

BD Cytometric Bead Array (CBA) Human Chemokine Kit

Instruction Manual

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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
23-11111-00 Rev. A	1/2010	Initial document
23-11111-01 Rev. 01	10/2010	Setup and acquisition instructions removed
23-11111-02 Rev. 01	4/2011	Updated chemokine labels in Figure 1

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About this kit

This section covers the following topics:

- [Purpose of the kit \(page 6\)](#)
- [Limitations \(page 8\)](#)
- [Kit contents \(page 10\)](#)
- [Storage and handling \(page 12\)](#)

Purpose of the kit

Use of the kit The BD™ CBA Human Chemokine Kit can be used to quantitatively measure interleukin-8 (CXCL8/IL-8), RANTES (CCL5/RANTES), monokine induced by interferon- γ (CXCL9/MIG), monocyte chemoattractant protein-1 (CCL2/MCP-1), and interferon- γ -induced protein-10 (CXCL10/IP-10) levels in a single sample. The kit's performance has been optimized for analysis of specific chemokines in tissue culture supernatants, EDTA-treated 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 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

Five bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1, and CXCL10/IP-10. The five bead populations are mixed together to form the bead array that is resolved in a red channel (eg, FL3 or FL4) of a flow cytometer (see Figure 1).

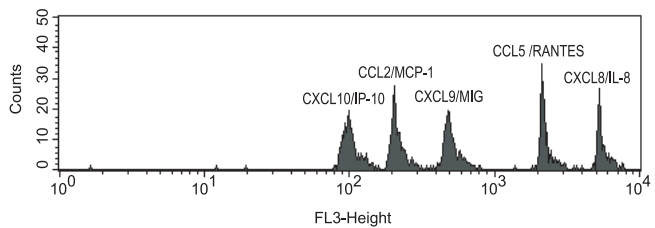


Figure 1

During the assay procedure, you will mix the chemokine 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-fifth the quantity necessary for conventional ELISA assays due to the detection of five 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 five 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 Chemokine 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 slightly. See [Theoretical limit of detection \(page 32\)](#).

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 and EDTA-plasma spike recoveries for CXCL9/MIG, CCL2/MCP-1, and CXCL10/IP-10 are lower than for other proteins in this assay. This variation is due to assay conditions and serum or EDTA-plasma proteins and may affect quantitation of these proteins in serum or plasma samples.

When testing serum samples for CXCL10/IP-10 and CCL2/MCP-1, it is recommended that the serum samples be diluted 1:10 and 1:4, respectively. This is to reduce the effects of serum interference with the quantitation of these proteins in serum samples.

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 Chemokine Kit assay has been shown to detect non-human primate CXCL8/IL-8, CCL5/RANTES, and CCL2/MCP-1 proteins produced by the activation of cells from rhesus and cynomolgus macaques. Direct quantitation of proteins from non-human primates 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	CXCL8/IL-8 Capture Beads	1 vial, 0.8 mL
A2	CCL5/RANTES Capture Beads	1 vial, 0.8 mL
A3	CXCL9/MIG Capture Beads	1 vial, 0.8 mL
A4	CCL2/MCP-1 Capture Beads	1 vial, 0.8 mL
A5	CXCL10/IP-10 Capture Beads	1 vial, 0.8 mL
B	Human Chemokine PE Detection Reagent	1 vial, 4 mL
C	Human Chemokine 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, 130 mL
G	Assay Diluent	1 bottle, 30 mL

Bead reagents

Human Chemokine Capture Beads (A1–A5): An 80-test vial of each specific capture bead (A1–A5). The specific capture beads, having discrete fluorescence intensity characteristics, are distributed from brightest (A1) to dimmest (A5).

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 μ L per test.

Antibody and standard reagents

Human Chemokine PE Detection Reagent (B): An 80-test vial of PE-conjugated anti-human CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1, and CXCL10/IP-10 antibodies, formulated for use at 50 μ L per test.

