BD™ Cytometric Bead Array (CBA)

Mouse Immunoglobulin Isotyping Kit
Instruction Manual

BD

Kit Contents

(Store the following items at 4°C)

A   Mouse Ig Capture Bead Array: 1 vial, 5 ml

B1   Anti-Mouse Kappa PE* Detector Antibody (10×): 1 vial, 0.5 ml

B2   Anti-Mouse Lambda FITC Detector Antibody (10×): 1 vial, 0.5 ml

C1   Mouse Ig Standards 1 (10×): 1 vial, 0.5 ml

C2   Mouse Ig Standards 2 (10×): 1 vial, 0.5 ml

D   Cytometer Setup Beads: 1 vial, 1.5 ml

E1   PE Positive Control Detector: 1 vial, 1 ml

E2   FITC Positive Control Detector: 1 vial, 1 ml

F   PBS Detergent Solution (10×): 1 vial, 30 ml

G   BSA Solution (10×): 1 vial, 30 ml

US Orders: **877.232.8995**

Table of Contents

Introduction

Flow cytometry is an analysis tool that allows for the discrimination of different particles on the basis of size and color. Multiplexing is the simultaneous assay of many analytes in a single sample. The BD™ Cytometric Bead Array (CBA) employs a series of spectrally discrete particles that can be used to detect soluble analytes, combining the powerful tools of flow cytometry and multiplexing.

The BD CBA system combines a conventional immunoassay with the sensitivity of fluorescence via flow cytometry. Each bead in a BD CBA provides a capture surface for a specific protein and is analogous to an individually coated well in an ELISA plate. The broad dynamic range of fluorescence detection, combined with multiple analyte detection, allows for small sample volume, fewer sample dilutions, and substantially less time overall to establish the value of an unknown versus a conventional ELISA approach. The Mouse Immunoglobulin Isotyping CBA Kit can be used to rapidly identify the heavy and light chain isotypes of a mouse monoclonal antibody in a single sample.

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.

Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.

US Orders: **877.232.8995**   5

Principle of the Test

Seven bead populations with distinct fluorescence intensities, which can be resolved in the FL3 channel, have been coated with monoclonal antibodies specific for IgA, IgE, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, or IgM mouse heavy-chain isotypes. The seven bead populations have been mixed together to form the BD™ CBA. *Figure 1* displays the FL3 intensity profile of the BD CBA; each discernable population corresponds to a heavy-chain specificity. For data analysis, the BD CBA Software automatically gates on the FL3 peak corresponding to each isotype.
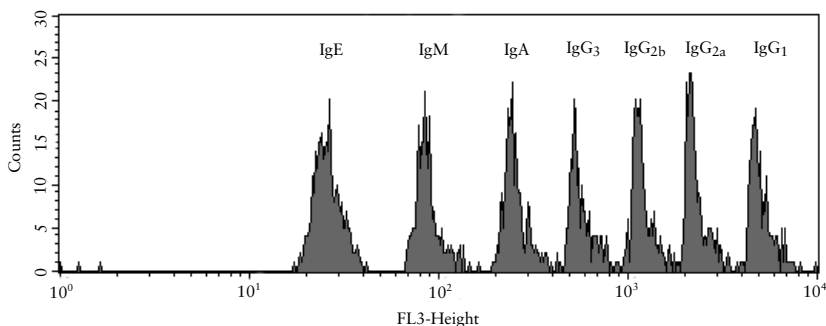
Figure 1

In the first step, a sample of the hybridoma supernatant or ascites is briefly incubated with the BD CBA. Subsequently, the isotype is revealed by a second incubation with a mixture of PE-labeled Anti-Mouse Kappa and FITC-labeled Anti-Mouse Lambda detector antibodies. Following this step, the beads are analyzed on a flow cytometer.

The heavy-chain isotype is easily identified by the FL3 intensity of the stained bead population, while the light chain is indicated by whether the positive beads fluoresce in FL1 (λ) or FL2 (κ). The determination is documented by simultaneous display of FL1 vs. FL3 and FL2 vs. FL3 dot plots as in the BD CellQuest™ Data Profiles in Section 10.2. The result is automatically calculated by the BD CBA Software.

Advantages

This assay provides several advantages compared with conventional microtiter ELISA assay:

- The required sample volume for this assay is 10 – 1000 fold less than for ELISA, considering that all 7 heavy-chain isotypes can be tested in one tube to discriminate 14 possible heavy/light-chain combinations.

- Greater sensitivity and reproducibility are achieved with the BD CBA Mouse Immunoglobulin Isotyping Kit.

- Multiple standards can be assayed in a single tube.

- Replicate tests are unnecessary, as each bead can be considered an individual test.

- Costs are reduced, as more samples can be measured per assay.

Limitations

- This is a qualitative assay and assay signal is not proportional to protein levels. This assay is not recommended for serum or plasma samples because they contain all seven Ig isotypes.

