BD™ Cytometric Bead Array

Human Active Caspase-3 CBA

Cat. No. 552124

BD Biosciences

Kit Contents

(Store the following items at 4°C)

A Human Caspase-3 Capture Beads: 1 vial, 5 ml

B Human Active Caspase-3 PE* Detection Reagent: 1 vial, 5 ml

C Human Active Caspase-3 Lysate Standard: 3 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

Protease Inhibitor Cocktail: 1 vial, 1 ml lyophilized

Cell Lysis Buffer: 1 bottle, 50 ml

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Introduction

Flow cytometry is an analysis tool that allows for the discrimination of different particles on the basis of size and color. The BD™ Cytometric Bead Array (CBA) employs a particle with a discrete fluorescence intensity to detect a soluble analyte. The BD CBA is combined with flow cytometry to create a powerful particle-based immunoassay.

The BD CBA system uses the sensitivity of amplified fluorescence detection by flow cytometry to measure a soluble analyte. Each bead in a CBA provides a capture surface for a specific protein and is analogous to an individually coated well in an ELISA plate. The BD CBA capture bead is in suspension to allow for the detection of an analyte in a small sample volume. The combined advantages of the broad dynamic range of fluorescent detection via flow cytometry and the efficient capturing of analytes via suspended particles enable CBA to use fewer sample dilutions and to obtain the value of an unknown in substantially less time (compared to conventional ELISA and western blot techniques).

The BD Human Active Caspase-3 CBA Kit can be used to quantitatively measure active caspase-3 protein levels in a single sample. The kit performance has been optimized for analysis of specific protein in cell lysates.

The BD CBA System, a product of BD Biosciences, was developed jointly by BD Biosciences Immunocytometry Systems and BD Biosciences Pharmingen. This kit incorporates the quality, reliability, and service that you have come to expect from BD Biosciences.

Principle of the Test

A single bead population with a distinct fluorescence intensity has been coated with a capture antibody specific for caspase-3 protein. The bead population is resolved in the FL3 channel of a BD FACS™ brand flow cytometer.

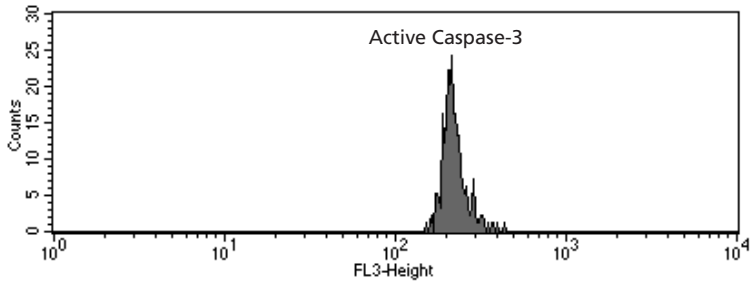


Figure 1

The capture bead, PE-conjugated detection antibody, and cell lysate standard or test samples are incubated together to form sandwich complexes. Following acquisition of sample data using the flow cytometer, the sample results are generated in graphical and tabular format using the BD CBA Analysis Software. The kit provides sufficient reagents for the quantitative analysis of 100 samples, including the generation of up to three standard curve sets.

Advantages

The CBA provides several advantages when compared with conventional ELISA and western blot methodologies:

- A CBA experiment takes significantly less time than a western blot assay and provides quantitative results.
- The CBA has a wider dynamic range than conventional ELISAs.

Limitations

The sensitivity of the Human Active Caspase-3 CBA is comparable to conventional ELISA, but due to the complexity and kinetics of this assay, actual sensitivity in a given experiment may vary slightly ***(see sensitivity and precision information in Sections 12.1 and 12.3)***.

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

Reagents Provided

Bead Reagents

Human Caspase-3 Capture Beads (A): A single 100-test vial of capture beads (A) is included in this kit. Store at 4°C. Do not freeze.

Note: The antibody-conjugated beads will settle out of suspension over time. It is necessary to vortex the vial vigorously for 3 - 5 seconds before taking a bead suspension aliquot.

Cytometer Setup Beads (D): A single 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 Active Caspase-3 PE Detection Reagent (B): A 100-test vial of PE-conjugated anti-human Active Caspase-3 antibody that is formulated for use at 50 µl/test. Store at 4°C. Do not freeze.

PE Positive Control Detector (E1): A 10-test vial of PE-conjugated antibody control that is formulated for use at 50 µl/test. This reagent is used with the Cytometer Setup Beads to set the initial instrument compensation settings. Store at 4°C. Do not freeze.

FITC Positive Control Detector (E2): A 10-test vial of FITC-conjugated antibody control that is formulated for use at 50 µl/test. This reagent is used with the Cytometer Setup Beads to set the initial instrument compensation settings. Store at 4°C. Do not freeze.

