BD Cytometric Bead Array (CBA) Human Anaphylatoxin Kit

Instruction Manual

Catalog No. 561418

Trademarks

FCAP Array is a registered trademark of Soft Flow Hungary Ltd.

Mac is a trademark of Apple Computer, Inc., registered in the US and other countries.

Falcon® is a registered trademark of Corning Incorporated.

History

Revision	Date	Change made
23-11624-00 Rev. 01	11/2010	New document
Rev. 2	4/2015	Update hazard statements

Contents

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About this kit

This section covers the following topics:

Purpose of the kit

Use of the kit

The BD™ CBA Human Anaphylatoxin Kit (Catalog No. 561418) can be used to quantitatively measure anaphylatoxin C3a, C4a, and C5a (bioactive cleavage products released from C3, C4, and C5 during complement activation) protein levels in a single EDTA plasma or serum sample. The kit performance has been optimized for analysis of specific proteins in 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 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

Three bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for C4a, C3a, and C5a plasma proteins and their desArg forms. In plasma and serum, C4a, C3a, and C5a are rapidly converted to their desArg forms ($C4a_{desArg}$, $C3a_{desArg}$, and $C5a_{desArg}$). The Human Anaphylatoxin kit measures both C4a, C3a, and C5a, and their desArg forms (since this kit will measure both forms of each protein, normal and desArg, this manual will use C4a, C3a, and C5a when referring to the measurement of either form). The three bead populations are mixed together to form the bead array, which is resolved in a red channel of a flow cytometer (see Figure 1).

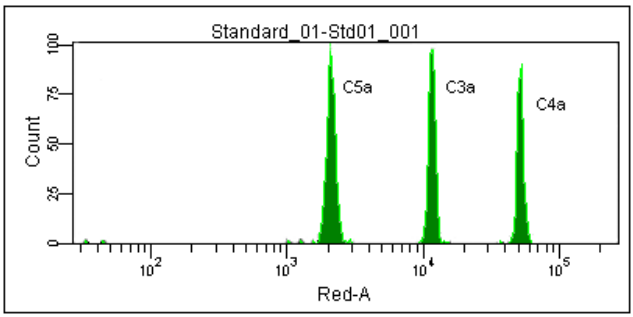


Figure 1

During the assay procedure, you will mix the anaphylatoxin capture beads with standards (purified from human plasma) or test samples (EDTA plasma or serum), incubate, wash, and then incubate with the PE-conjugated detection antibodies to form sandwich complexes. In this assay, the Human Anaphylatoxin Standards consist of purified $C3a_{desArg}$, $C4a_{desArg}$, and $C5a_{desArg}$. 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 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-third the quantity necessary for conventional ELISA assays due to the detection of three 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 three 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 Anaphylatoxin Kit is comparable to conventional ELISA, but due to the complexity and kinetics of this multi-analyte assay, the actual limit of detection of a given experiment may vary slightly. See Theoretical limit of detection (page 34).

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.

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 Anaphylatoxin assay has been shown to detect C3a produced by the activation of complement in EDTA plasma and serum from the non-human primate rhesus and cynomolgus models. In addition, the assay has been shown to detect C5a produced by the activation of complement in EDTA plasma and serum from the non-human primate baboon model. Direct quantitation of proteins from the rhesus, cynomolgus, and baboon models has not been validated using this kit and results may vary.

Kit contents

Contents

The BD CBA Human Anaphylatoxin Kit contains the following components sufficient for 80 tests.

Vial label	Reagent	Quantity
A1	Human C4a Capture Beads	1 vial, 1.6 mL
A2	Human C3a Capture Beads	1 vial, 1.6 mL
A3	Human C5a Capture Beads	1 vial, 1.6 mL
B	Human Anaphylatoxin PE Detection Reagent	1 vial, 4 mL
C	Human Anaphylatoxin 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
F	Wash Buffer	1 bottle, 260 mL
G	Assay Diluent	2 bottles, 30 mL each

Bead reagents

Human Anaphylatoxin Capture Beads (A1–A3): An 80-test vial of each specific capture bead (A1–A3). The specific capture beads, having discrete fluorescence intensity characteristics, are distributed from brightest (A1) to dimmest (A3).

