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

Instruction Manual

Catalog No. 551809

BD

Trademarks

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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 Th1/Th2 Cytokine Kit II (Catalog No. 551809) can be used to quantitatively measure Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor Necrosis Factor (TNF), and Interferon-γ (IFN-γ) protein levels in a single sample. The kit performance has been optimized for analysis of specific cytokines in tissue culture supernatants, EDTA plasma, and serum samples. The kit provides sufficient reagents for the quantitative analysis of 80 samples.

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 the assay

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

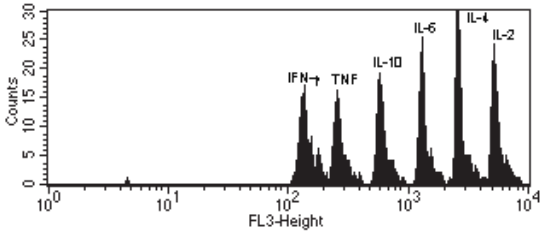

Figure 1

During the assay procedure, you will mix the cytokine capture beads with 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 fluorescence 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-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

The theoretical limit of detection of the BD CBA Human Th1/Th2 Cytokine Kit II 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 slightly. See Theoretical limit of detection (page 32) and Precision (page 38).

The BD CBA assay 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 Influx™, and BD FACSVantage™ flow cytometers (BD Biosciences, San Jose, CA).

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

This BD CBA Human Th1/Th2 Cytokine Kit II 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
B	Human Th1/Th2 - II PE Detection Reagent	1 vial, 4 mL
C	Human Th1/Th2 Cytokine Standards	2 vials 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–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/test.

Antibody and standard reagents

Human Th1/Th2 - II PE Detection Reagent (B): An 80-test vial of mixed PE-conjugated anti-human IL-2, IL-4, IL-6, IL-10, TNF, and IFN-γ antibodies formulated for use at 50 µL/test.

Human Th1/Th2 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 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 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 Cytokine Standards and to dilute test 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.

Appearance: The visual appearance of Assay Diluent may range in color from red to yellow/orange. The visual appearance of ELISA Dilution reagent may range in color from clear to cloudy white.

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. Human Th1/Th2 Cytokine Standards (component 51-2428KC) contains 0.02% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).
Hazard statements	May cause an allergic skin reaction.
Precautionary statements	Wear protective gloves / eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/ attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/ regional/national/international regulations.

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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	Preparation of Human Th1/Th2 Cytokine Standards (page 18)
2	Mixing Human Th1/Th2 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 Th1/Th2 Cytokine II Assay (page 24)
6	Acquiring samples (instructions can be found at bdbiosciences.com/cbasetup)
7	Data analysis (page 28)

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 Capture Beads	30 minutes (for serum/plasma samples only)
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 Th1/Th2 Cytokine Kit II, 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.		

- Falcon® 12 × 75-mm sample acquisition tubes (Catalog No. 352008), or equivalent
- 15-mL conical, polypropylene tubes (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:

Preparation of Human Th1/Th2 Cytokine Standards

Purpose of this procedure

The Human Th1/Th2 Cytokine Standards are lyophilized and must 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:

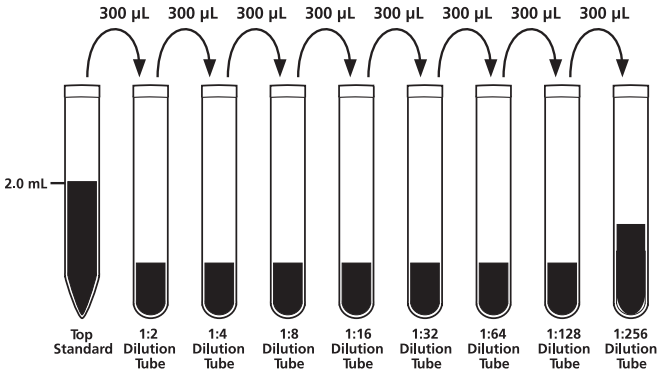
1. Open one vial of lyophilized Human Th1/Th2 Standards. Transfer the standard spheres to a 15-mL conical, polypropylene tube. Label the tube "Top Standard."
2. Reconstitute the standards with 2.0 mL of Assay Diluent.
   a. Allow the reconstituted standard to equilibrate for at least 15 minutes at room temperature.
   b. Gently mix reconstituted protein by pipet 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 to each of the remaining tubes.

