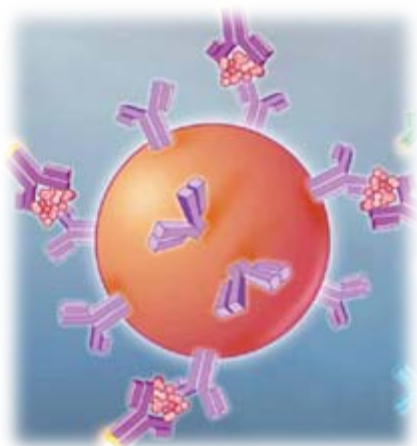


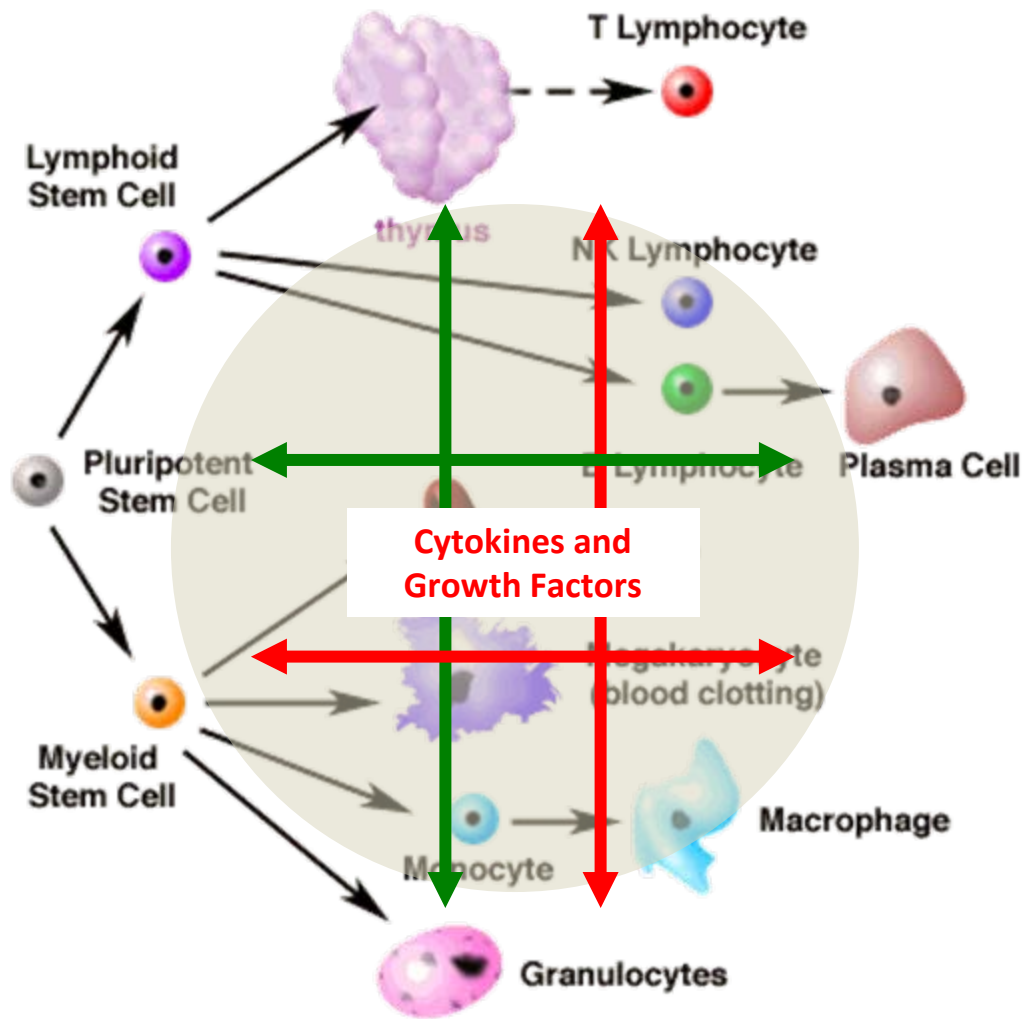
BD™ CBA on the BD Accuri™ C6: Bringing Multiplexed Cytokine Detection to the Benchtop



Maria Dinkelmann, PhD
Senior Marketing Applications Specialist
BD Biosciences, Ann Arbor, MI



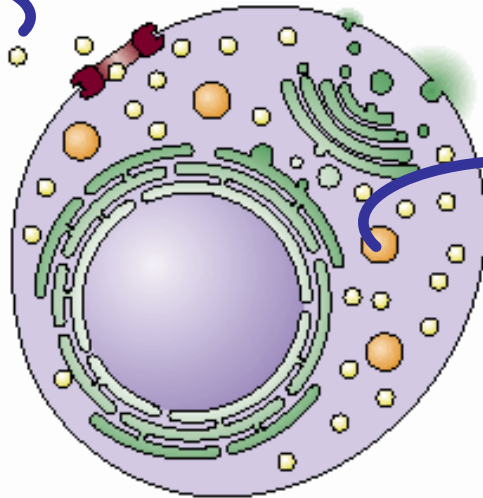
Cellular Communication



Techniques for Measurement of Cytokines

Soluble Proteins

- ELISA
- ELISPOT
- **BD CBA**

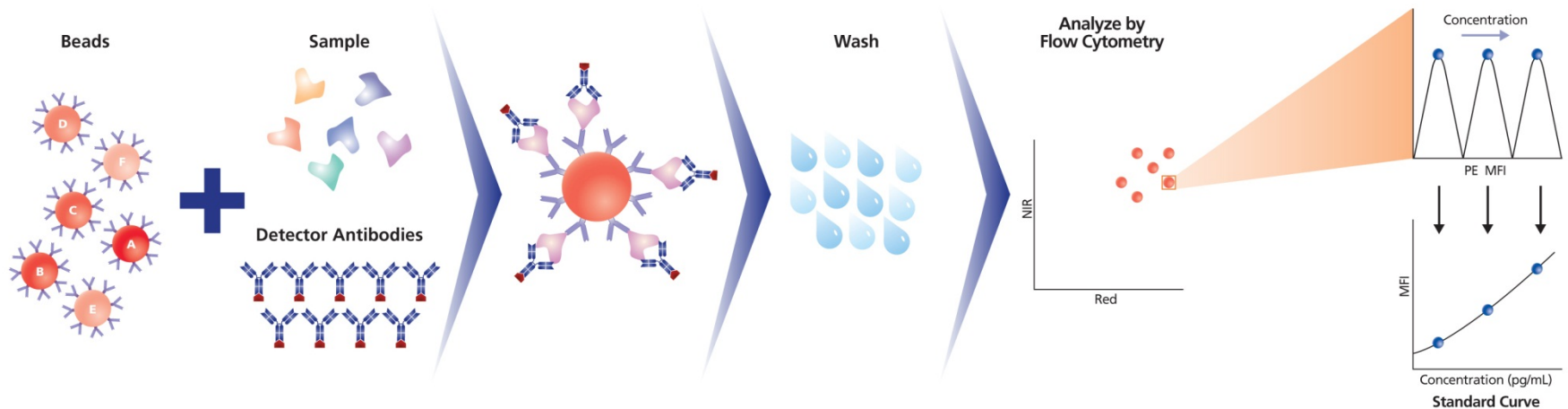


Intracellular Proteins

- Flow Cytometry
- Western Blot
- Immunohistochemistry

BD Cytometric Bead Array (CBA) Assay Overview

- Analytes are bound by specific capture antibodies conjugated to beads with distinct fluorescent properties
- The reporter in the assay is a PE-labeled detection antibody
- Analyte concentrations are estimated by comparison with a standard curve in FCAP Array™ software



Bead-Based Immunoassay Overview

- **Advantages**
 - Analyze multiple cytokines simultaneously (≤ 30)
 - Reduced sample volume requirements
 - Reduced hands-on time with parallel analysis of samples
 - Wide dynamic range (fluorescence)
 - Requires fewer sample dilutions
 - High statistical relevance
 - 300 beads measured per cytokine \rightarrow equivalent of 300 ELISA wells
- **BD CBA is like doing multiple ELISAs at the same time by flow cytometry**

The BD CBA Workflow

Stain

BD CBA Kits
BD CBA Flex Sets

Acquire

Flow cytometers:

BD Accuri™ C6
BD FACSVerse™
BD LSRFortessa™
BD™ LSR II
BD FACSCanto™ II
BD FACS Aria™ III
BD FACSArray™
BD FACSCalibur™

Analyze

FCAP Array v3.0.1 software
(Microsoft® Windows)



CBA Kit Workflow

- Experimental samples

PBMCs were cultured for several days with plate-bound anti-CD3, soluble anti-CD28, IL-2, and IL-4. Cells were stimulated with PMA and ionomycin for several hours prior to collecting culture supernatants.