Human Active Caspase-3 Lysate Standard (C): Three vials containing lyophilized camptothecin-treated Jurkat cell lysate. Each vial should be reconstituted in 0.2 ml of deionized water to prepare a 5× bulk standard. The reconstituted 5× bulk standard contains 30,000 Units/ml of Active Caspase-3 protein ***(see Section 6 for information regarding the unit values for Active Caspase-3)***. Store at 4°C.

Note: The Human Active Caspase-3 Standard vials are stable until the kit expiration date. Following reconstitution, store the freshly reconstituted 5× bulk standard at 2 – 8°C **and use within 12 hours.**

Buffer Reagents

Assay Diluent (G): A single 30 ml bottle of a buffered protein* solution (1×) used to reconstitute and dilute the Human Active Caspase-3 Lysate Standard and to dilute test samples. Store at 4°C.

Wash Buffer (F): Two 130 ml bottles of phosphate buffered saline (PBS) solution (1×), containing protein* and detergent, used for wash steps and to resuspend the washed beads for analysis. Store at 4°C.

Protease Inhibitor Cocktail: A single vial containing lyophilized protease inhibitors. Store lyophilized material at 4°C. The vial should be reconstituted in 1 ml of anhydrous ethanol to prepare a 50× bulk cocktail. The 50× bulk cocktail contains 800 µg/ml benzamidine HCl, 500 µg/ml each of phenanthroline, aprotinin, leupeptin, and pepstatin A, and 50 mM PMSF. Following reconstitution, aliquot and store excess freshly reconstituted 50× bulk cocktail at -20°C. Use within 12 months. Use each aliquot of the 50× bulk cocktail once and then discard.

Cell Lysis Buffer: A single 50 ml bottle of a phosphate buffered saline solution (1×) used to prepare cell samples. Keep sterile. Store at 4°C.

Hazardous Ingredients:

Sodium Azide:

Component D contains 0.1% sodium azide and is harmful if swallowed. Components A, B, E1 - E2, F, and G contain 0.09% sodium azide. Sodium azide yields a highly toxic hydrazoic acid under acidic conditions. Avoid exposure to skin and eyes, ingestion, and contact with heat, acids, and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute azide compounds in running water before discharging to avoid accumulation of potentially explosive deposits in plumbing.

PMSF:

The Protease inhibitor cocktail contains 75% phenylmethyl sulfonylfluoride (PMSF). PMSF is toxic by inhalation and if swallowed. Causes burns. Wear protective clothing, gloves, and eye/face protection. This material and its container must be disposed of as a hazardous waste.

* Source of all serum proteins is from the United States

Materials Required but not Provided

In addition to the reagents provided in the Human Active Caspase-3 CBA Kit, the following items are also required:

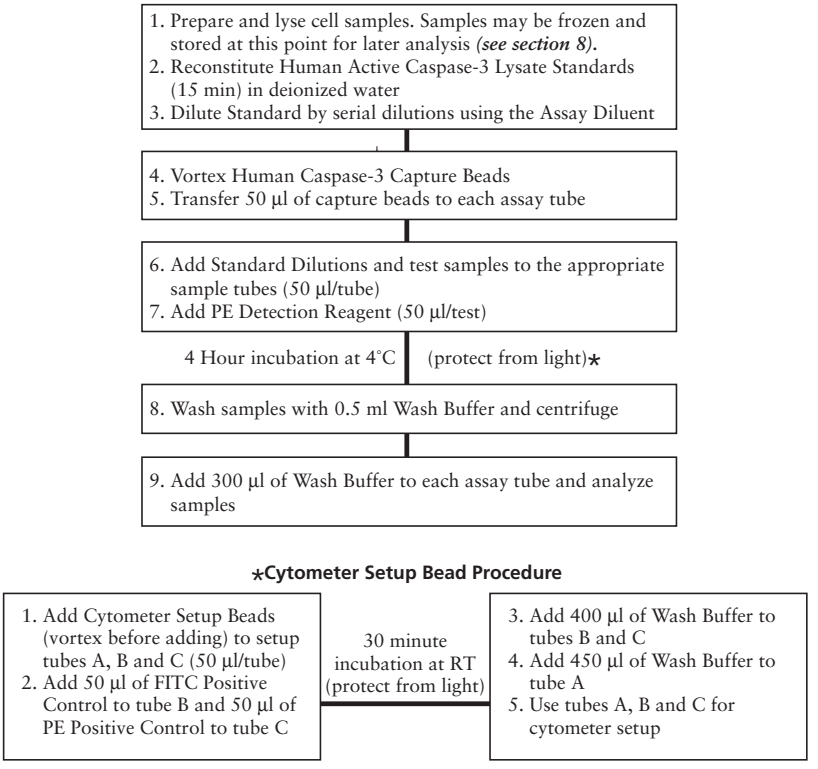
- A flow cytometer equipped with a 488 nm laser capable of detecting and distinguishing fluorescence emissions at 576 and 670 nm (eg, BD FACScan™ or BD FACSCalibur™ instruments) and BD CellQuest™ Software.
- 12 × 75 mm sample acquisition tubes for a flow cytometer (eg, BD Falcon™ Cat. No. 352008).