- Due to the use of a two-color detector, BD CBA analysis software is required to analyze this kit. This kit cannot be analyzed using FCAP Array™ software.

- Due to the use of FITC as a reporter molecule, this kit cannot be analyzed on the BD FACSArray™ bioanalyzer.

Reagents Provided

Contains sufficient reagents for 100 tests

A. **Mouse Ig Capture Bead Array :** One vial containing 5.0 ml premixed beads specific for mouse IgA, IgE, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, and IgM.

 Note: Mix well before use.

B1. **Anti-Mouse Kappa PE Detector Antibody (10×):** One vial containing 0.5 ml of PE-labeled anti-mouse Kappa Detector antibody.

 Note: 10X concentrate, dilute with **1X Master Buffer** before use.

B2. **Anti-Mouse Lambda FITC Detector Antibody (10×):** One vial containing 0.5 ml of FITC-labeled anti-mouse Lambda Detector antibody.

 Note: 10× concentrate, dilute with **1× Master Buffer** before use.

C1. **Mouse Ig Standards 1 (10×):** One vial containing 0.5 ml IgA κ and λ, IgE κ, IgG_1 κ and λ, and IgG_{2b} κ and λ.

 Note: 10× concentrate, dilute with **1× Master Buffer** before use.

C2. **Mouse Ig Standards 2 (10×):** One vial containing 0.5 ml IgG_{2a} κ and λ, IgG_3 κ and λ, and IgM κ and λ.

 Note: 10× concentrate, dilute with **1× Master Buffer** before use.

D. **Cytometer Setup Beads:** One vial containing 1.5 ml calibration beads for initial setup of PMT voltages and compensation settings.

    Note: Mix well before use.

E1. **PE Positive Control Detector:** One vial containing 1.0 ml PE-labeled positive control Ig.

E2. **FITC Positive Control Detector:** One vial containing 1.0 ml FITC-labeled positive control Ig.

F. **PBS Detergent Solution (10×):** One vial containing 30 ml 10× PBS Detergent Solution.

    Note: 10× concentrate; see Section 7 to prepare **Master Buffer.**

G. **BSA* Solution (10×):** One vial containing 30 mL 10× BSA Solution.

    Note: 10× concentrate; see *Reagent Preparation*, page 9, to prepare Master Buffer.

    Hazardous Ingredient:

Sodium Azide:
    Components A, B1, B2, C1, C2, D, 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.

* Source of all serum proteins is from USDA inspected abattoirs located in the United States.

Materials Required But Not Provided

In addition to standard laboratory equipment, the following items are required:

- BD™ CBA Software (Cat. No. 550065).

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

- Flow cytometer with 488-nm laser and three fluorescence parameters — FL1 (515-545 nm), FL2 (555-585 nm) FL3 (> 600 nm) and BD CellQuest Software, *e.g.*, BD FACScan™, BD FACSCalibur™.

- 96-well U-bottom plates (eg, BD Falcon™, Cat. No. 353910) Alternatively, a Protocol is provided for use of sample tubes instead of plates.

- Sample tubes for flow cytometer (eg, BD Falcon, Cat. No. 352008).

- BD CaliBRITE™ 3 Beads, (Cat. No. 340486).

Storage

All components of the BD™ CBA Mouse Immunoglobulin Isotyping Kit should be stored undiluted at 2 – 8°C. **Do not freeze.**

Warnings and Precautions

Although all reagents contain sodium azide as a preservative, care should be taken to avoid microbial contamination, which can cause erroneous results. Excessive changes in results can indicate contamination of the beads or changes in instrument conditions. If deterioration is suspected, a new bead suspension should be used and/or the instrument conditions should be checked.

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

Reagent Preparation

Bring all reagents to room temperature (20 – 25°C) before use.

Prepare Master Buffer

- Combine 1 part PBS Detergent Solution (10×), 1 part BSA Solution (10×), and 8 parts deionized water to yield the **Master Buffer**. For extended storage, use 0.1% sodium azide.

  Note 1: The **Master Buffer** is used throughout the assay for dilution of samples, detector antibodies, and positive controls.

  Note 2: Approximate volumes of **Master Buffer** needed for subsequent stages of a single assay run are 1 ml for Cytometer Setup, 2 ml for the assay Standards, and 1 ml per test sample.

Prepare Standards

- Mouse Ig Standards 1 (10×): Standard contains 2.5 µg/ml each of IgG_1 κ/λ, IgG_{2b} κ/λ, IgA κ/λ, and IgE κ. Dilute to 1× with **Master Buffer**.

- Mouse Ig Standards 2 (10×): Standard contains 2.5 µg/ml each of IgG_{2a} κ/λ, IgG_3 κ/λ, and IgM κ/λ. Dilute to 1× with **Master Buffer**.

Prepare Detector Antibodies

- PE- and FITC-labeled Detector Antibodies (10×): Together in one tube, dilute your required volume of Anti-Mouse Kappa PE and Anti-Mouse Lambda FITC detector antibodies to 1× with **Master Buffer**.

Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.

US Orders: **877.232.8995** 9

Sample Preparation Guidelines

Mouse hybridoma supernatants and ascites may be tested with the BD™ CBA Mouse Ig Isotyping Kit. Samples should be clear and all particulate matter should be removed before running the assay.

The sample concentration should be between 10 ng/ml and 0.5 mg/ml. The optimum concentration range for the BD CBA Mouse Ig Isotyping Kit is 10 – 200 ng/ml.

- For hybridoma supernatants: Dilute samples 1:100 in **Master Buffer** to assure the Ig concentration falls within the optimal assay range.

- For ascites: Perform multiple sample dilutions in **Master Buffer** (≥ 2 dilutions/sample), such as 1:10,000 and 1:100,000 to assure the Ig concentration falls within the optimal assay range.

Recommended dilutions are based on typical concentration ranges for hybridoma supernatants (1 – 50 µg/ml) and ascites (1 – 10 mg/ml). All isotypes might test positive in mouse ascites due to residual host Ig if samples are used at higher concentrations than recommended.

Assay Procedure

Bead Staining Using Tubes

1. Add 50 µl of Mouse Ig Capture Bead Array to each tube. Reserve tubes 1 and 2 for negative controls.

2. Add 50 µl **Master Buffer** to tubes 1 and 2. Add 50 µl of standards or samples to the appropriate remaining tubes. See *Reagent Preparation*, page 9, for **Master Buffer** preparation.

3. Incubate for 15 minutes at room temperature.

4. Add 50 µl **Master Buffer** to tube 2. Add 50 µl of PE/FITC Detector Antibodies mixture to each remaining tube.

5. Incubate for 15 minutes at room temperature in the dark.

6. Add 500 µl of **Master Buffer** to each tube.

7. Spin tubes at 1200 rpm for 5 minutes.

8. Carefully aspirate fluid from all tubes.

   Note: Keep tip against side of tube to avoid aspirating bead pellet.

9. Add 500 µl of **Master Buffer** to each tube and resuspend the beads.

10. Keep tubes at 2 – 8°C in the dark if data acquisition is not performed immediately.

11. Run samples within 2 – 3 hours after completion of the assay. Vortex tubes immediately prior to acquiring on the flow cytometer.

Bead Staining Using Plates

1. Add 50 µl of Mouse Ig Capture Bead Array to each well. Reserve wells 1 and 2 for negative controls.

2. Add 50 µl **Master Buffer** to wells 1 and 2. Add 50 µl of standards or samples to the appropriate remaining wells. See ***Reagent Preparation***, page 9, for **Master Buffer** preparation.

3. Incubate for 15 minutes at room temperature.

4. Add 50 µl **Master Buffer** to well 2. Add 50 µl of PE/FITC Detector Antibodies to each remaining well.

5. Incubate for 15 minutes at room temperature in the dark.

6. Add 100 µl of **Master Buffer** to each well.

7. Spin the plate at 1200 rpm for 5 minutes.

8. Carefully aspirate fluid from all wells.

9. Add 200 µl of **Master Buffer** to each well and resuspend the beads.

10. Transfer the suspended beads to tubes.

11. Add 300 µl of **Master Buffer** to each tube.

12. Keep tubes at 2 – 8°C in the dark if data acquisition is not performed immediately.

13. Run samples within 2 – 3 hours after completion of the assay. Vortex tubes immediately prior to acquiring on the flow cytometer.

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 **Master Buffer** to tube A and 400 µl of **Master Buffer** to tubes B and C.

6. Proceed to ***Instrument Setup With BD FACSComp™ Software and BD CaliBRITE™ Beads***, page 12.

US Orders: **877.232.8995**

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 *Instrument Setup With the Cytometer Setup Beads,* page 12.

   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 *Instrument Setup With the Cytometer Setup Beads,* Steps 3 - 5. Optimization of the fluorescence parameter settings is still required (i.e., PMT and compensation settings, see *Instrument Setup With the Cytometer Setup Beads,* 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: **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 2a*).
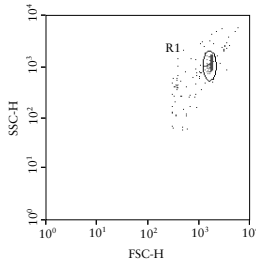

Figure 2a

Adjust FL3 PMT value so that the median of the top FL3 bead population's intensity is around 5000 (*Figure 2b*). Adjust gate R3 as necessary so that the dim FL3 bead population is located in gate R3 (*Figure 2b*). Do not adjust the R2 gate.
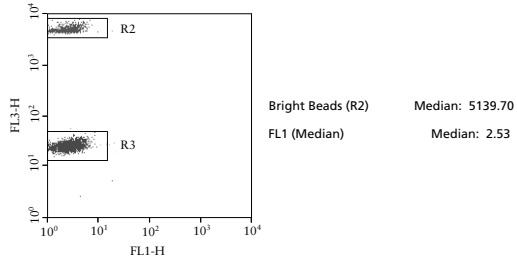

Bright Beads (R2)   Median: 5139.70

FL1 (Median)   Median: 2.53

Figure 2b

Adjust FL1 PMT value so that the median of FL1 is approximately 2.0 – 2.5 (*Figure 2b*).