- Staining

BD CBA Human Th1/Th2/Th17 Cytokine Kit

- IL-2
- IL-6
- TNF
- IL-4
- IL-10
- IFN- γ
- IL-17A

- Acquisition

BD Accuri C6 standard configuration

- Analysis

FCAP Array software v3.0.1 to measure cytokine concentrations



The BD CBA Kit Workflow



BD CBA Kits

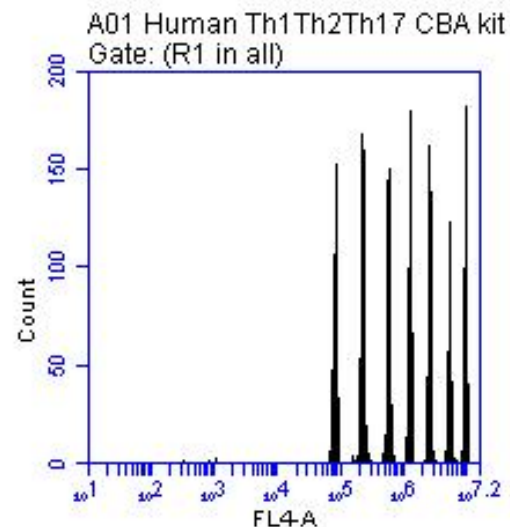
BD CBA Kits

Stain

- Preconfigured panels of 3 to 7 analytes
 - Cytokines
 - Inflammatory cytokines
 - Chemokines
 - Anaphylatoxins
 - Ig isotyping (mouse)
- 2-color assay
 - Red dye in beads
 - PE reporter

- Contents

- Diluents and wash buffers
- Setup beads
- 3–7 vials of capture beads
- 2 vials of standards
- 1 bottle of detection reagent



BD CBA Kits

Stain

- Reconstitute lyophilized standards in assay diluent
- Prepare 10 serial dilutions of standards (typically 20–5,000 pg/mL)
- Mix capture beads in a single tube
- Dilute samples if necessary with assay diluent
- Combine capture beads, test sample (or standard), and detection reagent for each sample
- Incubate for 3 hours
- Wash 1X with wash buffer



| | Concentration (pg/mL) | Dilution |
|----|-----------------------|----------|
| 1 | 0 | N/A |
| 2 | 20 | 1:256 |
| 3 | 40 | 1:128 |
| 4 | 80 | 1:64 |
| 5 | 156 | 1:32 |
| 6 | 312.5 | 1:16 |
| 7 | 625 | 1:8 |
| 8 | 1250 | 1:4 |
| 9 | 2500 | 1:2 |
| 10 | 5000 | neat |
| 11 | Sample A SUP | 1:100 |
| 12 | Sample A SUP | 1:10 |
| 13 | Sample A SUP | neat |
| 14 | Sample B SUP | 1:100 |
| 15 | Sample B SUP | 1:10 |
| 16 | Sample B SUP | neat |



The BD CBA Kit Workflow



The BD Accuri C6 Flow Cytometer System

Acquire



- An affordable, full-featured, easy-to-use flow cytometer
- Equipped with two lasers and six detectors

BD Accuri CBA Kit Template



Collect
Analyze
Statistics
Batch Analysis

A01

| | | | | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

3 blue 1 red
 2 blue 2 red
 4 blue

Run Settings

Run Unlimited
 Run with Limits

2100 events
 in R1

0 Min 0 Sec
 0 µL

Do not collect events outside R1

Fluidics

Slow Medium Fast

Flow Rate 35 µL/min
Core Size 16 µm

Custom

Flow Rate 14 µL/min
Core Size 10 µm

Threshold

500,000 on FSC-H
500,000 on SSC-H

RUN

Last Run

0 Events
 0:00.0 Time
 0 Microliters
 0 Events / Sec
 0 Events / µL

show warning

All
 Outside R1

Data Capacity Used
0% of 96,000,000 Events

Plot 1: A01 GATE [No Gating]

Plot 2: A01 GATE [No Gating]

Plot 3: A01 GATE R1

Plot 4: A01 GATE R1

Plot 5: A01 GATE R1

Select plot type to make a new plot.

| Plot 1: A01 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC-A | Median SSC-A |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| All | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | | |

| Plot 2: A01 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC-A | Median SSC-A |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| All | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | | |
| R1 | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | | |

| Plot 3: A01 | Count | Volume (µL) | % of This Plot | % of All | Mean FL4-A | CV FL4-A | Median FL4-A |
|-------------|-------|-------------|----------------|----------|------------|----------|--------------|
| Gated on R1 | | | | | | | |
| This Plot | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00% | |

| Plot 4: A01 | Count | Volume (µL) | % of This Plot | % of All | Mean FL2-A | Mean FL4-A | CV FL2-A | CV FL4-A | Median FL2-A | Median FL4-A |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|--------------|
| Gated on R1 | | | | | | | | | | |
| This Plot | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | | |



Human Th1/Th2/Th17 Cytokine Kit



Collect
Analyze
Statistics
Batch Analysis

A01 Human Th1Th2Th17 CBA kit

| | | | | | | | | | | | |
|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

3 blue 1 red
2 blue 2 red
4 blue

Run Settings

Run Unlimited
 Run with Limits

2100 events
in R1

0 Min 0 Sec
0 µL

Do not collect events outside R1

Backflush Unclog

Fluidics

Slow Medium Fast

Flow Rate 35 µL/min
Core Size 16 µm

Custom
Flow Rate 11 µL/min
Core Size 5 µm

Threshold
Set Threshold
500,000 on FSC-H
500,000 on SSC-H

ADD to A01

Set Color Compensation

Last Run

0 Events
0.00.0 Time

0 Microliters
0 Events / Sec
0 Events / µL

Cumulative

2,644
1:12.1

41
36
64

Delete Events show warning

All
 Outside R1

Data Capacity Used
<1% of 96,000,000 Events

Plot 1: A01 Human Th1Th2Th17 CBA...
GATE: [No Gating]

Plot 2: A01 Human Th1Th2Th17 CBA...
GATE: [No Gating]

Plot 3: A01 Human Th1Th2Th17 CBA...
GATE: R1

Plot 4: A01 Human Th1Th2Th17 CBA...
GATE: R1

Plot 5: A01 Human Th1Th2Th17 CBA...
GATE: R1

Select plot type to make a new plot.