Human Chemokine Standards (C): Two vials containing lyophilized recombinant human proteins. Each vial should be reconstituted in 4.0 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 μ L 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 μ L per 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 Chemokine Standards and to dilute the unknown 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–A5, B, D, E1–E2, F, and G 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 \(page 14\)](#)
- [Required materials \(page 15\)](#)

Workflow overview

Workflow The overall workflow consists of the following steps.

Step	Description
1	Preparing Human Chemokine Standards (page 18)
2	Mixing Human Chemokine Capture Beads (page 20)
3	Diluting samples (page 21)
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 Chemokine 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 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 Chemokine 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 FACSAArray™	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 Chemokine Standards \(page 18\)](#)
- [Mixing Human Chemokine Capture Beads \(page 20\)](#)
- [Diluting samples \(page 21\)](#)

Preparing Human Chemokine Standards

Purpose of this procedure

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

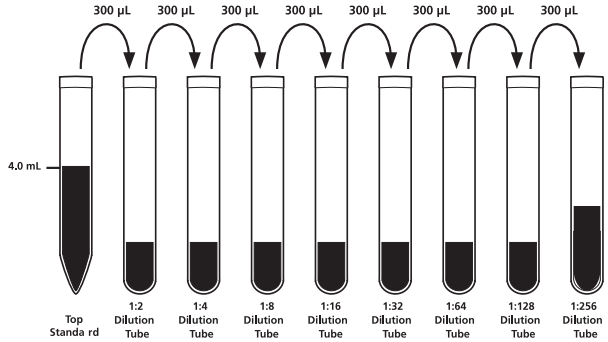
You must prepare fresh chemokine standards to run with each experiment. Do not store or reuse reconstituted or diluted standards.

Procedure

To reconstitute and serially dilute the standards:

1. Open one vial of lyophilized Human Chemokine Standards. Transfer the standard spheres to a 15-mL polypropylene tube. Label the tube “Top Standard.”
2. Reconstitute the standards with 4 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 [Procedure for tubes](#) section of [Performing the Human Chemokine Assay \(page 24\)](#) for a listing of the concentrations (pg/mL) of all five recombinant proteins in each standard.

Next step

Proceed to [Mixing Human Chemokine Capture Beads \(page 20\)](#).

Mixing Human Chemokine Capture Beads

Purpose of the procedure The Capture Beads are bottled individually (A1–A5). You must pool all five 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 chemokine standard dilutions + 1 negative control = 18 assay tubes).

Note: Extra tests of Capture Beads should be mixed to ensure that the necessary number of tests will be recovered from the mixed Capture Beads tube. Add an additional 2 to 3 assay tubes to the number determined in [step 1](#) above before calculating the amount to add to the mixed Capture Beads tube in [step 3](#).

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- μ L aliquot of each Capture Bead, for each assay tube to be analyzed, into a single tube labeled “Mixed Capture Beads” (for example, 10 μ L of CXCL8/IL-8 Capture Beads \times 18 assay tubes = 180 μ L of CXCL8/IL-8 Capture Beads required).
4. Vortex the bead mixture thoroughly.

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 Chemokine Assay \(page 24\)](#). If you need to dilute samples having a high-protein concentration, proceed to [Diluting samples \(page 21\)](#).

Diluting samples

Purpose of this procedure

The standard curve for each chemokine covers a defined set of concentrations from 10 to 2,500 pg/mL. It might be necessary to dilute test samples to ensure that their median fluorescence values fall within the range of the generated chemokine standard curve. For best results, dilute samples that are known or assumed to contain high levels of a given chemokine. 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.

Note: When testing serum samples for CXCL10/IP-10 or CCL2/MCP-1, it is recommended that the serum samples be diluted 1:10 and 1:4, respectively.