- BD CBA Software, BD Biosciences Pharmingen (Cat. No. 550065).

  Note: For use with BD CellQuest Software. Microsoft® Excel and a Macintosh or PC-compatible computer are required to utilize the BD CBA Software. See the *BD CBA Software User's Guide* for details.

- BD CaliBRITE™ 3 Beads, BD Biosciences (Cat. No. 340486).
- Deionized water
- Ethanol
- BD FACSComp™ Software version 4.2, BD Biosciences (Cat. No. 341738)
- Microcentrifuge

Overview: Human Active Caspase-3 CBA Assay Procedure



Preparation of Human Active Caspase-3 Lysate Standard

The Human Active Caspase-3 Lysate Standard is lyophilized and should be reconstituted and serially diluted before mixing with the Capture Beads and the PE Detection Reagent.

The active caspase-3 protein in the Human Active Caspase-3 Lysate Standard was assigned an arbitrary unit value. In the Human Active Caspase-3 CBA Assay, **a unit of active caspase-3 corresponds to the amount of active caspase-3 protein in 0.1 µg of total protein from a 4-hour camptothecin-treated Jurkat cell lysate. Due to variation in the activation of caspase-3 in treated Jurkat cells, the actual µg of total lysate protein per unit may vary.**

1. Reconstitute 1 vial of lyophilized Human Active Caspase-3 Lysate Standard with 0.2 ml of deionized water to prepare a 5× bulk standard. Allow the reconstituted standard to equilibrate **for at least 15 minutes** before making dilutions. Agitate vial to mix thoroughly.
2. Label 12 × 75 mm tubes (BD Falcon, Cat. No. 352008) and arrange them in the following order: Top Standard, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256.
3. Add 400 µl of Assay Diluent to the Top Standard tube.
4. Add 250 µl of Assay Diluent to each of the remaining tubes.
5. Transfer 100 µl of 5× bulk standard to the Top Standard tube and mix thoroughly.
6. Perform a serial dilution by transferring 250 µl from the Top Standard to the 1:2 dilution tube and mix thoroughly. Continue making serial dilutions by transferring 250 µl from the 1:2 tube to the 1:4 tube and so on to the 1:256 tube and mix thoroughly ***(see Figure 2.)*** The Assay Diluent serves as the negative control.

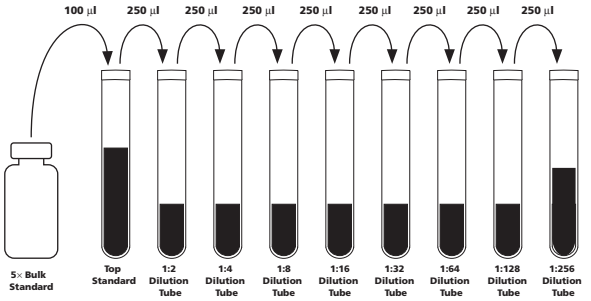


Figure 2. Preparation of Human Active Caspase-3 Lysate Standard Dilutions

The approximate concentration (Units/ml) of active caspase-3 in each dilution tube is shown in Table 1.

Table 1. Human Active Caspase-3 Lysate Standard concentrations after dilution

Protein (Units/ml)	Top Standard	1:2 Dilution Tube	1:4 Dilution Tube	1:8 Dilution Tube	1:16 Dilution Tube	1:32 Dilution Tube	1:64 Dilution Tube	1:128 Dilution Tube	1:256 Dilution Tube
Human Active Caspase-3	6000	3000	1500	750	375	187.5	94	47	23.4

Preparation of Cell Lysis Buffer Mixture

The Cell Lysis Buffer and Protease Inhibitor Cocktail are bottled individually, and it is necessary to mix them together before lysing cell samples.

1. Reconstitute the vial of lyophilized Protease Inhibitor Cocktail with 1 ml of ethanol to prepare a 50× bulk cocktail. Allow the reconstituted cocktail to equilibrate **for at least 15 minutes** before use. Agitate vial to mix thoroughly. Alternatively use an aliquot of 50× bulk Protease Inhibitor Cocktail.

   Note: Any excess reconstituted 50× bulk cocktail should be stored at -20°C in single-use aliquots. Use each aliquot of the reconstituted 50× bulk cocktail only once.

2. Determine the required amount of Cell Lysis Buffer mixture *(**see Section 8**)*. Do not prepare excess Cell Lysis Buffer mixture.

3. Dilute 50× bulk Protease Inhibitor Cocktail to a final 1× concentration in 1× Cell Lysis Buffer (eg, if 5 ml of Cell Lysis Buffer mixture is required, add 100 µl of 50× bulk Protease Inhibitor Cocktail to 5 ml of Cell Lysis Buffer).