| Plot 2: A01 Human Th1Th2Th17 CBA kit | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Medi |
|--------------------------------------|-------|-------------|----------------|----------|--------------|--------------|----------|----------|------|
| All | 2,644 | 41 | 100.00% | 100.00% | 1,019,630.24 | 1,456,376.63 | 22.23% | 80.07% | |
| R1 | 2,466 | 41 | 93.27% | 93.27% | 978,132.38 | 1,292,316.87 | 5.39% | 20.97% | |

| Plot 3: A01 Human Th1Th2Th17 CBA kit Gated on R1 | Count | Volume (µL) | % of This Plot | % of All | Mean FL4-A | CV FL4-A | Median FL4-A |
|---|-------|-------------|----------------|----------|--------------|----------|--------------|
| This Plot | 2,466 | 41 | 100.00% | 93.27% | 2,714,741.06 | 127.36% | |

| Plot 4: A01 Human Th1Th2Th17 CBA kit Gated on R1 | Count | Volume (µL) | % of This Plot | % of All | Mean FL2-A | Mean FL4-A | CV FL2-A | CV FL4-A | Mediar |
|---|-------|-------------|----------------|----------|------------|--------------|----------|----------|--------|
| This Plot | 2,466 | 41 | 100.00% | 93.27% | 21,234.70 | 2,714,741.06 | 160.53% | 127.36% | |

| Plot 5: A01 Human Th1Th2Th17 CBA kit Gated on R1 | Count | Volume (µL) | % of This Plot | % of All | Mean FL2-A | CV FL2-A | Median FL2-A |
|---|-------|-------------|----------------|----------|------------|----------|--------------|
| This Plot | 2,466 | 41 | 100.00% | 93.27% | 21,234.70 | 160.53% | |



The BD CBA Kit Workflow

Stain

BD CBA Kits

Acquire

BD Accuri C6

Analyze

FCAP Array v3.0.1 (Microsoft®
Windows)

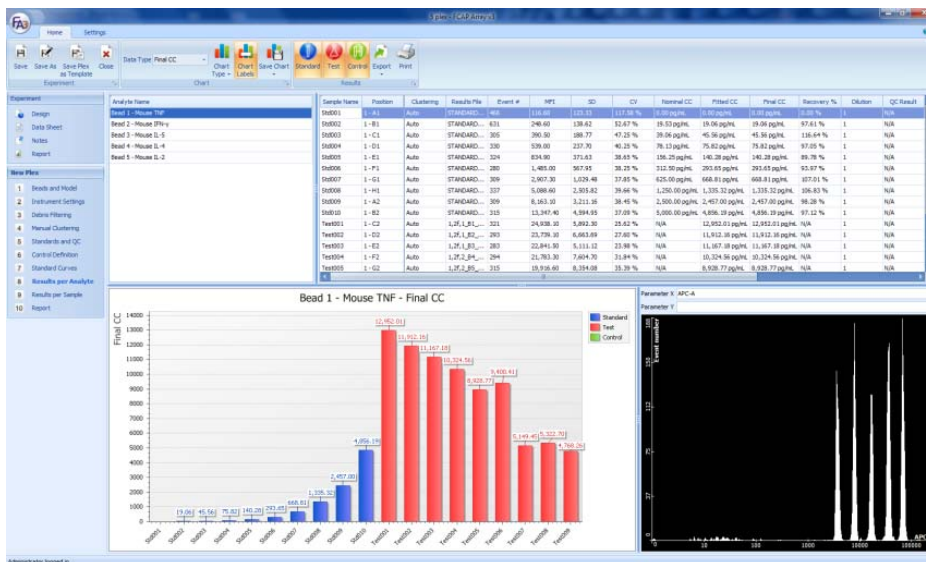


FCAP Array Analysis Software

Analyze



- Version 3.0.1 compatible with BD Accuri FCS files
- Compatible with FCS 2.0 or 3.0 files from any BD flow cytometer
- Results in graphical and tabular format
- Ability to save plex templates for routine panels
- Automatic and manual gating options



Design View



Workflow Group

- 1 Beads and Model
- 2 Instrument Settings
- 3 Debris Filtering
- 4 Manual Clustering
- 5 Standards and QC
- 6 Control Definition
- 7 Standard Curves
- 8 Results per Analyte
- 9 Results per Sample
- 10 Report

Plate layout:
Standards and Test Samples identified, files are assigned (floppy disc icon)

| Name | Date |
|-------------------------|----------------------|
| A01 std 1.fcs | 3/21/2012 4:03:16 PM |
| A02 std 1 to 256.fcs | 3/21/2012 4:04:40 PM |
| A03 std 1 to 128.fcs | 3/21/2012 4:07:19 PM |
| A04 std 1 to 64.fcs | 3/21/2012 4:09:15 PM |
| A05 std 1 to 32.fcs | 3/21/2012 4:11:06 PM |
| A06 std 1 to 16.fcs | 3/21/2012 4:12:52 PM |
| B01 std 1 to 8.fcs | 3/21/2012 4:15:03 PM |
| B02 std 1 to 4.fcs | 3/21/2012 4:16:52 PM |
| B03 std 1 to 2.fcs | 3/21/2012 4:18:59 PM |
| B04 std undiluted.fcs | 3/21/2012 4:20:45 PM |
| B05 sample 1 to 256.fcs | 3/21/2012 4:22:46 PM |

| Properties | File Header |
|-----------------------|-----------------------------------|
| Sample name | Std010 |
| Dilution | 1 |
| Result file | B04 std undiluted.fcs |
| Result file with path | C:\Users\10108273\Desktop\CBA ... |
| Position | 1 - B2 |
| Number of replicates | 1 |
| Plex | New Plex |
| Type | Standard |



Beads and Model



The screenshot shows the 'CBA cytokine kit - FCAP Array v3' software interface. The 'Selected Beads' table is highlighted with a red border. The 'Bead Library' table is also highlighted with a red border. The 'Bead selection' text box is on the left, and the 'Bead Library' text box is on the right.

| Selected Beads | | | | | | |
|----------------|------------|----------------|---------------------|--------------|--------------|--|
| Bead Name | Lot Number | Catalog Number | Analyte | | | |
| | | | Name | Model | 2nd Reporter | |
| Bead 1 | | | Human IL-17A | Quantitative | No | |
| Bead 2 | | | Human IFN- γ | Quantitative | No | |
| Bead 3 | | | Human TNF | Quantitative | No | |
| Bead 4 | | | Human IL-10 | Quantitative | No | |
| Bead 5 | | | Human IL-6 | Quantitative | No | |
| Bead 6 | | | Human IL-4 | Quantitative | No | |
| Bead 7 | | | Human IL-2 | Quantitative | No | |

| Bead Library | | |
|--|---------------------|--|
| Bead Group | Group Description | |
| All Beads | All Beads | |
| Human Soluble Protein | BD CBA Flex Sets | |
| Mouse Th1/Th2 Cytokine Kit - 551287 | BD CBA Kit | |
| Cell Signaling | BD CBA Flex Sets | |
| Human Enhanced Sensitivity (ES) | BD CBA Flex Sets | |
| Human Immunoglobulin | BD CBA Flex Sets | |
| Mouse Soluble Protein | BD CBA Flex Sets | |
| Rat Soluble Protein | BD CBA Flex Sets | |
| Human Anaphylatoxin Kit - 561418 | BD CBA Kit | |
| Human Chemokine Kit - 552990 | BD CBA Kit | |
| Human Th1/Th2 Cytokine Kit - 550749 | BD CBA Kit | |
| Human Th1/Th2 Cytokine Kit II - 551... | BD CBA Kit | |
| Human Th1/Th2/Th17 Cytokine Kit - ... | BD CBA Kit | |
| Bead 1 | Human IL-17A | |
| Bead 2 | Human IFN- γ | |
| Bead 3 | Human TNF | |
| Bead 4 | Human IL-10 | |
| Bead 5 | Human IL-6 | |
| Bead 6 | Human IL-4 | |
| Bead 7 | Human IL-2 | |
| Mouse Inflammation Kit - 552364 | BD CBA Kit | |
| Mouse Th1/Th2/Th17 Cytokine Kit - ... | BD CBA Kit | |

Bead selection:
Drag beads from right pane into the left pane

Bead Library:
BD provides XML file on website that includes groupings (eg, Human Soluble Protein or Th1/2/17 Kit)

Instrument Settings: Cytokine Kit



Selected File: C:\Users\10100847\Desktop\CFlow-FCS Exports\...

