BD™ Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine Kit

Instruction Manual

Catalog No. 560484

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Regulatory information

BD cytometers are class 1 laser products.

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

History

Revision	Date	Change made
647210	2/09	Initial release
23-12381-00 Rev. 01	10/2010	Setup and acquisition instructions removed
23-12381-01	2/2013	Updated patent information

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 BD CBA Human Th1/Th2/Th17 Cytokine Kit can be used to measure Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor Necrosis Factor (TNF), Interferon-γ (IFN-γ), and Interleukin-17A (IL-17A) protein levels in a single sample. The kit performance has been optimized for analysis of physiologically relevant concentrations (pg/mL levels) of specific cytokine proteins in tissue culture supernatants, EDTA plasma, and serum samples. 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 a BD CBA 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

The BD CBA BD CBA Human Th1/Th2/Th17 Cytokine Kit uses bead array technology to simultaneously detect multiple cytokine proteins in research samples. Seven bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A proteins. The seven bead populations are mixed together to form the bead array, which is resolved in a red channel (ie, FL3 or FL4) of a flow cytometer.

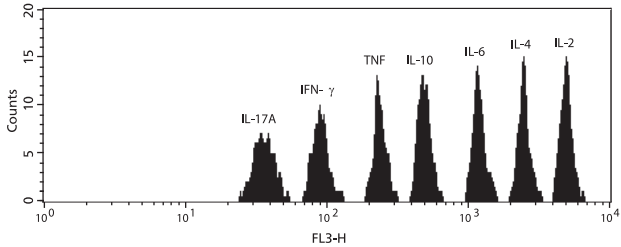

Figure 1

During the assay procedure, you will mix the 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 the BD CBA assay 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-seventh the quantity necessary for conventional ELISA assays due to the detection of seven 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 seven conventional ELISAs.

Limitations

Assay limitations

The theoretical limit of detection of the BD CBA BD CBA Human Th1/Th2/Th17 Cytokine Kit is comparable to conventional ELISA, but due to the complexity and kinetics of this multi-analyte assay, the actual limit of detection on a given experiment may vary. See Theoretical limit of detection (page 32) and Precision (page 39).

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).

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.

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

Kit contents

Contents

The BD CBA Human Th1/Th2/Th17 Cytokine Kit contains the following components sufficient for 80 tests.

Vial label	Reagent	Quantity
A1	Human IL-2 Capture Beads	1 vial, 0.8 mL
A2	Human IL-4 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 IFN-γ Capture Beads	1 vial, 0.8 mL
A7	Human IL-17A Capture Beads	1 vial, 0.8 mL
B	Human Th1/Th2/Th17 PE Detection Reagent	1 vial, 4 mL
C	Human Th1/Th2/Th17 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

Vial label	Reagent	Quantity
F	Wash Buffer	1 bottle, 130 mL
G	Assay Diluent	1 bottle, 30 mL
H	Serum Enhancement Buffer	1 bottle, 10 mL

Bead reagents

Human Cytokine Capture Beads (A1–A7): An 80-test vial of each specific capture bead (A1–A7). The specific capture beads, having discrete fluorescence intensity characteristics, are distributed from brightest (A1) to dimmest (A7).

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/test.

Antibody and standard reagents

Human Th1/Th2/Th17 PE Detection Reagent (B): An 80-test vial of PE-conjugated anti-human IL-2, IL-4, IL-6, IL-10, TNF, IFNγ, and IL-17A antibodies that is formulated for use at 50 µL/test.

Human Th1/Th2/Th17 Cytokine Standards (C): Two vials containing lyophilized recombinant human cytokine 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 that is formulated for use at 50 µL/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 that is formulated for use at 50 µL/test. This reagent is used with the Cytometer Setup Beads to set the initial instrument compensation settings.