5. Perform a serial dilution:

   a. Transfer 300 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly by pipet 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 pipet only. Do not vortex.



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

Concentration of standards

See the table in Performing the Human Th1/Th2 Cytokine II Assay (page 24) for a listing of the concentrations (pg/mL) of all six recombinant proteins in each standard dilution.

Next step

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

Mixing Human Th1/Th2 Cytokine Capture Beads

Purpose of this procedure

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

Follow the procedure to mix the Capture Beads for all sample types. Then perform additional steps to incubate the beads in Serum Enhancement Buffer, if analyzing serum and/or plasma samples, to reduce the chances of false-positive results due to the effects of serum or plasma proteins.

Procedure

To mix the Capture Beads when testing any sample type:

1. Determine the number of assay tubes (including standards and controls) that are 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 a few seconds before mixing.
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.
5. If you are testing serum or plasma samples, you will need to incubate the Capture Beads in Serum Enhancement Buffer. Proceed to Additional steps when testing serum and plasma samples (page 21).

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 Cytokine II Assay (page 24). If you need to dilute samples having high cytokine concentration, proceed to Diluting samples (page 22).

Additional steps when testing serum and plasma samples

Note: These steps can also be performed when testing cell culture supernatants.

When testing serum and/or plasma samples:

1. Perform the procedure to mix the beads in Procedure (page 20).
2. Centrifuge the mixed Capture Beads at 200*g* for 5 minutes.
3. Carefully aspirate and discard the supernatant.
4. Resuspend the mixed Capture Beads pellet in Serum Enhancement Buffer (equal volume to amount removed in step 3) and vortex thoroughly.
5. Incubate the mixed Capture Beads for 30 minutes at room temperature, protected from light.

Next step

To begin the assay, proceed to Performing the Human Th1/Th2 Cytokine II Assay (page 24). If you need to dilute samples having a high cytokine concentration, proceed to Diluting samples (page 22).

Diluting samples

Purpose of this procedure

The standard curve for each cytokine covers a defined set of concentrations from 20 to 5,000 pg/mL. It may be necessary to dilute test 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.

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.

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 Cytokine II Assay (page 24), and you can perform instrument setup during the 3-hour staining incubation.

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Assay procedure

Performing the Human Th1/Th2 Cytokine II Assay

Before you begin

- Prepare the standards as described in Preparation of Human Th1/Th2 Cytokine Standards (page 18).
- Mix the Capture Beads as described in Mixing Human Th1/Th2 Cytokine Capture Beads (page 20).
- If necessary, dilute the unknown samples. See Diluting samples (page 22).

Procedure for tubes

Follow these steps regardless of whether you are testing cell culture supernatants or serum/plasma samples. If testing serum and/or plasma samples, be sure to use the Capture Beads that you prepared specifically for the serum/plasma samples. See Additional steps when testing serum and plasma samples (page 21).

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 Cytokine Standard dilutions to the control assay 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

Tube label	Concentration (pg/mL)	Cytokine 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. Add 50 µL of the Human Th1/Th2 - II 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 - II 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 the 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 28).

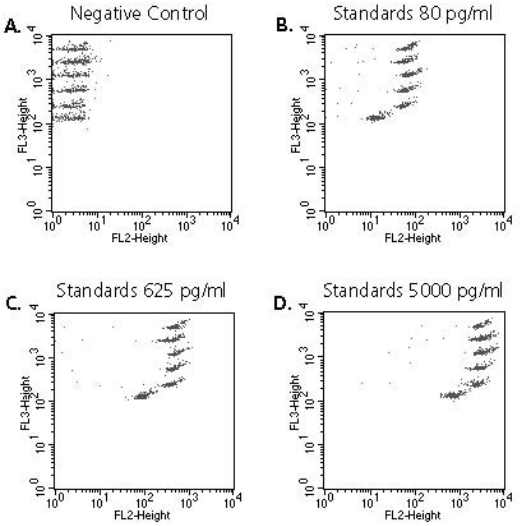
Data analysis

How to analyze

Analyze BD CBA Human Th1/Th2 Cytokine Kit II 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*.