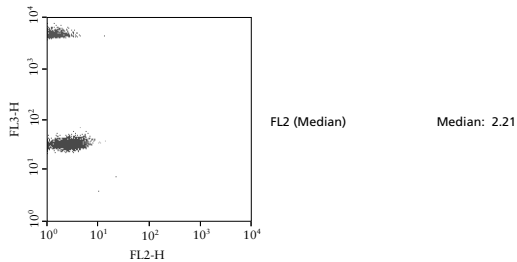Adjust FL2 PMT value so that the median of FL2 is approximately 2.0 – 2.5 (*Figure 2c*).

FL2 (Median)   Median: 2.21

Figure 2c

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

US Orders: **877.232.8995**

Adjust gate R5 as necessary so that the FL1 bright bead population is located in gate R5 (*Figure 2d*). Using FL2 – %FL1 control, adjust the median of R5 to equal the median of R4 (*Figure 2d*).
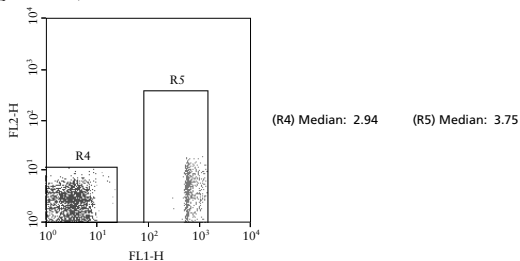

(R4) Median: 2.94   (R5) Median: 3.75

Figure 2d

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 2e*). Using the FL1 – %FL2 control, adjust the median of R7 to equal the median of R6 (*Figure 2e*).
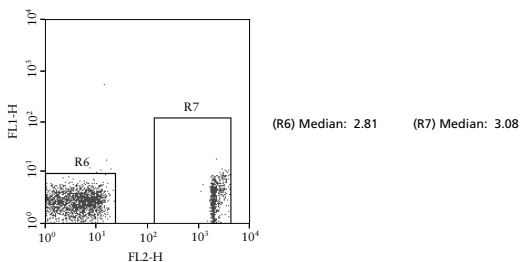

(R6) Median: 2.81   (R7) Median: 3.08

Figure 2e

Adjust gate R9 so that the FL2 bright bead population is located in gate R9 (*Figure 2f*). Using FL3 - %FL2 control, adjust the median of R9 to equal the median of R8 (*Figure 2f*).
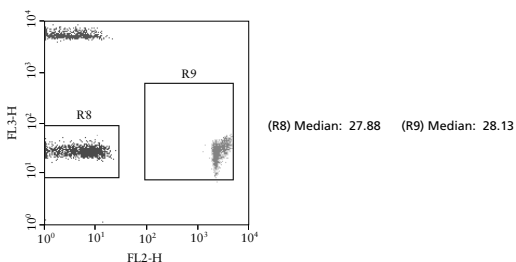

(R8) Median: 27.88   (R9) Median: 28.13

Figure 2f

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 can be found in the BD Applications/BD CBA folder/ Sample Files/Mouse Isotyping Files/Instrument Setup folder and is labeled "Isotype Kit Acquire Template". Alternatively, the Acquisition template may be downloaded via the internet from: **bdbiosciences.com/pharmingen/CBA/downloads.shtml**

2. Set acquisition mode and retrieve the optimized instrument settings from *Instrument Setup With the Cytometer Setup Beads*, page 12.

3. In the Acquisition & Storage window, set the resolution to 1024.

4. Set number of events to be counted at 3000 of R1 gated events.

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 2a*).