4. Mix Cell Lysis Buffer mixture thoroughly. Proceed to Section 8.

   Note: Do not store excess Cell Lysis Buffer mixture containing Protease Inhibitor Cocktail.

Preparation of Test Samples

The Human Active Caspase-3 CBA is designed to measure active caspase-3 protein from cell lysate samples. It is necessary to lyse cell samples using the Cell Lysis Buffer mixture *(**see Section 7**)* before testing in the CBA assay.

The standard curve for active caspase-3 covers a defined set of concentrations from 23 – 6000 Units/ml. It may be necessary to dilute test samples to ensure that their mean fluorescence values fall within the limits or range of the generated standard curve. For best results, samples that are known or assumed to contain high levels of a given protein should be diluted as described below.

1. Count cell number in each sample.
2. Pellet cell sample by centrifugation at 200 × *g* for 5 minutes. Resuspend cell pellet in 1× PBS.
3. Pellet cell sample by centrifugation at 200 × *g* for 5 minutes.
4. Add Cell Lysis Buffer mixture *(**see Section 7**)* to the cell pellet to bring cell concentration to 2×10^6/ml.

   Note: Numbers are representative for Jurkat cells. It may be necessary to lyse cells at higher or lower density depending on cell type.
5. Incubate cells for 30 minutes on ice, and vortex at 10-minute intervals.
6. Pellet cellular debris by centrifugation at 12,500 rpm (eg, in a microcentrifuge) for 10 minutes. Transfer cell lysate to a clean tube.
7. Cell lysates may be stored frozen at -70°C for up to 6 months at this point. If samples are stored frozen, thaw sample before proceeding to step 8. Avoid multiple freeze/thaw treatments of sample.
8. Dilute cell lysate sample by the desired dilution factor (ie, 1:2, 1:10, or 1:20) using the appropriate volume of Assay Diluent.
9. Mix sample dilutions thoroughly before transferring samples to the appropriate assay tubes containing Capture beads.

Human Active Caspase-3 CBA Assay Procedure

Following the preparation and dilution of the standard, transfer the standards, capture beads, PE Detection Reagent, and test samples to the appropriate assay tubes for incubation and analysis. **In order to calibrate the flow cytometer and quantitate test samples, it is necessary to run the Active Caspase-3 Lysate Standard and the Cytometer Setup controls in each experiment.** See Table 2 for a detailed description of the reagents added to the Active Caspase-3 Lysate Standard control assay tubes. The Cytometer Setup procedure is described in Section 10.

1. Add 50 µl of the Caspase-3 Capture beads to the appropriate assay tubes. Vortex the Capture beads before adding them to the assay tubes.
2. Add 50 µl of the Human Active Caspase-3 Lysate Standard dilutions to the control assay tubes.

3. Add 50 µl of each cell lysate test sample to the test assay tubes.
4. Add 50 µl of the Human Active Caspase-3 PE Detection Reagent to the assay tubes.
5. Incubate the assay tubes for 4 hours at 4°C and protect from direct exposure to light. During this incubation, perform the Cytometer Setup procedure described in Sections 10.1 - 10.3.
6. Add 0.5 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.
9. Begin analyzing samples on a flow cytometer. **Vortex each sample for 3 - 5 seconds immediately before analyzing on the flow cytometer.***

**Note:* It is necessary to analyze CBA samples on the day of the experiment. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

Table 2. Essential control assay tubes

Tube No.	Reagents (All reagents volumes are 50 µl)
1 (Negative Control 0 Units/ml Standard)	Capture Beads, Assay Diluent, PE Detection Reagent
2 (23.4 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:256 Dilution, PE Detection Reagent
3 (47 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:128 Dilution, PE Detection Reagent
4 (94 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:64 Dilution, PE Detection Reagent
5 (187.5 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:32 Dilution, PE Detection Reagent
6 (375 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:16 Dilution, PE Detection Reagent
7 (750 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:8 Dilution, PE Detection Reagent
8 (1500 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:4 Dilution, PE Detection Reagent
9 (3000 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard 1:2 Dilution, PE Detection Reagent
10 (6000 Units/ml Standard)	Capture Beads, Active Caspase-3 Lysate Standard "Top Standard", PE Detection Reagent

Cytometer Setup, Data Acquisition, and Analysis

The Cytometer setup information in this section is for the BD FACScan and BD FACSCalibur flow cytometers. The BD FACSComp software is useful for setting up the flow cytometer. BD CellQuest Software is required for analyzing samples and formatting data for subsequent analysis using the BD CBA Software.

Preparation of Cytometer Setup Beads

1. Add 50 µl of Cytometer Setup Beads to three cytometer setup tubes labeled A, B, and C.
2. Add 50 µl of FITC Positive Control Detector to tube B.
3. Add 50 µl of PE Positive Control Detector to tube C.
4. Incubate tubes A, B, and C for 30 minutes at room temperature and protect from direct exposure to light.
5. Add 450 µl of Wash Buffer to tube A and 400 µl of Wash Buffer to tubes B and C.
6. Proceed to Section 10.2.