Instrument Data: Accuri C6

Scatter Parameter: SSC-A

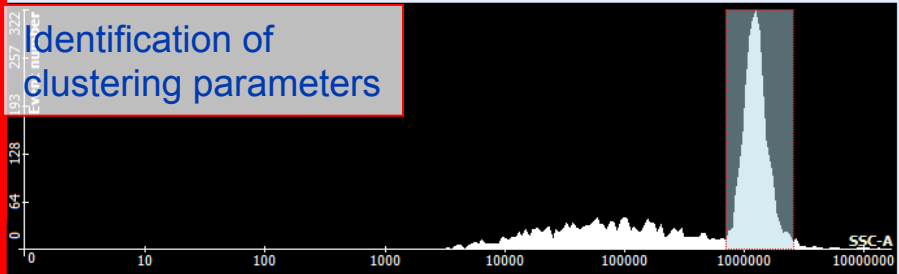
Scatter Peaks: 1

Clustering Parameters: FL4-A

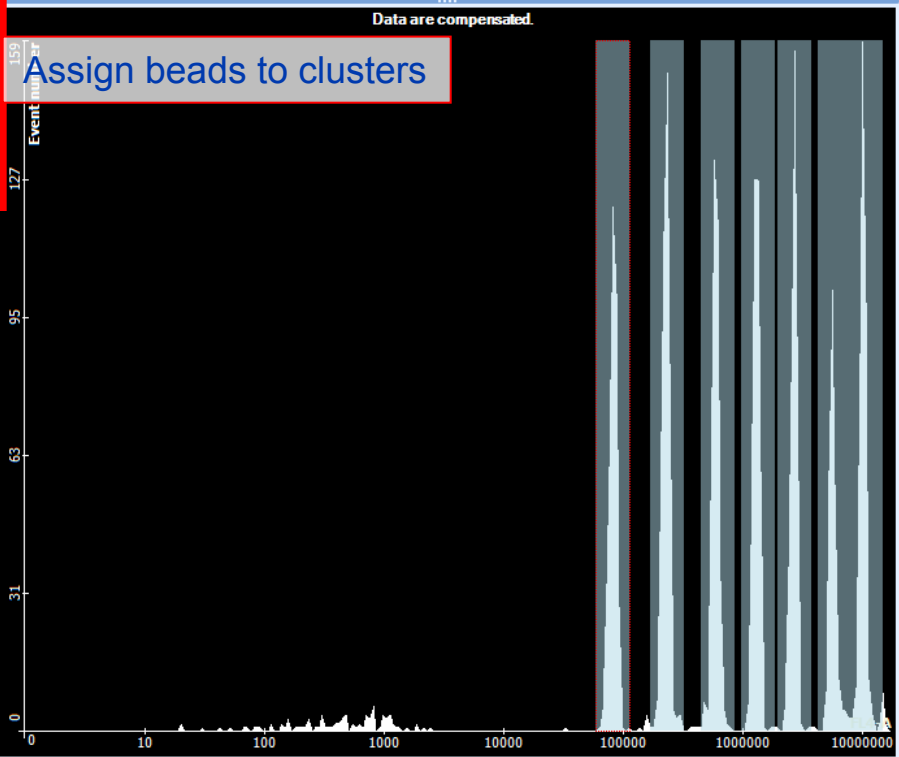
Reporter Parameter 1: FL2-A

Reporter Parameter 2: []

| Bead | Analyte |
|--------|--------------|
| Bead 1 | Human IL-17A |
| Bead 2 | Human IFN-γ |
| Bead 3 | Human TNF |
| Bead 4 | Human IL-10 |
| Bead 5 | Human IL-6 |
| Bead 6 | Human IL-4 |
| Bead 7 | Human IL-2 |



Identification of clustering parameters



Assign beads to clusters



Standards and QC

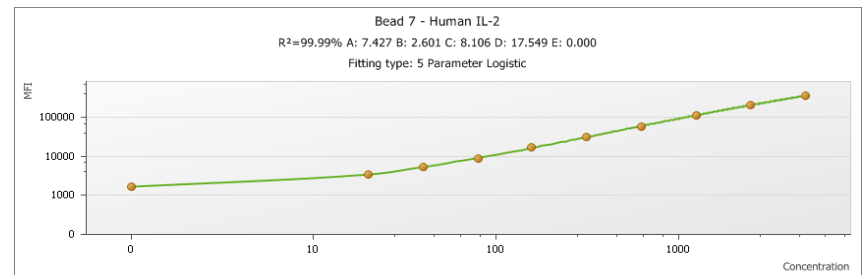
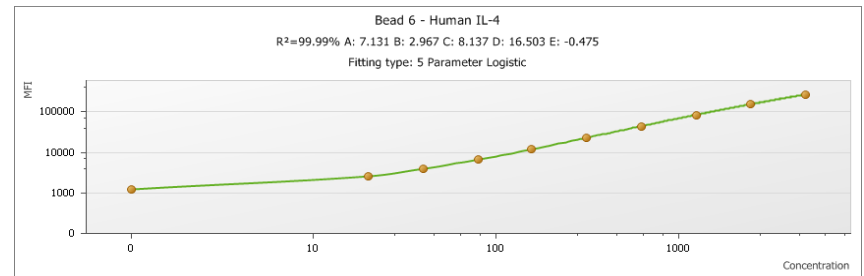
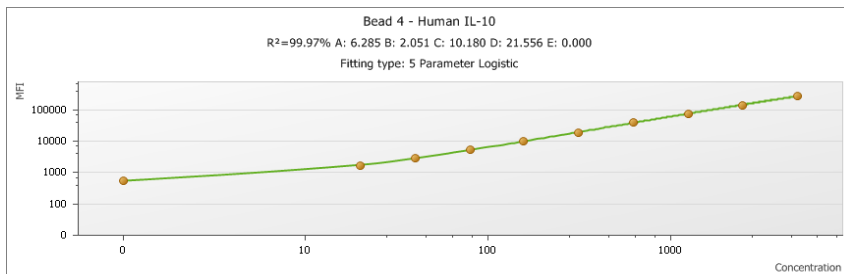
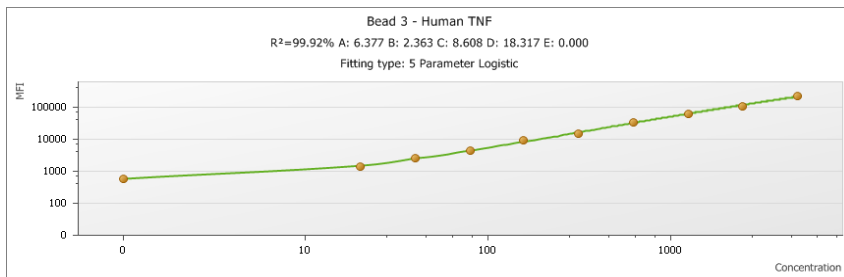
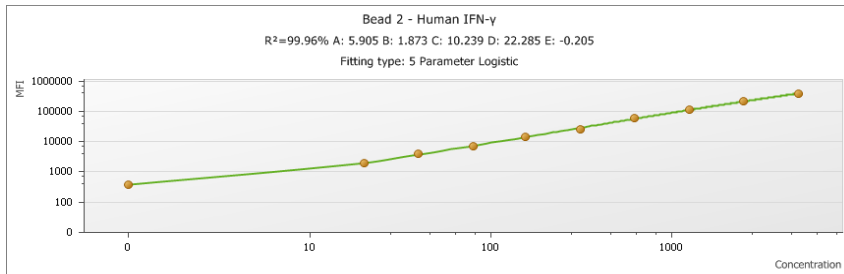
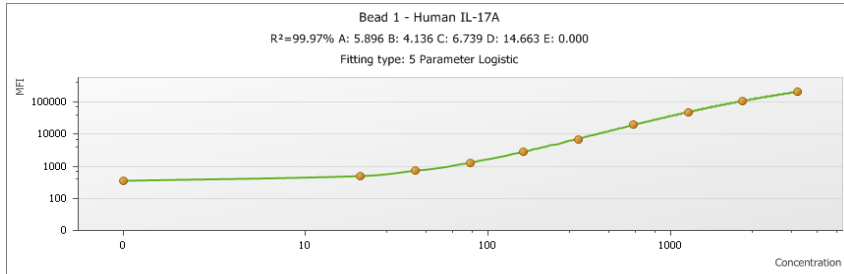