Buffer reagents

Wash Buffer (F): A 130-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 Th1/Th2/Th17 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–A7, B, D, E1, E2, F, G, and H contain sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding 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 Th1/Th2/Th17 Cytokine Standards (page 18)
2	Mixing Human Th1/Th2/Th17 Cytokine Capture Beads (page 20)
3	Diluting samples (page 21), if necessary
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 Th1/Th2/Th17 Cytokine Assay (page 24)
6	Acquiring samples (instructions can be found at bdbiosciences.com/cbasetup)
7	Data Analysis (page 27)

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 BD CBA Human Th1/Th2/Th17 Cytokine 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 (Catalog No. 352008), or equivalent
- 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 Th1/Th2/Th17 Cytokine Standards

Purpose of this procedure

The Human Th1/Th2/Th17 Cytokine 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 cytokine standards to run with each experiment. Do not store or reuse reconstituted or diluted standards.

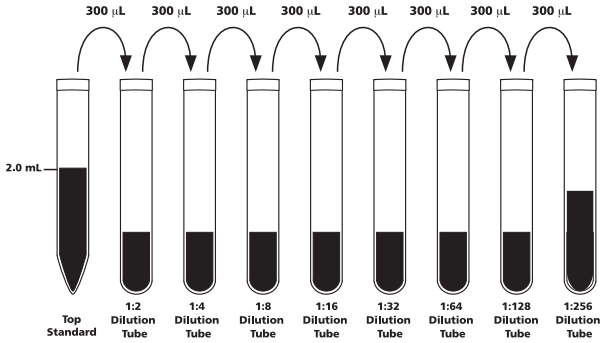
Procedure

To reconstitute and serially dilute the standards:

1. Open one vial of lyophilized Human Th1/Th2/Th17 Standards. Transfer the standard spheres to a 15-mL conical, 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 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 serial dilutions:
    a. Transfer 300 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly by pipette only.

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.

c. Mix thoroughly by pipette only. Do not vortex.



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 Procedure for tubes section of Performing the Human Th1/Th2/Th17 Cytokine Assay (page 24) for a listing of the concentrations (pg/mL) of all seven recombinant proteins in each standard dilution.

Next step

Proceed to Mixing Human Th1/Th2/Th17 Cytokine Capture Beads (page 20).

Mixing Human Th1/Th2/Th17 Cytokine Capture Beads

Purpose of this procedure

The Capture Beads are bottled individually (A1–A7). You must pool all seven 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) required for the experiment (eg, 8 unknowns, 9 cytokine standard dilutions, and 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. It is necessary to vortex the vial 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-2 Capture Beads × 18 assay tubes = 180 µL of IL-2 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. 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 Th1/Th2/Th17 Cytokine Assay (page 24). If you need to dilute samples having a high cytokine concentration, proceed to Diluting samples (page 21).

Diluting samples

Purpose of this procedure

The standard curve for each cytokine covers a defined set of concentrations from 20 to 5000 pg/mL. It might be necessary to dilute samples to ensure that their mean fluorescence values fall within the range of the generated cytokine standard curve. For best results, dilute samples that are known or assumed to contain high levels of a given cytokine. This procedure is not necessary for all samples.

Procedure

To dilute samples with a known high cytokine concentration:

1. Dilute the sample by the desired dilution factor (ie, 1:2, 1:10, or 1:100) using the appropriate volume of Assay Diluent.
2. Mix sample dilutions thoroughly.

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

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 Th1/Th2/Th17 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 Th1/Th2/Th17 Cytokine Assay

Before you begin

- Prepare the standards as described in Preparing Human Th1/Th2/Th17 Cytokine Standards (page 18).
- Mix the Capture Beads as described in Mixing Human Th1/Th2/Th17 Cytokine Capture Beads (page 20). Be sure to follow the appropriate procedure (cell culture supernatant vs serum/plasma) for your sample type.
- If necessary, dilute the unknown samples. See Diluting samples (page 21).

Procedure for tubes

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 Th1/Th2/Th17 Cytokine Standard dilutions to the control tubes as listed in the following table:

Tube label	Concentration (pg/mL)	Cytokine 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 Th1/Th2/Th17 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 filter plates

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 Th1/Th2/Th17 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.

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 27).