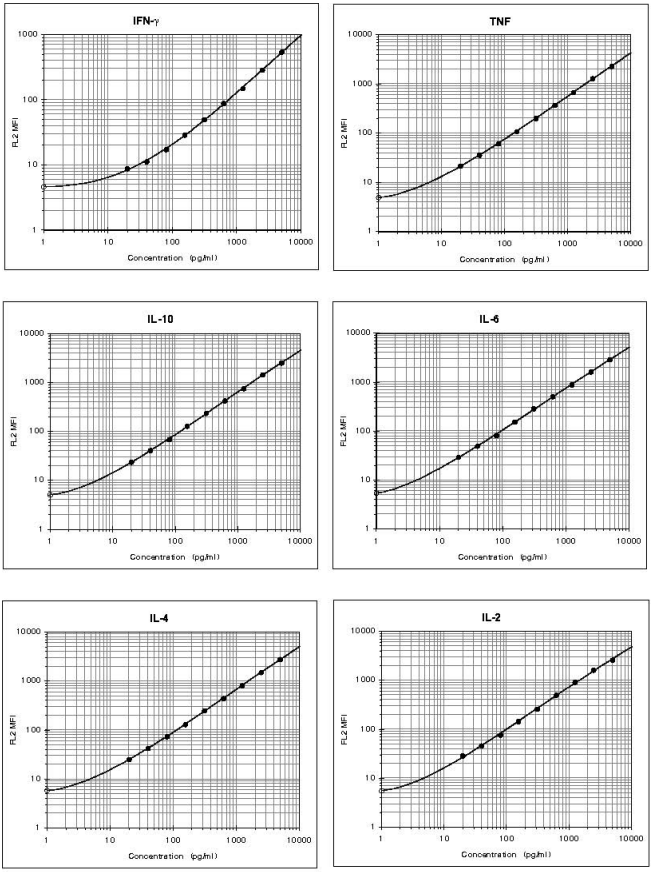
Typical Data

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



Standard curve examples

The following graphs represent standard curves from the BD CBA Human Th1/Th2 Cytokine Kit II.



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Performance

Theoretical limit of detection

Experiment details

The individual standard curve range for a given cytokine defines the minimum and maximum quantifiable levels (20 pg/mL and 5,000 pg/mL) using the BD CBA Human Th1/Th2 Cytokine Kit II. 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 Cytokine Kit II 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-2	3.3	0.2	2.6
IL-4	2.3	0.2	2.6
IL-6	2.6	0.2	3.0
IL-10	2.4	0.2	2.8
TNF	2.0	0.2	2.8
IFN-γ	2.1	0.3	7.1

Recovery

Experiment details

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

Recovery data

Cytokine	Matrix	Standard spike concentration (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
IL-2	Pooled donor sera (n=5)	2,500 625 80	2,028 439 67	81% 70% 84%
	Pooled donor plasma (n=5)	2,500 625 80	1,870 439 50	87% 70% 62%
	Cell culture supernatant	2,500 625 80	2,471 737 86	99% 118% 107%
IL-4	Pooled donor sera (n=5)	2,500 625 80	1,937 475 70	78% 76% 88%
	Pooled donor plasma (n=5)	2,500 625 80	1,863 785 58	76% 78% 73%
	Cell culture supernatant	2,500 625 80	2,367 684 84	95% 110% 104%

Cytokine	Matrix	Standard spike concentration (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
IL-6	Pooled donor sera (n=5)	2,500 625 80	2,187 533 73	88% 85% 91%
	Pooled donor plasma (n=5)	2,500 625 80	1,959 491 61	84% 79% 76%
	Cell culture supernatant	2,500 625 80	2,523 720 83	101% 115% 104%
IL-10	Pooled donor sera (n=5)	2,500 625 80	2,004 506 60	80% 81% 76%
	Pooled donor plasma (n=5)	2,500 625 80	1,967 499 62	80% 80% 77%
	Cell culture supernatant	2,500 625 80	2,530 719 83	101% 115% 104%
TNF	Pooled donor sera (n=5)	2,500 625 80	1,907 487 60	76% 78% 75%
	Pooled donor plasma (n=5)	2,500 625 80	1,773 477 58	70% 76% 72%
	Cell culture supernatant	2,500 625 80	2,552 743 85	102% 119% 107%