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

Antibody and standard reagents

Human Anaphylatoxin PE Detection Reagent (B): An 80-test vial of PE-conjugated anti-human C4a, C3a, and C5a antibodies, formulated for use at 50 µL per test.

Human Anaphylatoxin Standards (C): Two vials containing lyophilized human proteins purified from serum. 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 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 that is formulated for use at 50 µL per test. This reagent is used with the Cytometer Setup Beads to set the initial instrument compensation settings.

Buffer reagents

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

Assay Diluent (G): Two 30-mL bottles of a buffered protein solution (1X) used to reconstitute and dilute the Human Anaphylatoxin Standards and to dilute test 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–A3, 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.

This product contains human blood, serum, cells, or materials derived from them, which are potentially hazardous materials. Use universal precautions when handling. Handle as if product were capable of transmitting disease. Material used in this product has been tested using FDA approved methods and found negative for Human Immunodeficiency Virus (HIV-1/HIV-2), Hepatitis B Surface Antigen (HBSAG) and antibody to Hepatitis C Virus (HCV). However, no known test method can offer complete assurance that specimens of human origin will not transmit infectious disease. When handling or disposing, follow precautions described in CDC and FDA recommendations and OSHA Bloodborne Pathogen recommendations.

Human Anaphylatoxin Standards (component 51-9000045) 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.

2

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 Anaphylatoxin Standards (page 20)
2	Mixing Human Anaphylatoxin Capture Beads (page 22)
3	Diluting samples (page 23) and/or Treating plasma samples with Futhan (page 24)
4	Performing instrument setup with Cytometer Setup Beads (instructions can be found at bdbiosciences.com/cbasetup) **Note:** Can be performed during step 5 incubation.
5	Performing the Human Anaphylatoxin Assay (page 26)
6	Acquiring samples (instructions can be found at bdbiosciences.com/cbasetup)
7	Data analysis (page 30)

Incubation times

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

Procedure	Incubation time
Preparing standards	15 minutes
Preparing Cytometer Setup Beads	30 minutes
Staining samples for analysis	3 hours

Required materials

Materials required but not provided

In addition to the reagents provided in the BD CBA Human Anaphylatoxin 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.		

- 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)

3

Assay preparation

This section covers the following topics:

Preparing Human Anaphylatoxin Standards

Purpose of this procedure

The Human Anaphylatoxin 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 anaphylatoxin standards to run with each experiment. Do not store or reuse reconstituted or diluted standards.

Procedure

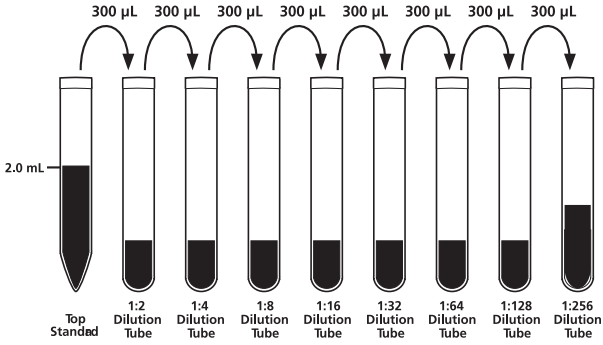
To reconstitute and serially dilute the standards:

1. Open one vial of lyophilized Human Anaphylatoxin Standards. Transfer the standard spheres to a 15-mL polypropylene tube. Label the tube "Top Standard."
2. Reconstitute the standards with 2 mL of Assay Diluent.
    a. Allow the reconstituted standard to equilibrate for at least 15 minutes at room temperature.
    b. Gently mix the reconstituted protein by 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 in each of the 12 x 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 pipet 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.



   c. 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 Procedure for tubes section of Performing the Human Anaphylatoxin Assay (page 26) for a listing of the concentrations (pg/mL) of all three recombinant proteins in each standard dilution.

Next step

Proceed to Mixing Human Anaphylatoxin Capture Beads (page 22).

Mixing Human Anaphylatoxin Capture Beads

Purpose of this procedure

The Capture Beads are bottled individually (A1–A3). You must pool all three 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 (eg, 8 unknowns, 9 anaphylatoxin standard dilutions, and 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 20-µL aliquot of each Capture Bead, for each assay tube to be analyzed, into a single tube labeled "Mixed Capture Beads" (for example, 20 µL of C4a Capture Beads × 18 assay tubes = 360 µL of C4a 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 Anaphylatoxin Assay (page 26). If you need to dilute samples having a high-protein concentration, proceed to Diluting samples (page 23).