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 Chemokine 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 Chemokine Assay \(page 24\)](#)
- [Data analysis \(page 27\)](#)

Performing the Human Chemokine Assay

- Before you begin**
1. Prepare the standards as described in [Preparing Human Chemokine Standards](#) (page 18).
 2. Mix the Capture Beads as described in [Mixing Human Chemokine Capture Beads](#) (page 20).
 3. 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 μ L to all assay tubes.
2. Add 50 μ L of the Human Chemokine Standard dilutions to the control tubes as listed in the following table.

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

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

4. Add 50 μL of the Human Chemokine 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 200g 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 Chemokine 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.
Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation.
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 μ L 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.

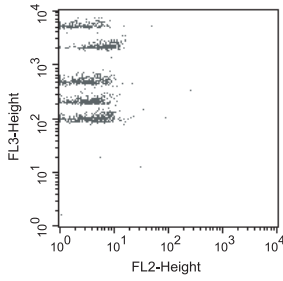
When you are finished acquiring samples, proceed to [Data analysis \(page 27\)](#).

Data analysis

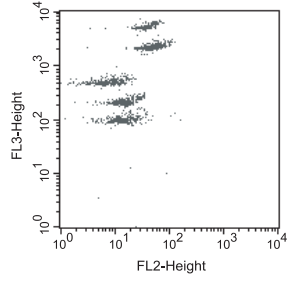
How to analyze Analyze BD CBA Human Chemokine Kit data using FCAP Array software. For instructions on analysis, go to bdbiosciences.com/cbasetup and see the *Guide to Analyzing Data from BD CBA Kits Using FCAP Array Software*.

Typical data

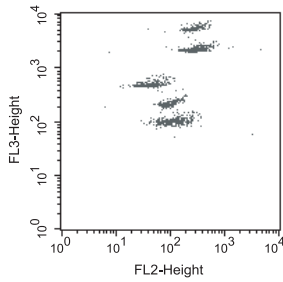
The following data, acquired using BD CellQuest software, shows standards and detectors alone.



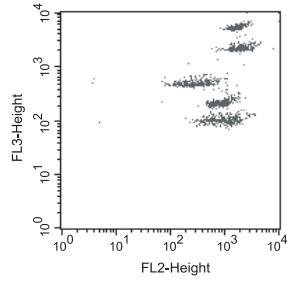
Negative control: 0 pg/mL



Standard: 20 pg/mL



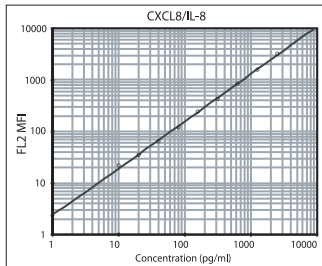
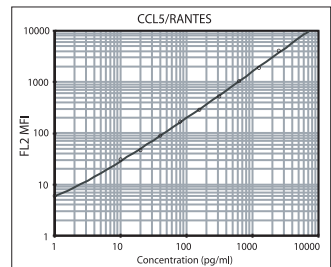
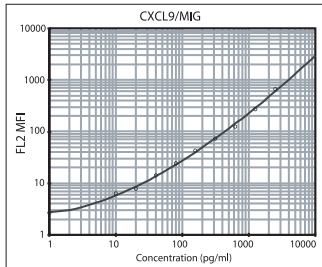
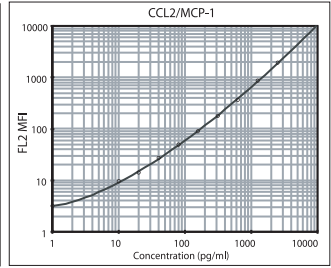
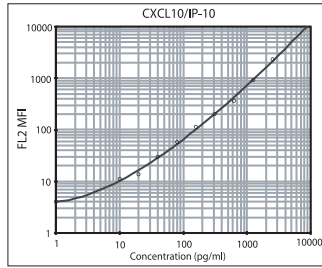
Standard: 156 pg/mL