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 before any other. We recommend using the Detector Ab-only sample (tube 1) as the negative control. The **Master Buffer**-only sample (tube 2) is an additional negative control for autofluorescence of the beads. It may be run along with the positive-control and unknown samples in any desired sequence. 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 could be saved as KT032598.001). The file name must be alphanumeric (ie, contain at least one letter).
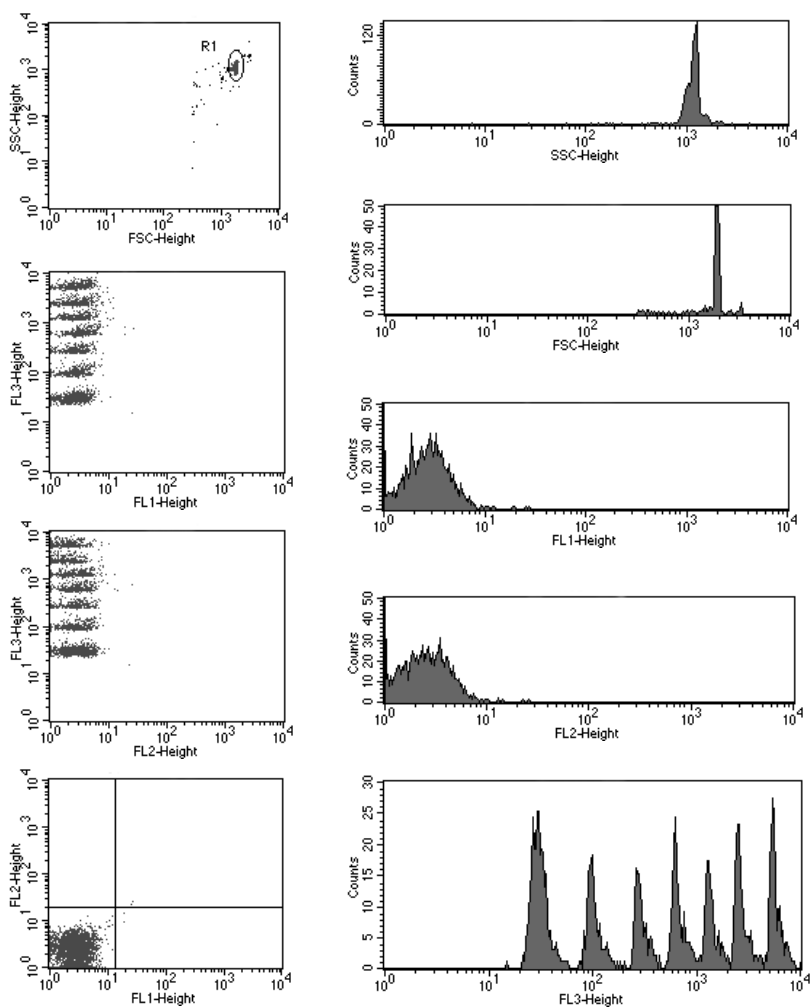
Figure 3. Acquisition Template Example

bdbiosciences.com

Analysis Results and Typical Data

BD™ CBA Data Report

Analyze the data with the BD™ CBA Software (Cat. No. 550065).

1. Install the BD CBA software following the instructions carefully. The BD CBA Software has been designed to automatically determine the isotype of samples analyzed in BD CellQuest™.

2. Analyze the data. For detailed information, refer to the *CBA Software User's Guide*.

3. Figures 4a-c on the following pages show parts of a single spreadsheet

   0 = Tube 1, negative control containing Detector Ab alone

   1 = Standards One

   2 = Standards Two

   3-14 = "unknown" samples, each containing a single mAb

   15 = Tube 2, **Master Buffer** alone

	Sample ID	Filename	Acq Date	Dilut Factor	FL1 MFI λ	λ	FL2 MFI κ	κ
		Negative Threshold (>= 3):		3	IgE			
0	Detector Ab only	011200S/B.003	01/12/00	1	2.50		2.74	
1	Standards 0ne	011200S/B.004	01/12/00	1	2.48	NEG	71.69	POS
2	Standards Two	011200S/B.006	01/12/00	1	2.53	NEG	2.67	NEG
3	sample #1	011200S/B.007	01/12/00	1	2.50	NEG	2.67	NEG
4	sample #2	011200S/B.008	01/12/00	1	2.55	NEG	2.64	NEG
5	sample #3	011200S/B.009	01/12/00	1	2.56	NEG	2.81	NEG
6	sample #4	011200S/B.010	01/12/00	1	2.44	NEG	2.64	NEG
7	sample #5	011200S/B.011	01/12/00	1	2.53	NEG	2.76	NEG
8	sample #6	011200S/B.012	01/12/00	1	2.55	NEG	2.59	NEG
9	sample #7	011200S/B.013	01/12/00	1	2.67	NEG	2.64	NEG
10	sample #8	011200S/B.014	01/12/00	1	2.56	NEG	2.76	NEG
11	sample #9	011200S/B.015	01/12/00	1	2.53	NEG	2.74	NEG
12	sample #10	011200S/B.016	01/12/00	1	2.48	NEG	2.62	NEG
13	sample #11	011200S/B.017	01/12/00	1	2.50	NEG	2.71	NEG
14	sample #12	011200S/B.018	01/12/00	1	2.48	NEG	283.87	POS
15	auto	011200S/B.auto	01/12/00	1	2.53	NEG	2.69	NEG