Instrument Setup with BD FACSComp™ Software and BD CaliBRITE™ Beads

1. Perform instrument start up.
2. Perform flow check.
3. Prepare tubes of BD CaliBRITE beads and open BD FACSComp software.
4. Launch BD FACSComp software.
5. Run BD FACSComp software in Lyse/No Wash mode.
6. Proceed to Section 10.3.

Note: For detailed information on using BD FACSComp with BD CaliBRITE beads to set up the flow cytometer, refer to the *BD FACSComp Software User's Guide* and the *BD CaliBRITE Beads* Package Insert. BD FACSComp Version 4.2 contains a BD CBA preference setting to automatically save a BD CBA calibration file at the successful completion of any Lyse/No Wash assay. The BD CBA calibration file provides the optimization for FSC, SSC, and threshold settings as described in Section 10.3 steps 3 – 5. Optimization of the fluorescence parameter settings is still required (ie, PMT and compensation settings, see Section 10.3 step 6).

Instrument Setup with the Cytometer Setup Beads

1. Launch BD CellQuest Software and open the CBA Instrument Setup template.

   Note: The CBA Instrument Setup template can be found on the BD CBA software or BD FACStation CD for Macintosh computers in the BD CBA folder. Following installation on Macintosh computers using BD CBA Software Version 1.0, the template can be found in the BD Applications/BD CBA folder/Sample Files/Mouse Isotyping Files/Instrument Setup folder. For BD CBA Software Version 1.1 or higher, the template can be found in the BD Applications/BD CBA folder. The template is not installed from the CD on PC-compatible computers. This file may also be downloaded via the internet from: http://www.bdbiosciences.com/pharmingen/CBA/downloads.shtml

2. Set the instrument to Acquisition mode.

   Note: The BD CBA Software will evaluate data in five parameters (FSC, SSC, FL1, FL2, and FL3). Turn off additional detectors.

3. Set SSC (side light scatter) and FSC (forward light scatter) to Log mode.
4. Decrease the SSC PMT voltage by 100 from what BD FACSComp set.
5. Set the Threshold to FSC at 650.
6. In setup mode, run Cytometer Setup Beads tube A. Follow the setup instructions on the following pages.

   Note: Pause and restart acquisition frequently during the instrument setup procedure in order to reset detected values after settings adjustments.

Adjust gate R1 so that the singlet bead population is located in gate R1 (**Figure 3a**).

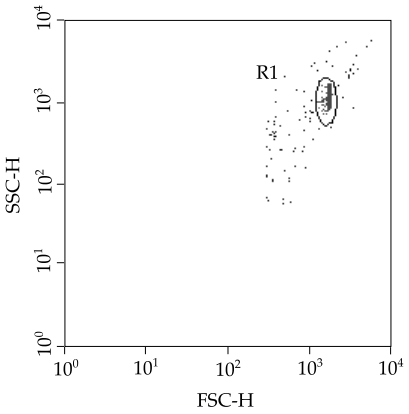


Figure 3a

Adjust the FL3 PMT so that the median of the top FL3 bead population's intensity is around 5000 (**Figure 3b**). Adjust gate R3 as necessary so that the dim FL3 bead population is located in gate R3 (**Figure 3b**). Do not adjust the R2 gate.

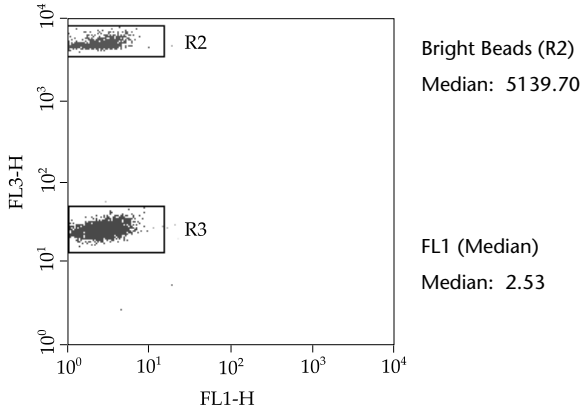


Figure 3b

Adjust the FL1 PMT so that the median of FL1 is approximately 2.0 – 2.5 (**Figure 3b**).

Adjust the FL2 PMT so that the median of FL2 is approximately 2.0 - 2.5 (**Figure 3c**).

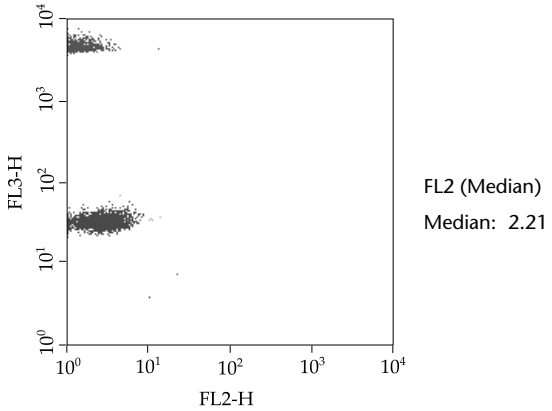


Figure 3c

Run Cytometer Setup Beads tube B to adjust the compensation settings for FL2 – %FL1.