Standard dilution calculator and units

| Standard Sample | Concentration |
|-----------------|----------------|
| Std001 | 0.00 pg/mL |
| Std002 | 20.00 pg/mL |
| Std003 | 40.00 pg/mL |
| Std004 | 80.00 pg/mL |
| Std005 | 156.00 pg/mL |
| Std006 | 312.50 pg/mL |
| Std007 | 625.00 pg/mL |
| Std008 | 1,250.00 pg/mL |
| Std009 | 2,500.00 pg/mL |
| Std010 | 5,000.00 pg/mL |

Assignment of concentration levels to Standards

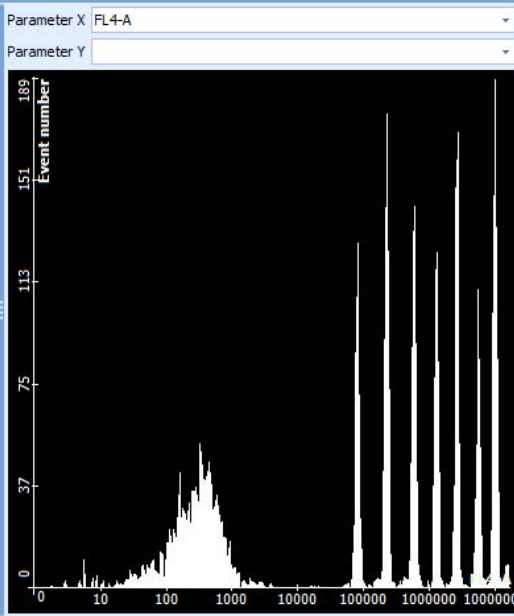
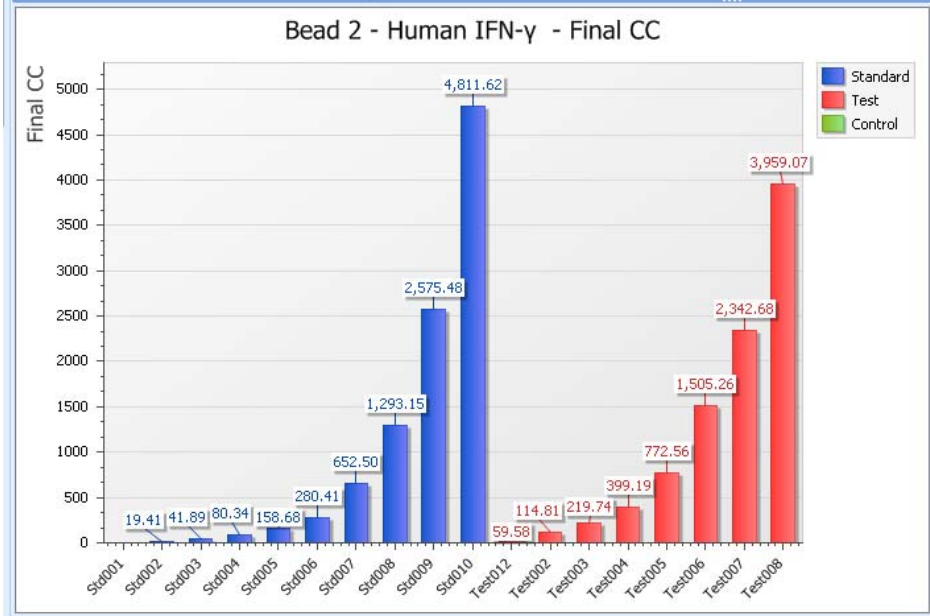
Standard Curves



Results per Analyte



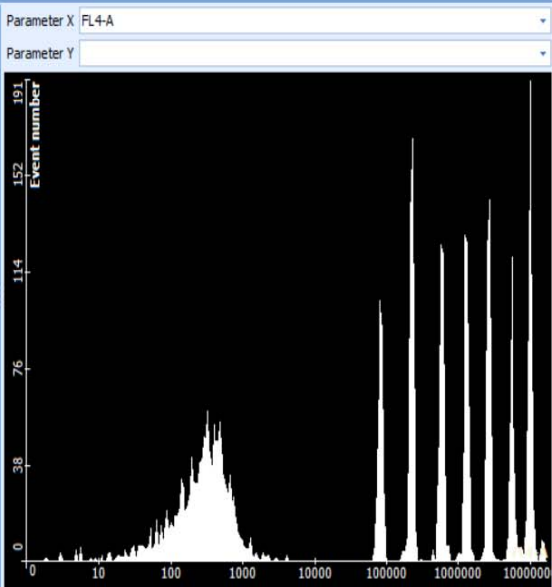
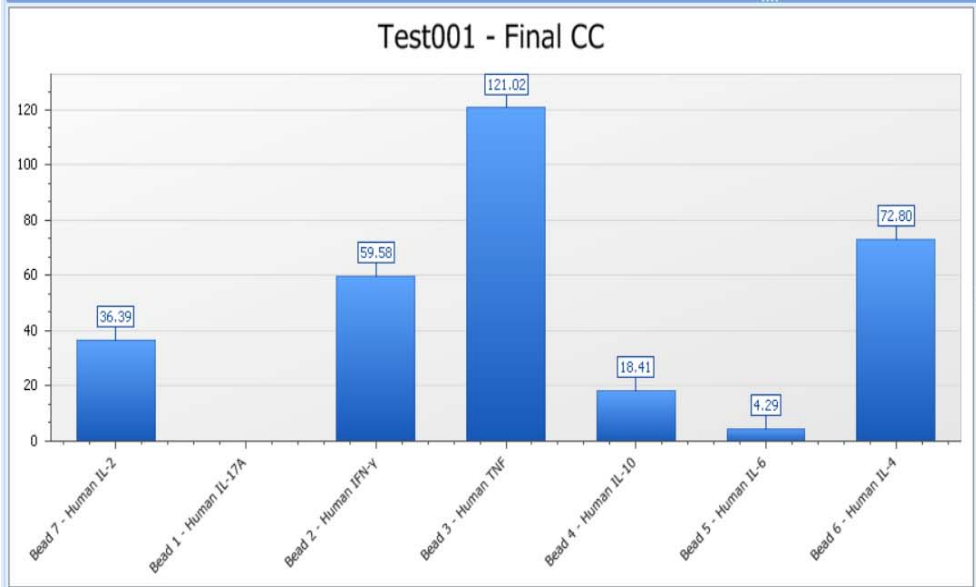
| Analyte Name | Sample Name | Position | Clustering | Results File | Event # | MFI | SD | CV | Nominal CC | Fitted CC |
|-----------------------|-------------|----------|------------|----------------|---------|------------|-----------|---------|----------------|----------------|
| Bead 1 - Human IL-17A | Std001 | 1 - A1 | Auto | A01 0 std. fcs | 443 | 370.00 | 313.57 | 69.01 % | 0.00 pg/mL | 0.00 pg/mL |
| Bead 2 - Human IFN-γ | Std002 | 1 - A2 | Auto | A02 std 1 t... | 300 | 1,976.00 | 1,226.48 | 53.31 % | 20.00 pg/mL | 19.41 pg/mL |
| Bead 3 - Human TNF | Std003 | 1 - A3 | Auto | A03 std 1 t... | 385 | 3,917.00 | 1,580.08 | 35.71 % | 40.00 pg/mL | 41.89 pg/mL |
| Bead 4 - Human IL-10 | Std004 | 1 - A4 | Auto | A04 std 1 t... | 453 | 7,328.00 | 3,111.61 | 36.87 % | 80.00 pg/mL | 80.34 pg/mL |
| Bead 5 - Human IL-6 | Std005 | 1 - A5 | Auto | A05 std 1 t... | 439 | 14,438.00 | 6,782.90 | 46.41 % | 156.00 pg/mL | 158.68 pg/mL |
| Bead 6 - Human IL-4 | Std006 | 1 - A6 | Auto | A06 std 1 t... | 413 | 25,600.00 | 14,198.49 | 48.04 % | 312.50 pg/mL | 280.41 pg/mL |
| Bead 7 - Human IL-2 | Std007 | 1 - A7 | Auto | B01 std 1 t... | 420 | 59,298.00 | 24,588.92 | 40.52 % | 625.00 pg/mL | 652.50 pg/mL |
| | Std008 | 1 - A8 | Auto | B02 std 1 t... | 406 | 114,732.00 | 31,456.51 | 31.66 % | 1,250.00 pg/mL | 1,293.15 pg/mL |
| | Std009 | 1 - B1 | Auto | B03 std 1 t... | 364 | 217,274.00 | 79,093.56 | 37.08 % | 2,500.00 pg/mL | 2,575.48 pg/mL |
| | Std010 | 1 - B2 | Auto | B04 std und... | 316 | 377,569.00 | 93,030.56 | 29.99 % | 5,000.00 pg/mL | 4,811.62 pg/mL |
| | Test012 | 1 - B3 | Auto | B05 sample ... | 440 | 5,476.00 | 1,453.69 | 30.93 % | N/A | 59.58 pg/mL |
| | Test002 | 1 - B4 | Auto | B06 sample ... | 406 | 10,440.00 | 2,284.50 | 22.81 % | N/A | 114.81 pg/mL |
| | Test003 | 1 - B5 | Auto | C01 sample... | 329 | 20,034.00 | 4,894.80 | 25.07 % | N/A | 219.74 pg/mL |
| | Test004 | 1 - B6 | Auto | C02 sample... | 340 | 36,461.00 | 11,728.11 | 36.00 % | N/A | 399.19 pg/mL |