Figure 4a. Raw data and results for detection of IgE κ/λ

		IgM				IgA			
	Sample ID	FL1 MFI λ	λ	FL2 MFI κ	κ	FL1 MFI λ	λ	FL2 MFI κ	κ
0	Detector Ab only	2.62		2.62		2.51		2.54	
1	Standards 0ne	2.62	NEG	2.62	NEG	181.06	POS	168.49	POS
2	Standards Two	107.46	POS	107.46	POS	2.48	NEG	2.41	NEG
3	sample #1	2.69	NEG	2.50	NEG	2.41	NEG	2.44	NEG
4	sample #2	2.78	NEG	2.56	NEG	2.46	NEG	2.41	NEG
5	sample #3	2.55	NEG	2.37	NEG	2.39	NEG	2.45	NEG
6	sample #4	2.61	NEG	2.59	NEG	2.33	NEG	2.35	NEG
7	sample #5	2.64	NEG	2.57	NEG	2.50	NEG	2.39	NEG
8	sample #6	2.69	NEG	2.54	NEG	2.38	NEG	2.43	NEG
9	sample #7	2.71	NEG	2.53	NEG	2.39	NEG	2.48	NEG
10	sample #8	2.75	NEG	2.68	NEG	2.54	NEG	2.48	NEG
11	sample #9	2.98	NEG	655.25	POS	2.48	NEG	2.74	NEG
12	sample #10	2.92	NEG	2.69	NEG	2.48	NEG	729.93	POS
13	sample #11	2.94	NEG	3.02	NEG	371.80	POS	2.86	NEG
14	sample #12	3.00	NEG	2.81	NEG	2.48	NEG	2.35	NEG
15	auto	2.59	NEG	2.55	NEG	2.37	NEG	2.38	NEG

Figure 4b. Raw data and results for detection of IgM κ/λ and IgA κ/λ

		IgG3				IgG2b			
	Sample ID	FL1 MFI λ	λ	FL2 MFI κ	κ	FL1 MFI λ	λ	FL2 MFI κ	κ
0	Detector Ab only	3.11		2.69		2.67		2.31	
1	Standards 0ne	3.18	NEG	2.34	NEG	243.62	POS	273.84	POS
2	Standards Two	19.90	POS	143.30	POS	2.97	NEG	2.48	NEG
3	sample #1	3.08	NEG	2.41	NEG	2.69	NEG	4.70	NEG
4	sample #2	3.00	NEG	2.62	NEG	2.74	NEG	2.35	NEG
5	sample #3	3.09	NEG	2.50	NEG	2.67	NEG	2.35	NEG
6	sample #4	2.98	NEG	2.57	NEG	2.79	NEG	2.50	NEG
7	sample #5	3.16	NEG	2.35	NEG	3.25	NEG	1197.09	POS
8	sample #6	3.05	NEG	2.62	NEG	629.26	POS	2.65	NEG
9	sample #7	2.86	NEG	441.09	POS	2.64	NEG	2.27	NEG
10	sample #8	32.20	POS	2.67	NEG	2.79	NEG	2.33	NEG
11	sample #9	3.13	NEG	2.70	NEG	2.74	NEG	2.32	NEG
12	sample #10	3.08	NEG	2.47	NEG	2.86	NEG	2.29	NEG
13	sample #11	3.05	NEG	3.68	NEG	2.50	NEG	2.81	NEG
14	sample #12	2.92	NEG	2.59	NEG	2.80	NEG	2.35	NEG
15	auto	2.50	NEG	2.23	NEG	2.48	NEG	2.17	NEG

Figure 4c. Raw data and results for detection of IgG$_3$ κ/λ, IgG$_{2b}$ κ/λ

	Sample ID	IgG2a				IgG1			
		FL1 MFI λ	λ	FL2 MFI κ	κ	FL1 MFI λ	λ	FL2 MFI κ	κ
0	Detector Ab only	2.57		1.57		2.59		1.64	
1	Standards 0ne	2.71	NEG	1.51	NEG	65.52	POS	120.79	POS
2	Standards Two	72.99	POS	129.80	POS	3.02	NEG	1.72	NEG
3	sample #1	2.69	NEG	1.63	NEG	2.53	NEG	784.39	POS
4	sample #2	2.73	NEG	1.55	NEG	291.64	POS	1.10	NEG
5	sample #3	2.59	NEG	349.12	POS	2.50	NEG	1.43	NEG
6	sample #4	130.97	POS	1.23	NEG	2.55	NEG	2.57	NEG
7	sample #5	2.71	NEG	1.47	NEG	2.57	NEG	1.35	NEG
8	sample #6	2.76	NEG	1.43	NEG	2.41	NEG	1.61	NEG
9	sample #7	2.67	NEG	1.47	NEG	2.57	NEG	1.42	NEG
10	sample #8	2.84	NEG	1.91	NEG	2.67	NEG	2.92	NEG
11	sample #9	2.76	NEG	1.50	NEG	2.59	NEG	1.36	NEG
12	sample #10	2.67	NEG	1.54	NEG	2.50	NEG	1.41	NEG
13	sample #11	2.69	NEG	1.54	NEG	2.50	NEG	3.00	NEG
14	sample #12	2.74	NEG	1.55	NEG	2.51	NEG	1.36	NEG
15	auto	2.64	NEG	1.44	NEG	2.44	NEG	1.18	NEG