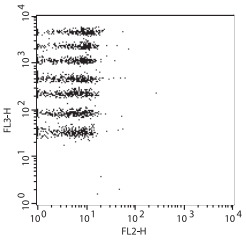
Data Analysis

How to analyze

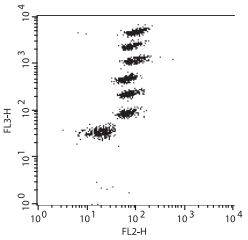
Analyze Human Th1/Th2/Th17 Cytokine data using FCAP Array software. For instructions on analysis, go to bdbiosciences.com/cbasetup and see the *Guide to Analyzing Data from BD CBA Kits Using FCAP Array Software*.

Typical data

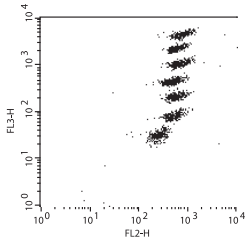
The following data, acquired using BD CellQuest software, shows standards and detectors alone.



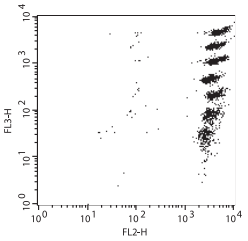
Negative control (0 pg/mL)



Standard 80 pg/mL



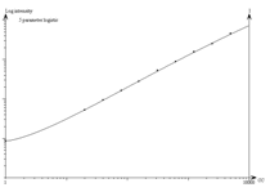
Standard 625 pg/mL



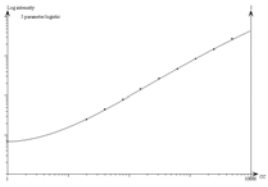
Standard 5000 pg/mL

Standard curve examples

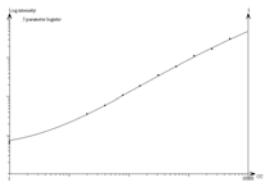
The following graphs represent standard curves from the Human Th1/Th2/Th17 Cytokine Standards.



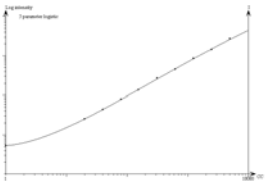
IL-2



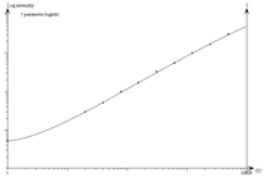
IL-4



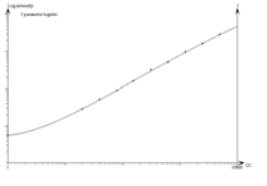
IL-6



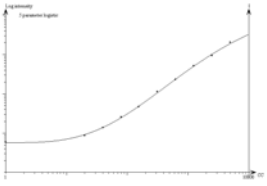
IL-10



TNF



IFN-γ



IL-17A

5

Performance

This section covers the following topics:

Theoretical limit of detection

Experiment details

The individual standard curve range for a given cytokine defines the minimum and maximum quantifiable levels using the BD CBA Human Th1/Th2/Th17 Cytokine Kit (ie, 20 pg/mL and 5000 pg/mL). By applying the 4-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 cytokine using the BD CBA Human Th1/Th2/Th17 Cytokine Kit is defined as the corresponding concentration at two standard deviations above the median fluorescence of 30 replicates of the negative control (0 pg/mL).

Limit of detection data

Cytokine	Limit of detection (pg/mL)
IL-2	2.6
IL-4	4.9
IL-6	2.4
IL-10	4.5
TNF	3.8
IFN-γ	3.7
IL-17A	18.9

Recovery

Experiment details

Individual cytokine protein was spiked into various matrices at three different levels within the assay range. The spiked samples were assayed and the results were compared with the expected values. The cell culture medium used in these experiments was not diluted before addition of the cytokine protein. Pooled human serum and pooled human plasma samples were diluted 1:4 in Assay Diluent before addition of cytokine protein. The plasma samples in these experiments were EDTA treated.

Recovery data

Cytokine	Matrix	Average % Recovery	Range (%)
IL-2	Media	83	72–95
	Serum	86	83–91
	Plasma	84	78–92
IL-4	Media	81	75–87
	Serum	91	87–94
	Plasma	87	85–88
IL-6	Media	86	79–92
	Serum	90	88–92
	Plasma	93	91–98
IL-10	Media	86	80–92
	Serum	95	93–96
	Plasma	91	89–94
TNF	Media	88	81–95
	Serum	95	93–97
	Plasma	95	92–98
IFN-γ	Media	80	72–89
	Serum	84	82–87
	Plasma	76	76–77
IL-17A	Media	84	74–93
	Serum	74	60–92
	Plasma	73	55–93

Linearity

Experiment details

In two experiments, the following matrices were spiked with IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A and then were serially diluted with Assay Diluent.

