation times were appropriate.
All samples are positive or above the high standard mean fluorescence value	Dilute the samples further. The samples may be too concentrated.
Biohazardous samples	It is possible to treat samples briefly with 1% paraformaldehyde before acquiring on the flow cytometer. However, this may affect assay performance and should be validated.

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United States
877.232.8995

Canada
800.268.5430

Europe
32.2.400.98.95

Japan
0120.8555.90

Asia/Pacific
65.6861.0633

Latin America/Caribbean
55.11.5185.9995

Becton, Dickinson and Company
BD Biosciences
San Jose, CA 95131
Toll free: 877.232.8995 (US)
Tel: 408.432.9475
Fax: 408.954.2347
bdbiosciences.com

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