Diluting samples

Purpose of this procedure

The standard curve for each anaphylatoxin protein covers a defined set of concentrations from 10 to 2,500 pg/mL or 4 to 1,000 pg/mL. It might be necessary to dilute EDTA plasma or serum test samples to ensure that their mean fluorescence values fall within the range of the generated standard curve. For best results, dilute samples that are known or assumed to contain high levels of a given protein. This procedure is not necessary for all samples.

Note: We recommend that plasma samples be used whenever possible instead of serum samples, since plasma samples have been shown to have less variability in the level of these proteins. See Serum and plasma anaphylatoxin levels (page 37).

Procedure

1. Dilute test samples by the desired dilution factor (for example, 1:200, 1:400, or higher) 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 Anaphylatoxin Assay (page 26), and you can perform instrument setup during the 1-hour PE Detection Reagent incubation.

Treating plasma samples with Futhan

Purpose of this procedure

Treatment of EDTA plasma samples prevents the activation of a number of plasma proteases. However, in vitro cleavage of certain complement components can still occur. This residual cleavage makes measurements of in vivo-generated anaphylatoxins less accurate. Add Futhan (FUT-175) to EDTA plasma samples at the time of sample collection to provide additional inhibition of ex vivo complement activation. Treatment of samples with Futhan may provide more accurate measurements of in vivo anaphylatoxin levels.

Procedure

See the product insert for FUT-175 (Futhan) (Catalog No. 552035) for instructions on its use for treatment of EDTA plasma samples.

4

Assay procedure

This section covers the following topics:

Performing the Human Anaphylatoxin Assay

Before you begin

- Prepare the standards as described in Preparing Human Anaphylatoxin Standards (page 20).
- Mix Capture Beads as described in Mixing Human Anaphylatoxin Capture Beads (page 22).
- If necessary, dilute the unknown samples. See Diluting samples (page 23).

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 Anaphylatoxin Standard dilutions to the control tubes as listed in the following table.

Tube label	Concentration (pg/mL)		Anaphylatoxin Standard dilution
	C3a and C4a	C5a	
1	0 (negative control)	0 (negative control)	no standard dilution (Assay Diluent only)
2	10	4	1:256
3	20	8	1:128
4	40	16	1:64
5	80	32	1:32
6	156	62.5	1:16
7	312	125	1:8
8	625	250	1:4
9	1250	500	1:2
10	2500	1000	Top Standard

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

4. Incubate the assay tubes for 2 hours at room temperature, protected from light.
5. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.
6. Carefully aspirate and discard the supernatant from each assay tube.
7. Add 50 µL of the Human Anaphylatoxin PE Detection Reagent to all assay tubes.
8. Incubate the assay tubes for 1 hour at room temperature, protected from light.

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

Procedure for filter plates

To perform the assay:

1. Wet the plate by adding 100 µL of Wash Buffer to each well.
2. Place the plate on the vacuum manifold.
3. Aspirate for 2 to 10 seconds until the wells are drained.
4. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.

5. Add 50 µL of each of the following to the wells in the filter plate:
   - Capture Beads (vortex before adding)
   - Standard or sample (add standards from the lowest concentration to the highest, followed by samples)
6. Cover the plate and shake it for 5 minutes at 600 rpm on a plate shaker.
7. Incubate the plate for 2 hours at room temperature on a non-absorbent, dry surface.

   Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.
8. Remove the cover from the plate and apply the plate to the vacuum manifold.
9. Vacuum aspirate for 2 to 10 seconds until the wells are drained.
10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.
11. Add 200 µL of Wash Buffer to each well. Cover the plate and shake for 2 minutes at 600 rpm.
12. Repeat step 8 through step 10.
13. Add 50 µL of Human Anaphylatoxin PE Detection Reagent to each well.
14. Cover the plate and shake it for 5 minutes at 600 rpm on a plate shaker.
15. Incubate the plate for 1 hour 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.

16. Repeat step 8 through step 10.
17. Add 120 µL of wash buffer to each well to resuspend the beads.
18. Cover the plate and shake it for 2 minutes at 600 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.