Standard: 1,250 pg/mL

Standard curve examples

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



5

Performance

This section covers the following topics:

- [Theoretical limit of detection \(page 32\)](#)
- [Recovery \(page 33\)](#)
- [Linearity \(page 35\)](#)
- [Specificity \(page 37\)](#)
- [Precision \(page 38\)](#)

Theoretical limit of detection

Experiment details

The individual standard curve range for a given protein defines the minimum and maximum quantifiable levels (for example, 10 pg/mL and 2,500 pg/mL) using the BD CBA Human Chemokine 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 Chemokine 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

Protein	Median fluorescence	Standard deviation	Limit of detection (pg/mL)
CXCL8/IL-8	2.6	0.2	0.2
CCL5/RANTES	5.5	0.3	1.0
CXCL9/MIG	2.9	0.2	2.5
CCL2/MCP-1	3.3	0.2	2.7
CXCL10/IP-10	4.1	0.3	2.8

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
CXCL8/IL-8	Pooled donor sera (n=5)	1,250	966.4	77%
		312.5	217.3	68%
		40	28.7	72%
	Pooled donor plasmas (n=5)	1,250	1,007.8	81%
		312.5	242.8	76%
		40	28.7	72%
	Cell culture supernatant	1,250	1,291.7	103%
		312.5	349.0	109%
		40	33.5	84%
CCL5/RANTES	Pooled donor sera (n=5)	ND ^a	—	—
	Pooled donor plasmas (n=5)	ND ^a	—	—
	Cell culture supernatant	1,250	1,273.3	102%
		312.5	356.1	111%
		40	29.8	75%

Protein	Matrix	Standard spike conc. (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
CXCL9/MIG	Pooled donor sera (n=5)	1,250	779.4	62%
		312.5	186.4	58%
		40	35.8	90%
	Pooled donor plasmas (n=5)	1,250	773.6	62%
		312.5	212.5	66%
		40	15.7	39%
	Cell culture supernatant	1,250	1,447.8	116%
		312.5	363.6	113%
		40	36.4	91%
CCL2/MCP-1 ^b	Pooled donor sera (n=5)	1,250	597.0	48%
		312.5	114.5	36%
		40	5.9	15%
	Pooled donor plasmas (n=5)	1,250	543.3	43%
		312.5	110.4	34%
		40	8.9	22%
	Cell culture supernatant	1,250	1,345.7	108%
		312.5	353.2	110%
		40	31.9	80%
CXCL 10/IP-10	Pooled donor sera (n=5)	1,250	521.6	42%
		312.5	101.5	32%
		40	26.4	66%
	Pooled donor plasmas (n=5)	1,250	969.5	78%
		312.5	279.7	87%
		40	24.4	61%
	Cell culture supernatant	1,250	1,170.2	94%
		312.5	341.5	106%
		40	38.8	97%

a ND = Not determined. Due to the high levels of CCL2/RANTES in normal serum and EDTA-plasma samples, recovery information could not be generated for this protein.

b Recovery for these proteins is much lower than for other proteins in this assay. Variation is likely due to assay conditions and serum or plasma proteins and

may affect quantitation of these proteins in serum or plasma samples.

Linearity

Experiment details

In two experiments, the following matrices were spiked with CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1, and CXCL10/IP-10 and were then serially diluted with Assay Diluent.