Adjust gate R5 as necessary so that the FL1 bright bead population is located in gate R5 (Figure 3d). Using the FL2 – %FL1 control, adjust the median of R5 to equal the median of R4 (**Figure 3d**).

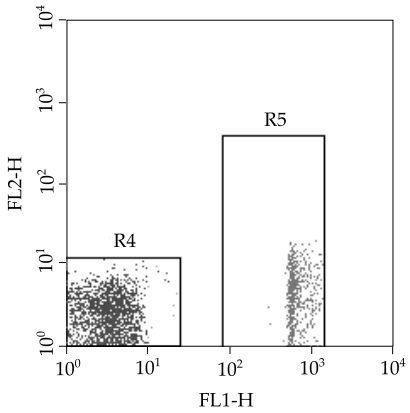


Figure 3d

Run Cytometer Setup Beads tube C to adjust the compensation settings for FL1 – %FL2 and FL3 – %FL2.

Adjust gate R7 so that the FL2 bright bead population is located in gate R7 (**Figure 3e**). Using the FL1 – %FL2 control, adjust the median of R7 to equal the median of R6 (**Figure 3e**).

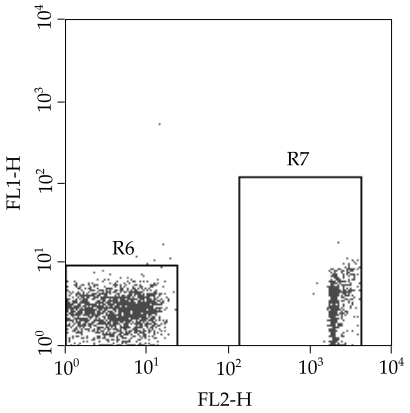


Figure 3e

Adjust gate R9 so that the FL2 bright bead population is located in gate R9 (**Figure 3f**). Using the FL3 – %FL2 control, adjust the median of R9 to equal the median of R8 (**Figure 3f**).

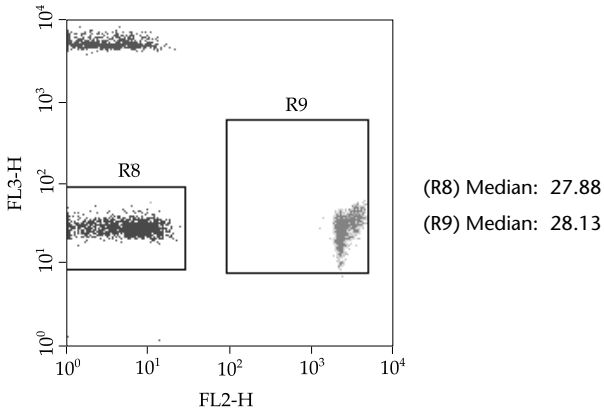


Figure 3f

Set the FL2 – %FL3 to 0.1 if necessary. Save and print the optimized instrument settings.

Data Acquisition

1. Open the Acquisition template on the BD CBA Software.

   Note: Following installation of the BD CBA Software, the Acquisition template is located in the BD Applications/BD CBA folder/Sample Files/Mouse Isotyping Files/Instrument Set Up folder and is labeled "Isotype Kit Acquire Template". Alternatively, the Acquisition template may be downloaded via the internet from: http://www.bdbiosciences.com/pharmingen/CBA/downloads.shtml

2. Set acquisition mode and retrieve the optimized instrument settings from Section 10.3.
3. In the Acquisition and Storage window, set the resolution to 1024.
4. Set number of events to be counted at 500 of R1 gated events. (This will ensure that the sample file contains approximately 500 events of the Capture Beads).
5. Set number of events to be collected to "all events". Saving all events collected will ensure that no true bead events are lost due to incorrect gating.
6. In setup mode, run tube no.1 and using the FSC vs. SSC dot plot, place the R1 region gate around the singlet bead population ***(See Figure 3a)***.
7. Samples are now ready to be acquired.
8. Begin sample acquisition with the flow rate set at HIGH.

Note: Run the negative control tube (0 Units/ml standard) before any of the recombinant standard tubes. Run the control assay tubes before any unknown test assay tubes. Run the tubes in the order listed in Table 2 of Section 9.

To facilitate analysis of data files using the BD CBA Software and to avoid confusion, add a numeric suffix to each file that corresponds to the assay tube number (ie, Tube No.1 containing 0 Units/ml could be saved as KT032598.001). The file name must be alphanumeric (ie, contain at least one letter).