Cytokine	Matrix	Standard spike concentration (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
IFN-γ	Pooled donor sera (n=5)	2,500 625 80	1,390 403 46	56% 65% 57%
	Pooled donor plasma (n=5)	2,500 625 80	1,494 441 61	67% 71% 77%
	Cell culture supernatant	2,500 625 80	2,184 711 79	87% 114% 99%

Linearity

Experiment details

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

Linearity data

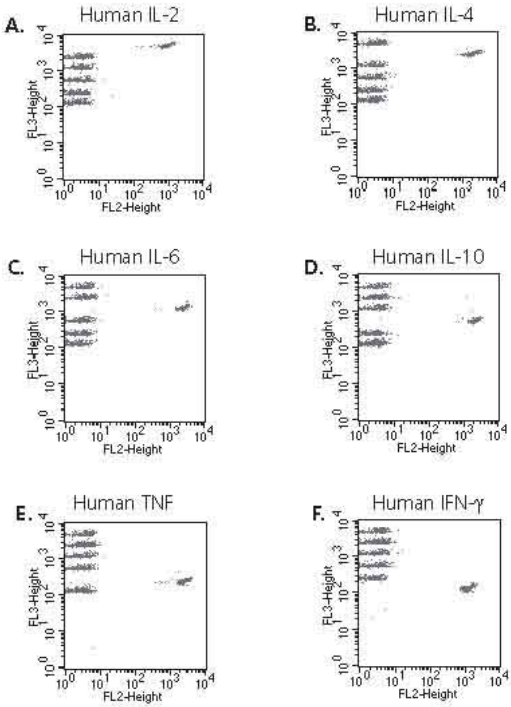
Matrix	Dilution	Observed (pg/mL)					
		IL-2	IL-4	IL-6	IL-10	TNF	IFN-γ
Pooled donor sera (n=5)	Neat	3,733	3,926	4,017	3,886	3,765	2,462
	1:2	2,020	2,147	2,175	2,169	1,920	1,407
	1:4	1,025	1,210	1,166	1,122	1,144	821
	1:8	497	611	600	582	630	450
	1:16	237	303	305	289	303	220
	1:32	120	153	141	147	155	112
	1:64	61	82	78	79	86	59
	1:128	28	40	40	39	43	42
	1:256	15	21	20	19	21	19
	Slope	1.09	1.00	1.00	0.99	0.95	1.19
Pooled donor plasma (n=5)	Neat	3,600	3,752	3,656	3,788	3,523	2,980
	1:2	1,981	2,225	2,092	2,166	2,088	1,630
	1:4	922	1,103	1,043	1,070	1,072	824
	1:8	448	596	547	558	600	435
	1:16	216	316	294	300	328	219
	1:32	100	163	159	154	174	113
	1:64	50	79	75	75	86	63
	1:128	23	40	38	36	45	36
	1:256	14	21	20	19	23	28
	Slope	1.12	0.99	0.97	0.99	0.93	1.17
Cell culture medium	Neat	4,376	4,468	4,706	4,561	4,554	4,350
	1:2	2,622	2,499	2,610	3,109	2,679	2,186
	1:4	1,277	1,212	1,289	1,288	1,345	1,028
	1:8	616	622	634	657	668	508
	1:16	286	307	316	331	335	251
	1:32	137	156	160	160	171	128
	1:64	69	83	78	83	87	66
	1:128	33	40	43	41	44	37
	1:256	15	19	21	20	21	27
	Slope	1.11	1.02	1.00	1.00	0.99	1.11

Specificity

Experiment details

The antibody pairs used in the BD CBA Human Th1/Th2 Cytokine Kit II assay 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. The following data shows the detection of individual cytokines.

Specificity data



Precision

Intra-assay precision

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

Cytokine	Actual Mean Conc (pg/mL)	Standard deviation	%CV
IL-2	64 514 2,172	3 25 51	4 5 2
IL-4	73 590 2,477	3 21 71	4 3 3
IL-6	70 571 2,383	2 14 77	3 2 3
IL-10	70 577 2,447	2 10 70	3 2 3
TNF	73 581 2,422	3 12 71	4 2 3
IFN-γ	64 493 1,916	3 17 85	4 3 4

Inter-assay

Three different levels of IL-2, IL-4, IL-6, IL-10, TNF, and IFN-γ (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.