Figure 4d. Raw data and results for detection of IgG_{2a} κ/λ, and IgG_1 κ/λ

	Sample ID	Positive Results	Comments
0	Detector Ab only		
1	Standards 0ne	IgE-K, IgA-λ, IgA-K, IgG2b-λ, IgG2b-K, IgG1-λ, IgG1-K	
2	Standards Two	IgM-λ, IgM-K, IgG3-λ, IgG3-K, IgG2a-λ, IgG2a-K	
3	sample #1	IgG1-K	
4	sample #2	IgG1-λ	
5	sample #3	IgG2a-K	
6	sample #4	IgG2a-λ	
7	sample #5	IgG2b-K	
8	sample #6	IgG2b-λ	
9	sample #7	IgG3-K	
10	sample #8	IgG3-λ	
11	sample #9	IgM-K	
12	sample #10	IgA-K	
13	sample #11	IgA-λ	
14	sample #12	IgE-K	
15	auto		

Figure 4e. Tabulation of positive results

US Orders: **877.232.8995**

BD CellQuest™ Data Profiles

For more detailed analysis, use BD CellQuest™ software. Display the data as FL1 vs. FL3 and FL2 vs. FL3 dot plots. The following BD CellQuest dot plots show the individual samples displayed on the preceding BD CBA Data Report. For Samples 1 – 12, only the positive dot plots (FL2 or FL1 vs. FL3) are displayed.
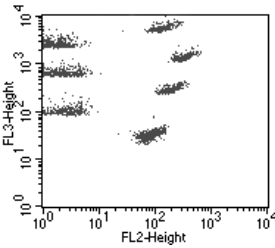
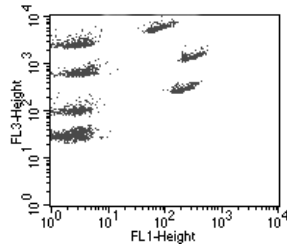## Detector Antibodies Only
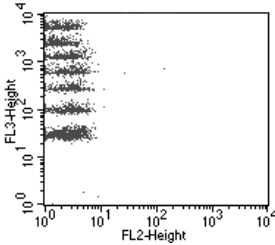
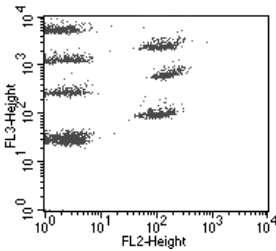All isotypes are negative.
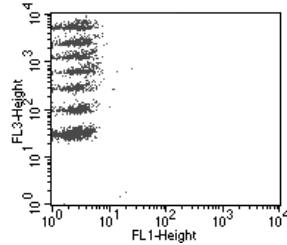
All isotypes are negative.

Standards
One

IgG$_1$ κ, IgG$_{2b}$ κ, IgA κ, and IgE κ are detected.

IgG$_1$ λ, IgG$_{2b}$ λ, and IgA λ are detected. No IgE λ is supplied.

Standards
Two

IgG$_{2a}$ κ, IgG$_3$ κ, and IgM κ are detected.
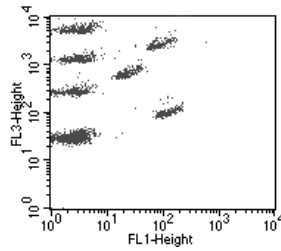
IgG$_{2a}$ λ, IgG$_3$ λ, and IgM λ are detected.

Sample #1
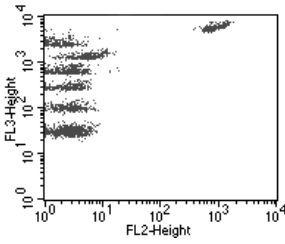
IgG$_1$, κ is detected.

Sample #2
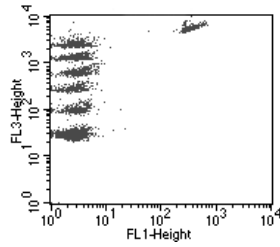
IgG$_1$, λ is detected.

Sample #3
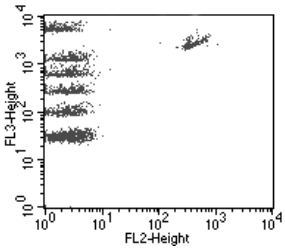
IgG$_{2a}$, κ is detected.

Sample #4
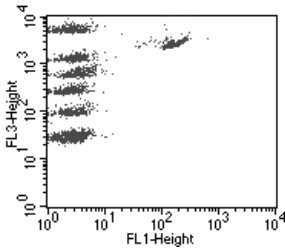
IgG$_{2a}$, λ is detected.

Sample #5
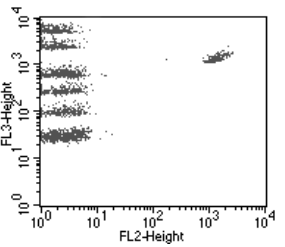
IgG$_{2b}$, κ is detected.