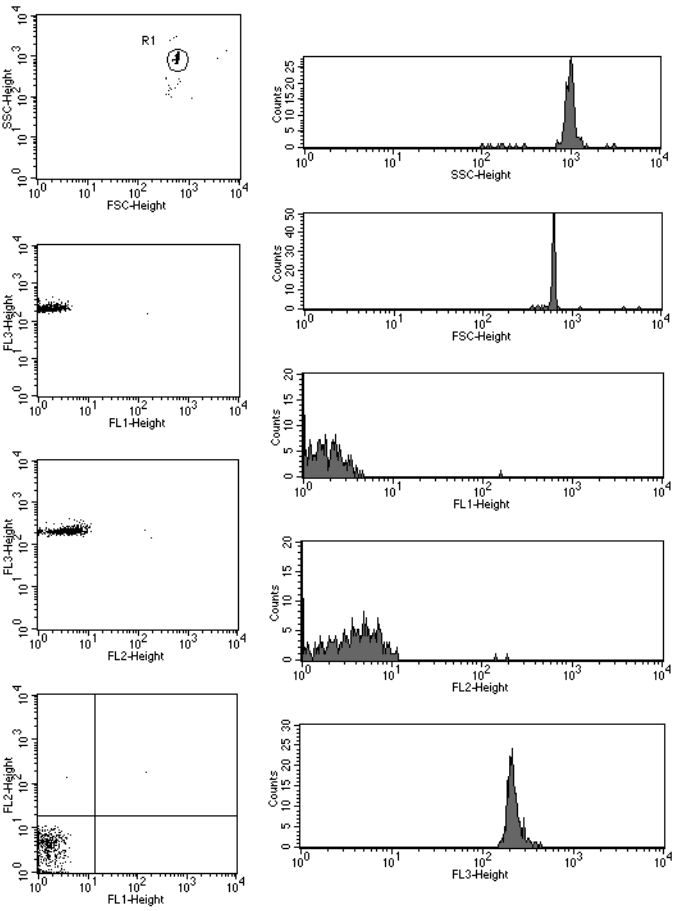


Figure 4. Acquisition Template Example

Analysis of Sample Data

The analysis of BD CBA data is optimized when using the BD CBA Software. Install the software according to the instructions in the *Software User's Guide*.

1. Transfer the FCS file data for the experiment to the computer with the BD CBA Software.
2. Create two new file folders and label one "Standards" and the other "Samples".
3. Move data files to the appropriate folders.

   Note: Only the files for control assay tubes no. 1 – 10 (the PE Detection Reagent alone and the dilution of standards) should be moved to the "Standards" file folder. All other samples should be moved to the "Samples" file folder.

Follow the instructions for analysis given in the *BD CBA Software User's Guide*.

Note: When entering analyte concentrations for the standards used in the experiment, it is necessary to give names to each analyte. **For the Human Active Caspase-3 CBA, analyte 1 is Active Caspase-3.**

Note: The default concentration setting for the BD CBA Software is pg/ml. To change this setting to Units/ml, please refer to the *BD CBA Software User's Guide*, Chapter 2: Quantitative Analysis.