Linearity data

Cytokine	Matrix	Sample dilution	Detected (pg/mL)	Average % of expected
IL-2	Media	1:2 1:4 1:8	1020.8 469.4 208.2	100 92 82
	Serum	1:2 1:4 1:8	1161.5 514.0 241.0	100 89 83
	Plasma	1:2 1:4 1:8	1001.4 480.2 232.9	100 96 93
IL-4	Media	1:2 1:4 1:8	958.4 464.2 222.0	100 97 93
	Serum	1:2 1:4 1:8	1119.9 523.8 263.7	100 94 94
	Plasma	1:2 1:4 1:8	937.5 494.6 250.3	100 106 107
IL-6	Media	1:2 1:4 1:8	1101.5 498.1 236.7	100 90 86
	Serum	1:2 1:4 1:8	1203.6 567.8 275.7	100 94 92
	Plasma	1:2 1:4 1:8	1063.0 577.2 272.5	100 109 103

Cytokine	Matrix	Sample dilution	Detected (pg/mL)	Average % of expected
IL-10	Media	1:2 1:4 1:8	1049.2 506.0 240.9	100 96 92
	Serum	1:2 1:4 1:8	1167.6 543.3 270.1	100 93 93
	Plasma	1:2 1:4 1:8	1013.6 506.8 250.1	100 100 99
TNF	Media	1:2 1:4 1:8	1077.0 518.4 238.5	100 96 89
	Serum	1:2 1:4 1:8	1277.8 574.2 273.2	100 90 86
	Plasma	1:2 1:4 1:8	1084.1 573.3 273.5	100 106 101
IFN-γ	Media	1:2 1:4 1:8	946.1 441.6 219.7	100 93 93
	Serum	1:2 1:4 1:8	1039.1 466.9 219.0	100 90 84
	Plasma	1:2 1:4 1:8	857.7 451.3 214.6	100 105 100

Cytokine	Matrix	Sample dilution	Detected (pg/mL)	Average % of expected
IL-17A	Media	1:2 1:4 1:8	1106.7 521.8 244.8	100 94 88
	Serum	1:2 1:4 1:8	937.3 496.4 256.8	100 106 110
	Plasma	1:2 1:4 1:8	752.9 466.0 226.4	100 124 120

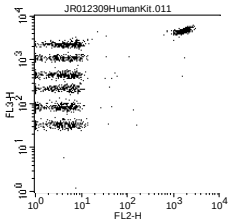
Specificity

Experiment details

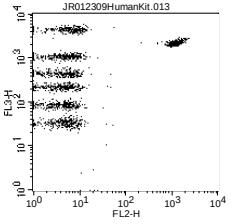
The antibodies used in the BD CBA Human Th1/Th2/Th17 Cytokine Kit have been screened for specific reactivity with their specific cytokines. Analysis of samples containing only a single recombinant cytokine protein found no cross-reactivity or background detection of cytokine in other Capture Bead populations using this assay.

Specificity data

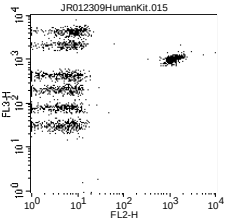
Sample data containing only a single recombinant cytokine protein was acquired using BD CellQuest software.



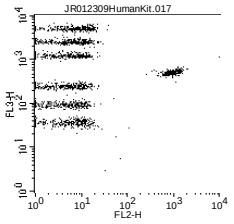
Human IL-2



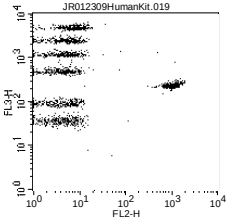
Human IL-4



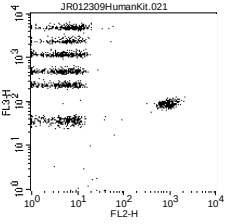
Human IL-6



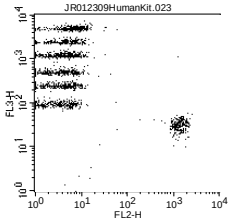
Human IL-10



Human TNF



Human IFN-γ



Human IL-17A

Precision

Intra-assay precision

Ten replicates of each of three different levels of IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A were tested.