Results per Sample



| Sample Name | Clustering | Results File | Analyte Na... | Event # | MFI | SD | CV | Nominal CC | Fitted CC | Final CC | Recovery % | Dilution | QC Result | Qualitative... | Message |
|-------------|------------|----------------------|----------------|---------|----------|----------|---------|------------|--------------|-------------|------------|----------|-----------|----------------|---------|
| Std006 | Auto | A06 std 1 to 16.fcs | Bead 7 - Hu... | 393 | 5,095.00 | 1,413.66 | 32.41 % | N/A | 36.39 pg/mL | 36.39 pg... | N/A | 1 | N/A | N/A | |
| Std007 | Auto | B01 std 1 to 8.fcs | Bead 1 - Hu... | 307 | 314.00 | 338.77 | 77.38 % | N/A | 0.00 pg/mL | 0.00 pg/mL | N/A | 1 | N/A | N/A | |
| Std008 | Auto | B02 std 1 to 4.fcs | Bead 2 - Hu... | 440 | 5,476.00 | 1,453.69 | 30.93 % | N/A | 59.58 pg/mL | 59.58 pg... | N/A | 1 | N/A | N/A | |
| Std009 | Auto | B03 std 1 to 2.fcs | Bead 3 - Hu... | 339 | 6,506.00 | 2,372.16 | 33.05 % | N/A | 121.02 pg... | 121.02 p... | N/A | 1 | N/A | N/A | |
| Std010 | Auto | B04 std undiluted... | Bead 4 - Hu... | 369 | 1,657.00 | 569.32 | 31.90 % | N/A | 18.41 pg/mL | 18.41 pg... | N/A | 1 | N/A | N/A | |
| Test001 | Auto | B05 sample 1 to 2... | Bead 5 - Hu... | 364 | 1,075.00 | 367.31 | 33.35 % | N/A | 4.29 pg/mL | 4.29 pg/mL | N/A | 1 | N/A | N/A | |
| Test002 | Auto | B06 sample 1 to 1... | Bead 6 - Hu... | 242 | 6,195.00 | 1,905.14 | 30.95 % | N/A | 72.80 pg/mL | 72.80 pg... | N/A | 1 | N/A | N/A | |
| Test003 | Auto | C01 sample 1 to ... | | | | | | | | | | | | | |
| Test004 | Auto | C02 sample 1 to ... | | | | | | | | | | | | | |
| Test005 | Auto | C03 sample 1 to ... | | | | | | | | | | | | | |
| Test006 | Auto | C04 sample 1 to ... | | | | | | | | | | | | | |
| Test007 | Auto | C05 sample 1 to ... | | | | | | | | | | | | | |
| Test008 | Auto | C06 sample 1 to ... | | | | | | | | | | | | | |



Report



FA3
CBA cytokine kit - FCAP Array v3

Home Settings Print Preview

Print
Quick Print

Options
Header
Scale
Margins
Orientation
Size

Find
First Page
Previous Page
Next Page
Last Page

Multiple Pages
Zoom Out
Zoom
Zoom In

Page Color
Watermark

Export To
E-Mail As

Report options

Experiment

- Design
- Data Sheet
- Notes
- Report

New Plex

- 1 Beads and Model
- 2 Instrument Settings
- 3 Debris Filtering
- 4 Manual Clustering
- 5 Standards and QC
- 6 Control Definition
- 7 Standard Curves
- 8 Results per Analyte
- 9 Results per Sample
- 10 Report

Layout 1

Plex Components

| Name | Lot Number | Analyte | | |
|--------|------------|--------------|--------------|------------|
| | | Name | Model | 2nd Respon |
| Bead 7 | | Human IL-2 | Quantitative | No |
| Bead 1 | | Human IL-17A | Quantitative | No |
| Bead 2 | | Human IFN-γ | Quantitative | No |
| Bead 3 | | Human INF | Quantitative | No |
| Bead 4 | | Human IL-10 | Quantitative | No |
| Bead 5 | | Human IL-6 | Quantitative | No |
| Bead 6 | | Human IL-4 | Quantitative | No |

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 Printed by Administrator, 5/12/2012 5:55:16 PM

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Standard Samples of Quantitative Analysis

| Sample Name | Reporter Parameter 1 | Concentration |
|-------------|----------------------|---------------|
| Std001 | 0.00 pg/mL | |
| Std002 | 20.00 pg/mL | |
| Std003 | 40.00 pg/mL | |
| Std004 | 80.00 pg/mL | |
| Std005 | 158.00 pg/mL | |
| Std006 | 312.50 pg/mL | |
| Std007 | 625.00 pg/mL | |
| Std008 | 1,250.00 pg/mL | |
| Std009 | 2,500.00 pg/mL | |
| Std010 | 5,000.00 pg/mL | |

Bead 7-Human IL-2
 $R^2=99.99\%$ A: 7.427 B: 2.601 C: 8.106 D: 17.549 E: 0.000
 Fitting type: 5 Parameter Logistic

| Name | Event # | MFI | SD | CV% (MFI) | Nominal CC | Fitted CC | Recovery % |
|--------|---------|----------|----------|-----------|------------|------------|------------|
| Std001 | 458 | 1,689.00 | 453.49 | 2581 % | 0.00 pg/mL | 0.52 pg/mL | 0.00 % |
| Std002 | 282 | 3,444.00 | 1,164.21 | 2983 % | 2000 pg/mL | 1935 pg/mL | 9675 % |

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The BD CBA Flex Set Workflow



BD CBA Flex Sets

CBA Flex Set Workflow

- Experimental samples

PBMCs were cultured for several days with plate-bound anti-CD3, soluble anti-CD28, IL-2, and IL-4. Cells were stimulated with PMA and ionomycin for several hours prior to harvesting.