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

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

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

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

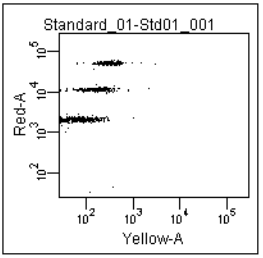
Data analysis

How to analyze

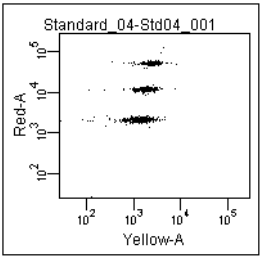
Analyze BD CBA Human Anaphylatoxin Kit data using FCAP Array software. For instructions on using the software, go to bdbiosciences.com/cbasetup and see the *Guide to Analyzing Data from BD CBA Kit Using FCAP Array Software*.

Typical data

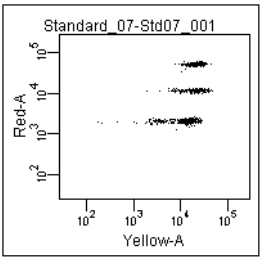
The following data, acquired using the BD FACSArray bioanalyzer, shows standards and detectors alone.



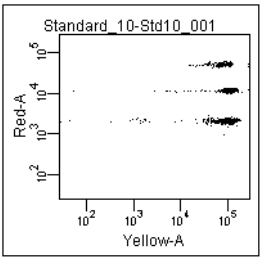
Negative control



Standards: 40 pg/mL (C4a, C3a) and 16 pg/mL (C5a)



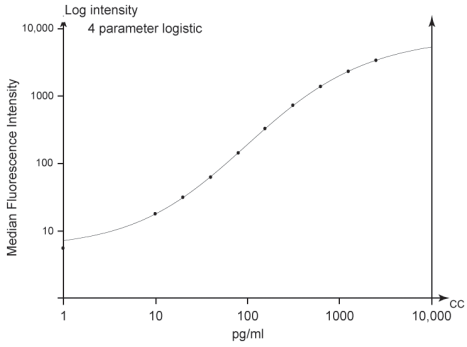
Standards: 312.5 pg/mL (C4a, C3a) and 125 pg/mL (C5a)



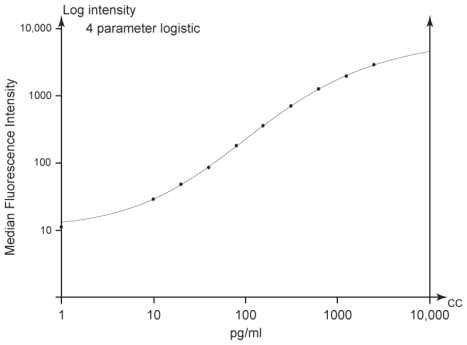
Standards: 2,500 pg/mL (C4a, C3a) and 1,000 pg/mL (C5a)

Standard curve examples

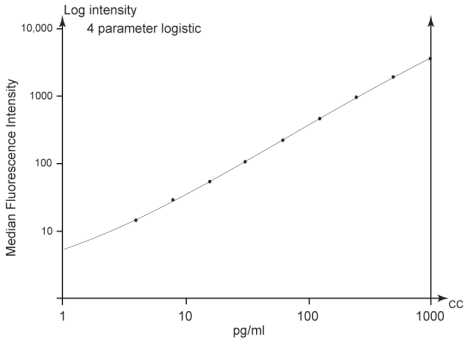
The following graphs represent standard curves from the BD CBA Human Anaphylatoxin Standards.



C3a



C4a



C5a

5

Performance

This section covers the following topics:

Theoretical limit of detection

Experiment details

The individual standard curve range for a given protein defines the minimum and maximum quantifiable levels using the BD CBA Human Anaphylatoxin Kit (for example, 10 pg/mL and 2,500 pg/mL or 4 pg/mL and 1,000 pg/mL). 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 cytokine using the BD CBA Human Anaphylatoxin 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