Sample #6
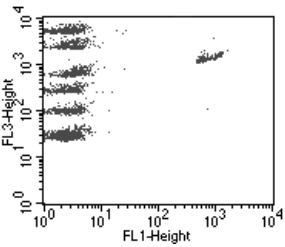
IgG$_{2b}$, λ is detected.

Sample #7
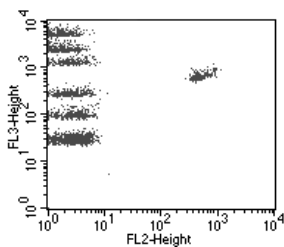
IgG$_3$, κ is detected.

Sample #8
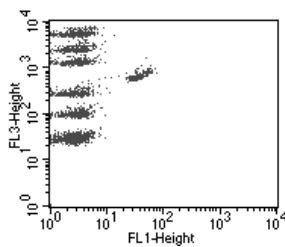
IgG$_3$, λ is detected.

Sample #9
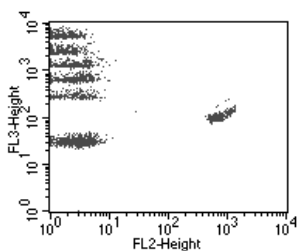
IgM, κ is detected.

Sample #10
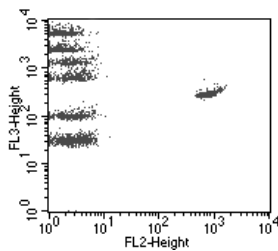
IgA, κ is detected.

Sample #11
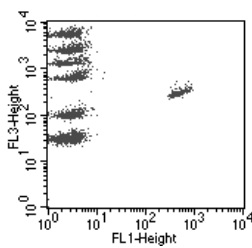
IgA, λ is detected.

Sample #12
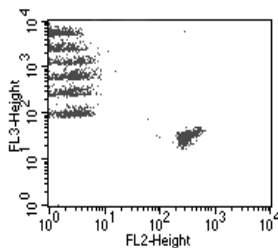
IgE, κ is detected.

Performance

Specificity

Each capture and detector antibody was generated from rats immunized with mouse Ig antigen and has been extensively tested for reactivity toward myeloma proteins and other Ig isotypes from mouse strains of both a and b allotypes. Each mAb is specific for its stated isotype. The standards are two mixtures of purified monoclonal mouse immunoglobulins of the indicated isotype combinations (see **Reagent Preparation**, page 9).

Sensitivity

This assay allows the detection of mouse Igs from 10 ng/ml to 0.5 mg/ml.

Troubleshooting Tips

Problem	Suggested Solution
Variation between duplicate samples.	Vortex Capture Beads before pipetting. Do not sonicate.
Low bead number in samples.	Avoid aspiration of beads during wash step. Do not wash or resuspend beads in volumes higher than recommended volumes.
High background.	Test various sample dilutions, the sample may be too concentrated. Remove excess PE- and FITC-labeled detection antibodies by decreasing non-specific, unbound detection antibodies.
Little or no detection of protein in sample.	Sample may be too dilute. Try various sample dilutions.
All samples are positive or above the high standard mean fluorescence value.	Dilute the samples further. The samples might be too concentrated.

References

1. Stall, A., Q. Sun, R. Varro, L. Lowe, E. Crowther, B. Abrams, J. Bishop, and K. Davis. 1998. A single tube flow cytometric multibead assay for isotyping mouse monoclonal antibodies. Abstract LB77. Experimental Biology Meeting 1998 (late-breaking abstracts).

2. Chen, R., L. Lowe, J.D. Wilson, E. Crowther, K. Tzeggai, J.E. Bishop, and R. Varro. 1999. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin. Chem.* 9: 1693-1694.

3. Cook, E.B., J.L. Stahl, L. Lowe, R. Chen, E. Morgan, J. Wilson, R. Varro, A. Chan, F.M. Graziano, and N.P. Barney. 2001. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. *J. Immunol. Methods* 254: 109-118.

4. Dotti, G., B. Salvodo, S. Takahashi, T. Goltsova, M. Brown, D. Rill, C. Rooney, and M. Brenner. 2001. Adenovector-induced expression of human-CD40-ligand (hCD40L) by multiple myeloma cells: A model for immunotherapy. *Exp. Hematol.* 29: 952-961.

Acknowledgements

Notes

Notes

United States
877.232.8995

Canada
888.259.0187

Europe
32.53.720.550

Japan
0120.8555.90

Asia/Pacific
65.6861.0633

Latin America/Caribbean
55.11.5185.9645

BD

BD Biosciences
10975 Torreyana Road
San Diego, CA 92121
Customer/Technical Service
Tel 877.232.8995 (US)
Fax 800.325.9637
bdbiosciences.com

01-8100055-550026E