Cytokine	Number of replicates	Actual Mean Conc (pg/mL)	Standard deviation	%CV
IL-2	8	64	6	9
	8	538	34	6
	8	2,446	100	4
IL-4	8	70	5	7
	8	576	17	3
	8	2,535	86	3
IL-6	8	71	5	7
	8	581	17	3
	8	2,502	88	3
IL-10	8	74	5	6
	8	586	22	4
	8	2,562	95	4
TNF	8	77	5	6
	8	613	21	3
	8	2,562	88	3
IFN-γ	8	56	6	11
	8	542	33	6
	8	2,260	121	5

6

Reference

Troubleshooting

Recommended actions

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

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

Note: The BD CBA Human Th1/Th2 Cytokine Kit II assay has been shown to detect IL-4, IL-6, TNF, and IFN-γ produced by the activation of cells from the non-human primate rhesus and cynomolgus models. Direct quantitation of cytokines from the rhesus and cynomolgus models has not been validated using this kit and results may vary.

Problem	Suggested solution
Variation between duplicate samples.	Vortex 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 recommended volumes.
High background.	Test various sample dilutions. The sample may be too concentrated. Remove excess Human Th1/Th2 - II PE Detection Reagent by increasing the number of wash steps since 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	Suggested solution
Debris (FSC/SSC) during sample acquisition. Also for plasma samples.	Increase the FSC threshold or further dilute samples. Increase the number of wash steps if necessary. Make a tighter FSC/SSC region gate around the bead population.
Overlap of bead population fluorescence (FL3) during acquisition.	This may occur in samples with very high cytokine concentration. Ensure that instrument settings have been optimized using the Cytometer Setup Beads.
Standards assay tubes show low fluorescence or poor standard curve.	Check that all components are properly prepared and stored. Use a 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 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 analyzing on the flow cytometer. However, this may affect assay performance and should be validated by the user.

References

Related publications

1. Bishop JE, Davis KA. A flow cytometric immunoassay for beta2-microglobulin in whole blood. *J Immunol Methods.* 1997;210:79-87.
2. Camilla C, Defoort JP, Delaage M, et al. A new flow cytometry-based multi-assay system. 1. Application to cytokine immunoassays. *Cytometry Suppl.* 1998;8:132.
3. Carson R, Vignali D. Simultaneous quantitation of fifteen cytokines using a multiplexed flow cytometric assay. *J Immunol Methods.* 1999;227:41-52.
4. Chen R, Lowe L, Wilson JD, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin Chem.* 1999;45:1693-1694.
5. Collins DP, Luebering BJ, Shaut DM. T-lymphocyte functionality assessed by analysis of cytokine receptor expression, intracellular cytokine expression, and femtomolar detection of cytokine secretion by quantitative flow cytometry. *Cytometry.* 1998;33:249-255.
6. Cook EB, Stahl JL, Lowe L, et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. *J Immunol Methods.* 2001;254:109-118.
7. Dotti G, Savoldo B, Takahashi S, et al. Adenovector-induced expression of human-CD40-ligand (hCD40L) by multiple myeloma cells: A model for immunotherapy. *Exp Hematol.* 2001;29:952-961.

8. Fulton RJ, McDade RI, Smith PI, Kienker LJ, Kettman JR Jr. Advanced multiplexed analysis with the FlowMetrix system. *Clin Chem*. 1997;43:1749-1756.

9. Kricka LJ. Simultaneous multianalyte immunoassays. In: Immunoassay. Diamandis EP, Christopoulos TK, eds. Academic Press. 1996:389-404.

10. Lund-Johansen F, Davis K, Bishop J, de Waal Malefyt R. Flow cytometric analysis of immunoprecipitates: High-throughput analysis of protein phosphorylation and protein-protein interactions. *Cytometry*. 2000;39:250-259.

11. McHugh TM. Flow microsphere immunoassay for the quantitative and simultaneous detection of multiple soluble analytes. *Methods Cell Bio*. 1994;42:575-595.

12. Oliver KG, Kettman JR, Fulton RJ. Multiplexed analysis of human cytokines by use of the FlowMetrix system. *Clin Chem*. 1998;44:2057-2060.

13. Stall A, Sun Q, Varro R, et al. A single tube flow cytometric multibead assay for isotyping mouse monoclonal antibodies. Abstract LB77. Experimental Biology Meeting 1998 (late-breaking abstracts).

Notes

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