Typical Data

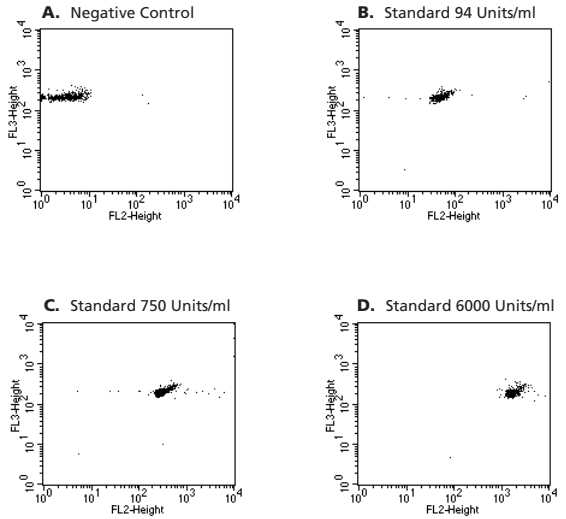


Figure 5. BD CellQuest Data Examples for Standards and Detectors Alone

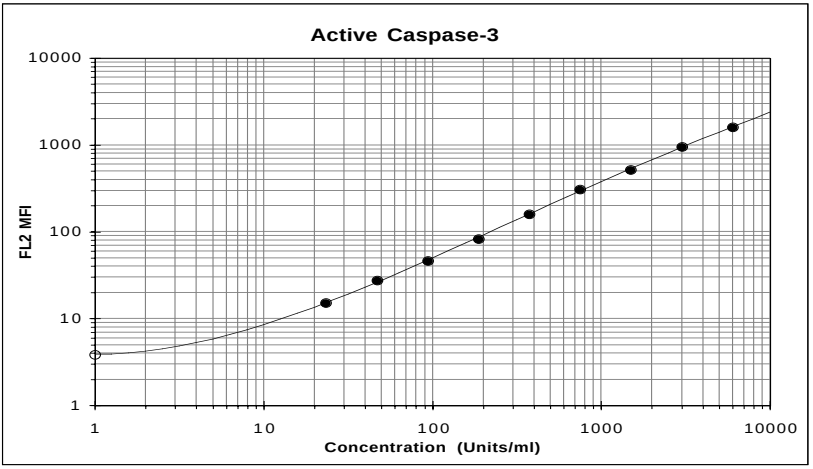


Figure 6. BD CBA Software Example of Standard Curve

Results

BD Cytometric Bead Array Analysis

12/2001

					Active Caspase-3		
	Filename	SampleID	Acq Date	Dilut Factor	FL2 MFI	Tube Units/ml	Sample Units/ml
1	CBA120401.039	35434	4-Dec-01	1	4.3	2.3	2.3
2	CBA120401.040	35439	4-Dec-01	1	3.4	<=0	
3	CBA120401.041	35449	4-Dec-01	1	2.7	<=0	
4	CBA120401.042	35434	4-Dec-01	5	388.9	1044.0	5220.2
5	CBA120401.043	35439	4-Dec-01	10	228.8	565.9	5659.4
6	CBA120401.044	35449	4-Dec-01	20	112.4	253.0	5059.6
7	CBA120401.045	35434	4-Dec-01	1	3.4	<=0	
8	CBA120401.046	35439	4-Dec-01	1	3.0	<=0	
9	CBA120401.047	35449	4-Dec-01	1	3.4	<=0	
10	CBA120401.048	35434	4-Dec-01	5	3.3	<=0	
11	CBA120401.049	35439	4-Dec-01	10	2.8	<=0	
12	CBA120401.050	35449	4-Dec-01	20	2.8	<=0	

Figure 7. BD CBA Software Example of Sample Results

Performance

The Human Active Caspase-3 CBA assay has been rigorously tested for performance characteristics including sensitivity, specificity, and intra- and inter-assay precision.

Sensitivity

The individual standard curve range for active caspase-3 defines the minimum and maximum quantifiable levels using the Human Active Caspase-3 CBA (ie, 23.4 Units/ml and 6000 Units/ml). By applying the 4-parameter curve fit option, it is possible to extrapolate values for sample intensities not falling within the limits of the standard curve. It is up to the researcher to decide the best method for calculating values for unknown samples using this assay. The sensitivity for active caspase-3 using the Human Active Caspase-3 CBA is defined as the corresponding concentration at two standard deviations above the median fluorescence of 20 replicates of the negative control (0 Units/ml).

Protein	Median Fluorescence	Standard Deviation	Assay Sensitivity (Units/ml)
Active Caspase-3	3.5	0.4	5.7 Units/ml

Specificity

The antibody pairs used in the Human Active Caspase-3 CBA assay have been screened for specific reactivity with active caspase-3. Analysis of camptothecin-treated cell samples expressing (Jurkat) or deficient (MCF-7) in active caspase-3 found no cross-reactivity or background detection of protein in populations deficient in active caspase-3 using this assay.

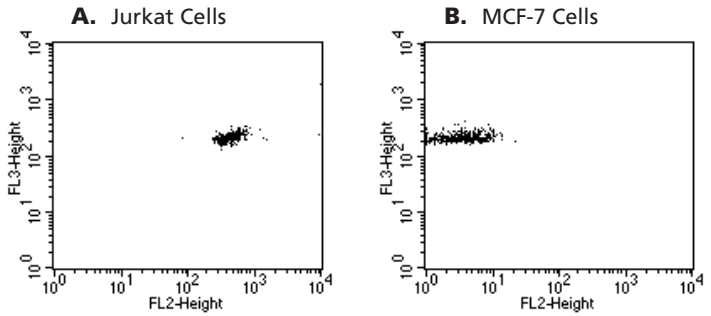


Figure 8. BD CellQuest Data Examples for Detection of Active Caspase-3

Precision

Intra-assay: Ten replicates of each of three different levels of active caspase-3 were tested.

Protein	Active Caspase-3		
Actual Mean Conc. (Units/ml):	58.7	638.9	1947.8
SD	5.1	65.6	140.7
% CV	8.7%	10.3%	7.2%

Inter-assay: Three different levels of active caspase-3 (94, 750, and 3000 Units/ml) were tested in four experiments conducted by different operators.

Protein	Active Caspase-3		
Number of Replicates:	8	8	8
Actual Mean Conc. (Units/ml):	68.9	695.5	2792.3
SD	6.9	88.5	328.2
% CV	10.1%	12.7%	11.8%

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

Troubleshooting Tips

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 wash step. Do not wash or resuspend beads in volumes higher than recommended.
High background.	Remove excess Human Active Caspase-3 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.
Debris (FSC/SSC) during sample acquisition.	Increase 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.
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.

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

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