Alternatively, PBMCs were stimulated for several hours with IFN- γ . LPS was added to the culture overnight.

- Staining

Master Buffer Kit = all buffers needed for assay

Flex Set = capture beads, detection reagent, standard (2 curves)

- IL-1 β
- IL-2
- IL-4
- IL-5
- IL-6
- IL-10
- IL-12p70
- IL-17A
- IFN- γ
- TNF

- Acquisition

BD Accuri C6

Selectable Lasers: 2 Blue, 2 Red option with 780 BP in FL3

- Analysis

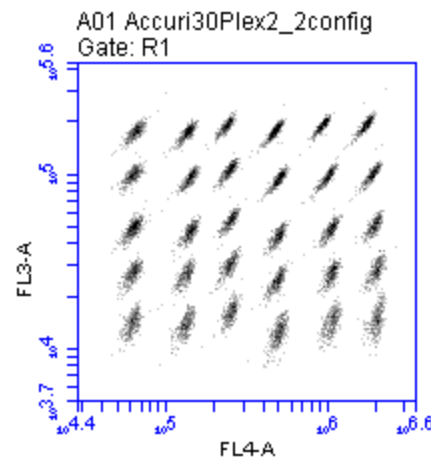
FCAP Array software to measure cytokine concentrations



BD CBA Flex Sets

Stain

- Build your own multiplex
 - Human soluble protein
 - Mouse or rat soluble protein
 - Enhanced sensitivity
 - (<1.0 pg/mL)
 - Cell signaling
 - Human Ig
- 3-color assay
 - Two red dyes in beads
 - PE reporter
- Master Buffer Kit
 - Includes buffers and setup reagents
- Flex Set
 - Capture beads
 - Detector reagents
 - Standard (x2)



BD CBA Flex Sets

Stain



- Combine flex set standards
- Combine flex set beads
- Combine flex set detection reagent
- Perform assay

| Tube | Concentration (pg/mL) | Dilution |
|------|-----------------------|----------|
| 1 | 0 | N/A |
| 2 | 10 | 1:256 |
| 3 | 20 | 1:128 |
| 4 | 40 | 1:64 |
| 5 | 80 | 1:32 |
| 6 | 156 | 1:16 |
| 7 | 312.5 | 1:8 |
| 8 | 625 | 1:4 |
| 9 | 1250 | 1:2 |
| 10 | 2500 | neat |
| 11 | test treatment A | 1:10 |
| 12 | test treatment A | neat |
| 13 | test treatment B | 1:10 |
| 14 | test treatment B | neat |