Protein	Limit of detection (pg/mL)
C3a	0.45
C4a	0.70
C5a	1.15

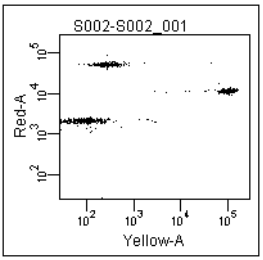
Specificity

Experiment details

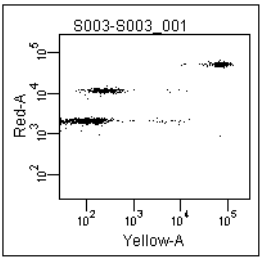
The antibody pairs used in the BD CBA Human Anaphylatoxin assay have been screened for specific reactivity with their specific proteins. Analysis of samples containing only a single purified protein found no cross-reactivity or background detection of protein in other Capture Bead populations using this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

Specificity data

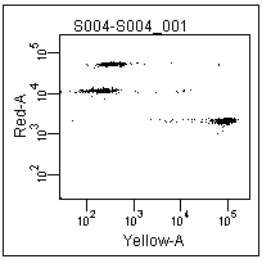
Data for the detection of individual anaphylatoxin proteins was analyzed using the BD FACSArray bioanalyzer.



C3a



C4a



C5a

Precision

Intra-assay precision

Ten replicates of each of three different levels of C3a, C4a, and C5a were tested. (C3a and C4a: 40, 156, and 625 pg/mL; C5a: 16, 62.5, and 250 pg/mL).

Protein	Sample	Mean (pg/mL)	Standard deviation	%CV
C3a	1	36.9	2.2	6%
	2	142.2	6.2	4%
	3	575.8	31.1	5%
C4a	1	38.5	2.0	5%
	2	151.3	3.1	2%
	3	582.4	33.1	6%
C5a	1	14.2	0.8	6%
	2	53.0	2.2	4%
	3	223.9	17.3	8%

Inter-assay precision

Three replicates each of three different levels of C3a, C4a, and C5a (C3a and C4a: 40, 156, and 625 pg/mL; C5a: 16, 64, and 250 pg/mL) were tested in four experiments conducted by different operators.

Protein	Sample	Mean (pg/mL)	Standard deviation	%CV
C3a	1	36.1	2.0	6%
	2	142.1	9.0	6%
	3	606.9	39.6	7%
C4a	1	36.6	1.9	5%
	2	149.3	8.4	6%
	3	589.9	32.2	5%
C5a	1	13.9	0.8	6%
	2	54.6	3.7	7%
	3	236.0	21.3	9%

Serum and plasma anaphylatoxin levels

Experiment details

Serum and Futhan-treated EDTA plasma samples were collected and immediately stored on ice. Serum samples were diluted 1:1600 and Futhan-treated EDTA plasma samples were diluted 1:400, then analyzed using the BD CBA Human Anaphylatoxin Kit.

	C3a	C4a	C5a
Plasma (n=5)			
Average (ng/mL)	52.0	177.9	4.0
SD	24.9	56.6	0.9
Serum (n=5)			
Average (ng/mL)	790.1	782.4	5.9
SD	419.8	312.0	2.2

6

Reference

This section covers the following topics:

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.

Note: The BD CBA Human Anaphylatoxin assay has been shown to detect C3a produced by the activation of complement in EDTA plasma and serum from the non-human primate rhesus and cynomolgus models. In addition, the assay has been shown to detect C5a produced by the activation of complement in EDTA plasma and serum from the non-human primate baboon model. Direct quantitation of proteins from the rhesus, cynomolgus, and baboon models has not been validated using this kit and results may vary.

Problem	Recommended action
Variation between duplicate samples	Vortex the Capture Beads before pipetting. Beads can aggregate.
Low bead number in samples	• Avoid aspiration of beads during the wash step. • Do not wash or resuspend beads in volumes higher than the recommended volumes.
High background	• Test various sample dilutions. The sample may be too concentrated. • Remove excess Human Anaphylatoxin 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.

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

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 15 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. Fulton RJ, McDade RL, Smith PL, Kienker LJ, Kettman JR Jr. Advanced multiplexed analysis with the FlowMetrix system. *Clin Chem*. 1997;43:1749–1756.
7. Kricka LJ. Simultaneous multianalyte immunoassays. Diamandis EP, Christopoulos TK, eds. *Immunoassay*. Academic Press. 1996:389–404.
8. 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.

9. Fulwyler M, McHugh TM. Flow microsphere immunoassay for the quantitative and simultaneous detection of multiple soluble analytes. *Methods Cell Biol.* 1994;33:613–629.

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

11. 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).

12. 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.

13. 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.

Notes

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