Cytokine	Sample	Mean (pg/mL)	Standard deviation	%CV
IL-2	Sample 1	67.8	2.9	4
	Sample 2	279.1	14.8	5
	Sample 3	1238.5	46.0	4
IL-4	Sample 1	78.1	3.8	5
	Sample 2	294.5	10.2	3
	Sample 3	1231.2	25.8	2
IL-6	Sample 1	75.8	4.5	6
	Sample 2	303.1	14.1	5
	Sample 3	1284.1	55.5	4
IL-10	Sample 1	76.2	4.1	5
	Sample 2	296.3	11.3	4
	Sample 3	1272.2	46.8	4
TNF	Sample 1	75.1	6.0	8
	Sample 2	302.6	20.4	7
	Sample 3	1316.9	74.5	6
IFN-γ	Sample 1	69.6	2.4	3
	Sample 2	280.9	11.4	4
	Sample 3	1233.7	53.1	4
IL-17A	Sample 1	76.9	3.6	5
	Sample 2	305.7	7.8	3
	Sample 3	1308.9	52.8	4

Inter-assay precision

Three different levels of IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A were tested in four experiments conducted by four different operators.

Cytokine	Sample	Mean (pg/mL)	Standard deviation	%CV
IL-2	Sample 1 Sample 2 Sample 3	71.0 290.0 1230.3	6.3 20.3 82.0	9 7 7
IL-4	Sample 1 Sample 2 Sample 3	76.9 297.7 1217.6	8.1 18.6 57.6	11 6 5
IL-6	Sample 1 Sample 2 Sample 3	77.7 297.8 1254.4	10.4 25.3 89.0	13 8 7
IL-10	Sample 1 Sample 2 Sample 3	77.8 296.0 1245.8	8.3 18.8 93.7	11 6 8
TNF	Sample 1 Sample 2 Sample 3	77.8 300.0 1263.7	9.4 26.9 96.8	12 9 8
IFN-γ	Sample 1 Sample 2 Sample 3	74.4 291.2 1239.8	8.3 26.6 95.1	11 9 8
IL-17A	Sample 1 Sample 2 Sample 3	75.1 303.7 1272.8	9.4 22.7 76.8	12 7 6

6

Reference

This section covers the following topics:

Troubleshooting

Recommended actions

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

Problem	Recommended actions
Variation between duplicate samples	Vortex Capture Beads before pipetting. Beads can aggregate.
Low bead number in samples	Avoid aspiration of beads during wash step. Do not wash or resuspend beads in volumes higher than recommended volumes.
High background	Test various sample dilutions. The sample might be too concentrated. Remove excess Human Th1/Th2/TH17 II PE Detection Reagent by increasing the number of wash steps, since background may be due to non-specific binding.
Little or no protein detected in sample	Sample may be too dilute. Try various sample dilutions.
Less than seven bead populations 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 beads are distributed throughout solution.
Debris (FSC/SSC) during sample acquisition	Increase FSC threshold or further dilute samples. Increase number of wash steps, if necessary.
Overlap of bead fluorescence (FL3) during acquisition	Samples might have very high cytokine concentration. Ensure instrument settings have been optimized using Cytometer Setup Beads.

Problem	Recommended actions
Standards show low fluorescence or poor standard curve	Ensure that all components are properly prepared and stored. Use new vial of standard with each experiment and once reconstituted, do not use after 12 hours. Ensure that incubation times were of proper length.
All samples are positive or above high standard mean fluorescence value	Dilute samples further. Samples might be too concentrated.
Biohazardous samples	You may treat samples briefly with 1% paraformaldehyde before acquiring samples on the flow cytometer. However, this may affect assay performance and should be validated by the user.

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