Linearity data

Matrix	Dilution	Observed				
		CXCL8/IL-8 (pg/mL)	CCL5/RANTES (pg/mL)	CXCL9/MIG (pg/mL)	CCL2/MCP-1 (pg/mL) ^b	CXCL10/IP-10 (pg/mL) ^b
Cell culture media	Neat	2,383	2,360	2,558	2,329	2,374
	1:2	1,264	1,256	1,397	1,308	1,275
	1:4	600	585	550	583	558
	1:8	306	315	327	297	317
	1:16	146	137	159	128	158
	1:32	71	62	74	68	70
	1:64	34	27	33	30	36
	1:128	20	16	18	15	19
	1:256	9	7	9	7	10
	Slope	1.00	1.07	1.03	1.06	1.00
Pooled human sera (n=5)	Neat	2,115	ND ^a	1,853	1,479	1,324
	1:2	1,212		1,290	968	1,087
	1:4	689		731	557	778
	1:8	292		355	260	422
	1:16	132		182	126	242
	1:32	63		85	65	129
	1:64	34		46	31	71
	1:128	17		18	14	34
	1:256	9		11	7	17
	Slope	1.01		0.97	0.99	0.81

Matrix	Dilution	Observed				
		CXCL8/IL-8 (pg/mL)	CCL5/ RANTES (pg/mL)	CXCL9/ MIG (pg/mL)	CCL2/ MCP-1 (pg/mL) ^b	CXCL10/ IP-10 (pg/mL) ^b
Pooled human plasma (n=5)	Neat	2,226	ND ^a	2,355	1,295	3,772
	1:2	1,290		1,393	860	2,007
	1:4	717		794	510	1,095
	1:8	293		365	234	584
	1:16	129		168	100	264
	1:32	63		82	44	124
	1:64	33		43	18	68
	1:128	18		21	9	34
	1:256	10		11	5	17
	Slope	1.02		0.99	1.06	0.98

- a ND = Not determined. Due to high levels of CCL5/RANTES in normal serum and EDTA-plasma samples, linearity information could not be generated for this protein.
- b When testing serum samples for CXCL10/IP-10 or CCL2/MCP-1, it is recommended that the serum sample be diluted 1:10 or 1:4, respectively. This is to reduce the effects of serum interference with the quantitation of these proteins in serum samples.

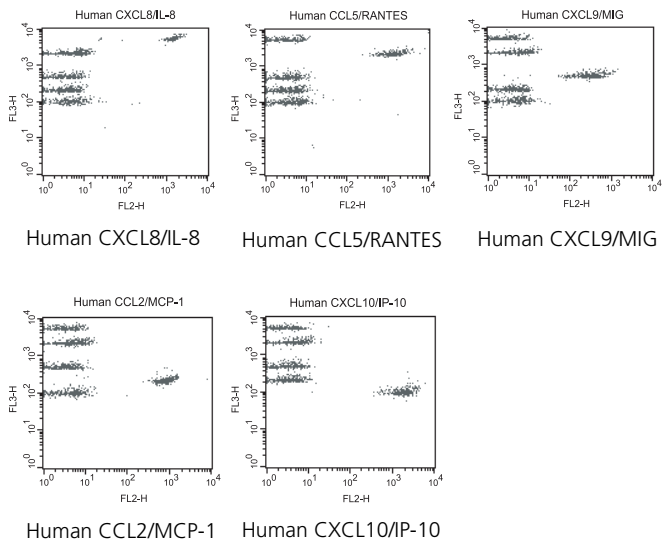
Specificity

Experiment details

The antibody pairs used in the BD CBA Human Chemokine Kit assay have been screened for specific reactivity with their corresponding 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 CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1, and CXCL10/IP-10 (40, 312.5, and 1,250 pg/mL) were tested.

CXCL8/IL-8	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Actual mean conc. (pg/mL)	38.7	299.0	1,301.8
SD	2.0	16.0	45.3
%CV	5.1%	5.3%	3.5%

CCL5/RANTES	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Actual mean conc. (pg/mL)	35.3	302.9	1,319.8
SD	3.2	36.0	78.7
%CV	9.1%	11.9%	6.0%

CXCL9/MIG	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Actual mean conc. (pg/mL)	35.9	300.9	1,299.4
SD	3.1	33.5	150.1
%CV	8.7%	11.1%	11.5%

CCL2/MCP-1	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Actual mean conc. (pg/mL)	37.4	291.3	1,274.0
SD	3.6	19.7	42.5
%CV	9.8%	6.8%	3.3%

CXCL10/IP-10	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Actual mean conc. (pg/mL)	40.0	326.4	1,303.1
SD	4.4	37.3	130.8
%CV	11.0%	11.4%	10.0%

Inter-assay precision

Three different levels of CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1, and CXCL10/IP-10 (40, 312.5, and 1,250 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.

CXCL8/IL-8	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Number of replicates	10	10	10
Actual mean conc. (pg/mL)	36.0	297.9	1,234.6
SD	1.2	24.0	82.1
%CV	3.4%	8.1%	6.7%

CCL5/RANTES	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Number of replicates	10	10	10
Actual mean conc. (pg/mL)	32.4	308.8	1,238.1
SD	1.4	41.7	163.6
%CV	4.2%	13.5%	13.2%

CXCL9/MIG	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Number of replicates	10	10	10
Actual mean conc. (pg/mL)	39.2	317.5	1,255.3
SD	2.0	50.5	184.3
%CV	5.2%	15.9%	14.7%

CCL2/MCP-1	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Number of replicates	10	10	10
Actual mean conc. (pg/mL)	34.7	297.5	1,275.2
SD	3.2	20.6	98.9
%CV	9.2%	6.9%	7.8%

CXCL10/IP-10	40 pg/mL	312.5 pg/mL	1,250 pg/mL
Number of replicates	10	10	10
Actual mean conc. (pg/mL)	39.2	317.5	1,255.3
SD	2.0	50.5	184.3
%CV	5.2%	15.9%	14.7%

6

Reference

This section covers the following topics:

- [Troubleshooting \(page 42\)](#)
- [Correlation with NIBSC/WHO standards \(page 44\)](#)
- [References \(page 45\)](#)

Troubleshooting

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

Note: 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	<ul style="list-style-type: none"> ● Avoid aspiration of beads during the wash step. ● Do not wash or resuspend beads in volumes higher than the recommended volumes.
High background	<ul style="list-style-type: none"> ● Test various sample dilutions. The sample may be too concentrated. ● Remove excess Human Chemokine 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 five bead populations are observed during analysis, or distribution is unequal	<ul style="list-style-type: none"> ● 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	<ul style="list-style-type: none"> ● 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 Chemokine Kit.
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 a poor standard curve	<ul style="list-style-type: none"> ● 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.

Correlation with NIBSC/WHO standards

Purpose of the procedure

This topic describes how to convert the CXCL8/IL-8, CCL5/RANTES, and CCL2/MCP-1 results obtained from the BD CBA Human Chemokine Kit to the equivalent NIBSC/WHO Standard.

Standards

The NIBSC/WHO First International Standard for CXCL8/IL-8 (89/520), CCL5/RANTES (92/520) NIBSC Reference Reagent, and CCL2/MCP-1 (92/794) NIBSC Reference Reagent were evaluated using the BD CBA Human Chemokine Kit.

The dose-response curves of these First International Standards paralleled the BD CBA Human Chemokine Kit standard curves for each protein.

To convert sample pg/mL values determined using the BD CBA Human Chemokine Kit to the equivalent NIBSC/WHO Standard approximate pg/mL concentrations, use the conversion factors listed below:

- NIBSC/WHO CXCL8/IL-8 (89/520) equivalent value (pg/mL) = $0.74 \times$ BD CBA CXCL8/IL-8 value (pg/mL)
- NIBSC/WHO CCL5/RANTES (92/520) equivalent value (pg/mL) = $2.26 \times$ BD CBA CCL5/RANTES value (pg/mL)
- NIBSC/WHO CCL2/MCP-1 (92/794) equivalent value (pg/mL) = $0.95 \times$ BD CBA CCL2/MCP-1 value (pg/mL)

Note: NIBSC/WHO Reference Reagents were not available for CXCL9/MIG and CXCL10/IP-10.

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