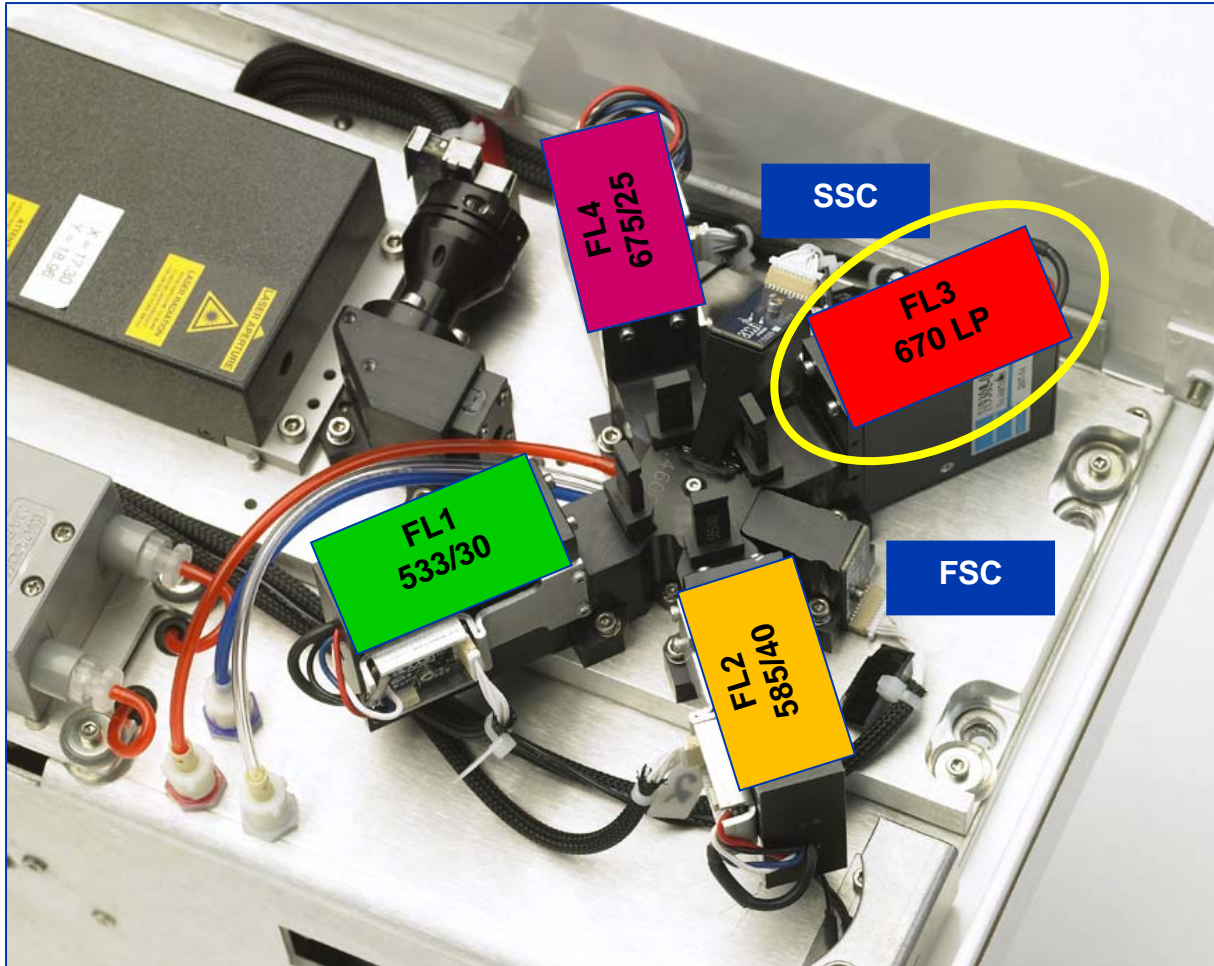


The BD CBA Flex Set Workflow



Optical Configuration

Acquire



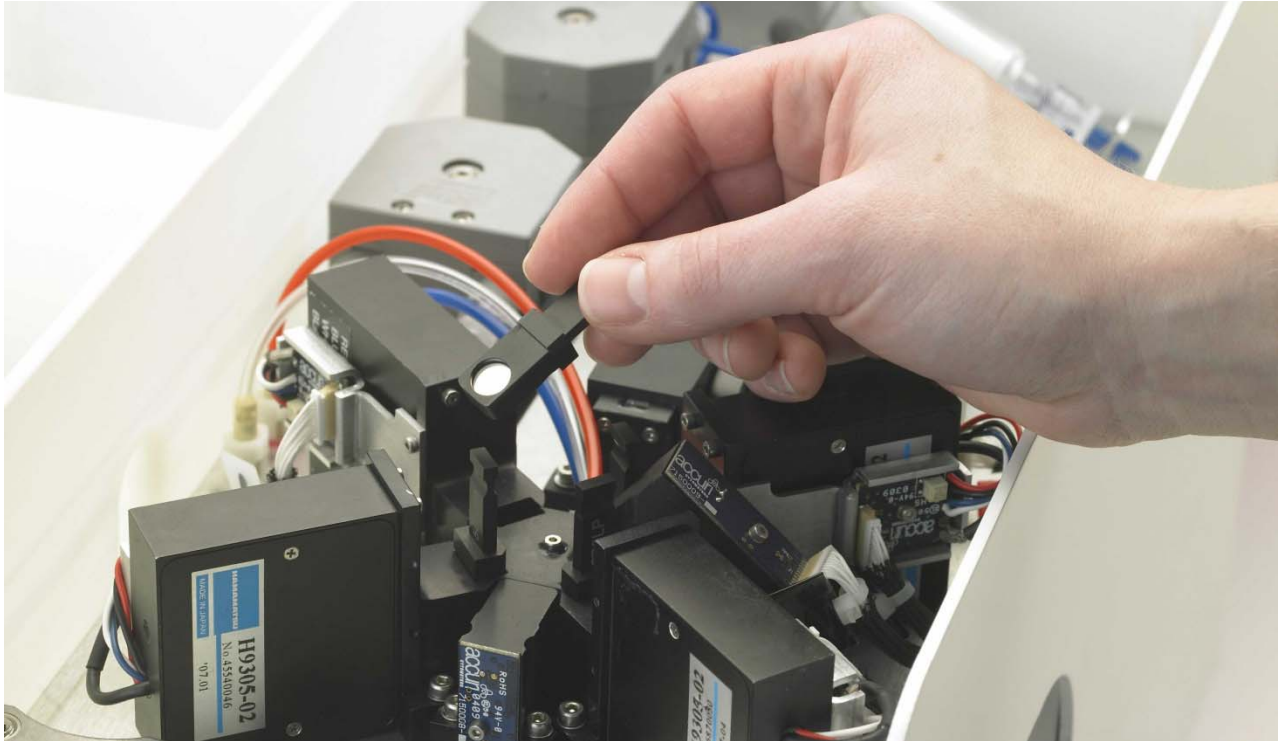
User changeable optical filters

510/15
540/20
565/20
610/20
780/60

Selectable lasers

3 blue 1 red
2 blue 2 red
4 blue

Selectable Lasers



- User changeable optical filters**
- 510/15
 - 540/20
 - 565/20
 - 610/20
 - 780/60**
- Selectable lasers**
- 3 blue 1 red
 - 2 blue 2 red**
 - 4 blue



C6 is connected and ready.

- 3 blue 1 red
- 2 blue 2 red
- 4 blue

BD Accuri CBA Flex Set Template



Collect
Analyze
Statistics
Batch Analysis

A01

| | | | | | | | | | | | |
|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

3 blue 1 red
 2 blue 2 red
 4 blue

Run Settings

Run Unlimited
 Run with Limits

2000 events
 in R1

2 Min 0 Sec
 0 µL

Do not collect events outside R1

Fluidics

Slow Medium Fast

Flow Rate 35 µL/min
 Core Size 16 µm

Custom

Flow Rate 14 µL/min
 Core Size 10 µm

Threshold

500,000 on FSC-H
 500,000 on SSC-H

RUN

Set Color Compensation

Last Run

Cumulative

0 Events
 0.00.0 Time
 0 Microliters
 0 Events / Sec
 0 Events / µL

Delete Events show warning

All
 Outside R1

Data Capacity Used
 0% of 96,000,000 Events

Plot 1: A01 GATE [No Gating]

Plot 2: A01 GATE [No Gating]

Plot 3: A01 GATE R1

Plot 4: A01 GATE R1

Plot 5: A01 GATE R1

Select plot type to make a new plot.

| Plot 1: A01 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|------------|
| All | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | |

| Plot 2: A01 | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC |
|-------------|-------|-------------|----------------|----------|------------|------------|----------|----------|------------|
| All | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | |
| R1 | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | |

| Plot 3: A01 Gated on R1 | Count | Volume (µL) | % of This Plot | % of All | Mean FL4-A | Mean FL3-A | CV FL4-A | CV FL3-A | Median FL4-A |
|-------------------------|-------|-------------|----------------|----------|------------|------------|----------|----------|--------------|
| This Plot | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | |
| Q1-UL | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | |
| Q1-LR | 0 | 0 | 100.00% | 100.00% | 0.00 | 0.00 | 0.00% | 0.00% | |



Set Color Compensation



Collect
Analyze
Statistics
Batch Analysis

A02 F1 + F9 set up beads

| | | | | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

C6 is connected and ready.

3 blue 1 red
 2 blue 2 red
 4 blue

Run Settings

Run Unlimited
 Run with Limits

2000 events
 in R1

2 Min 0 Sec
 0 µL

Do not collect events outside R1

Fluidics

Slow Medium Fast

Flow Rate 35 µL/min
 Core Size 16 µm

Custom
 Flow Rate 11 µL/min
 Core Size 5 µm

Threshold

500,000 on FSC-H
 500,000 on SSC-H

ADD to A02

Last Run

| | | |
|----------------|--------|---|
| 0 Events | 1,670 | 0 |
| 0:00.0 Time | 0:29.9 | 0 |
| 0 Microliters | 17 | 0 |
| 0 Events / Sec | 55 | 0 |
| 0 Events / µL | 98 | 0 |

show warning
 All
 Outside R1

Data Capacity Used <1% of 98,000,000 Events

Plot 1: A02 F1 + F9 set up beads
GATE [No Gating]

Plot 2: A02 F1 + F9 set up beads
GATE [No Gating]

Plot 3: A02 F1 + F9 set up beads
GATE R1

Plot 4: A02 F1 + F9 set up beads
GATE R1

Plot 5: A02 F1 + F9 set up beads
GATE R1

Select plot type to make a new plot.

| Plot | Count | Volume (µL) | % of This Plot | % of All | Mean FSC-A | Mean SSC-A | CV FSC-A | CV SSC-A | Median FSC |
|---|-------|-------------|----------------|----------|--------------|--------------|----------|----------|------------|
| Plot 1: A02 F1 + F9 set up beads | | | | | | | | | |
| All | 1,670 | 17 | 100.00% | 100.00% | 945,488.31 | 1,016,781.49 | 19.72% | 67.20% | |
| Plot 2: A02 F1 + F9 set up beads | | | | | | | | | |
| All | 1,670 | 17 | 100.00% | 100.00% | 945,488.31 | 1,016,781.49 | 19.72% | 67.20% | |
| R1 | 1,557 | 17 | 93.23% | 93.23% | 913,799.14 | 919,731.43 | 6.11% | 14.31% | |
| Plot 3: A02 F1 + F9 set up beads Gated on R1 | | | | | | | | | |
| This Plot | 1,557 | 17 | 100.00% | 93.23% | 510,149.05 | 8,035.99 | 110.11% | 157.35% | |
| Q1-UL | 6 | 17 | 0.39% | 0.36% | 306.83 | 208,519.50 | 160.69% | 7.45% | |
| Q1-UR | 1 | 17 | 0.06% | 0.06% | 3,079,023.00 | 32,900.00 | 0.00% | 0.00% | |



The BD CBA Flex Set Workflow

Stain

BD CBA Flex Sets

Acquire

BD Accuri C6

Analyze

FCAP Array v3.0.1 (Microsoft®
Windows)

Instrument Settings: 10-plex Flex Set



Selected File: C:\Users\10100847\Desktop\CFlow-FCS Exports\...

Instrument Data: Accuri C6

Scatter Parameter: SSC-A

Scatter Peaks: 1

Clustering Parameters: FL4-A FL3-A

Reporter Parameter 1: FL2-A

Reporter Parameter 2: []

| Bead | Analyte |
|------|----------------|
| A4 | Human IL-2 |
| A5 | Human IL-4 |
| A6 | Human IL-5 |
| A7 | Human IL-6 |
| A8 | Human IL-7 |
| B4 | Human IL-1β |
| B7 | Human IL-10 |
| B8 | Human IFN-γ |
| C4 | Human TNF |
| E5 | Human IL-12p70 |

Event number

SSC-A

Identification of clustering parameters

Data are compensated.

FL3-A

FL4-A

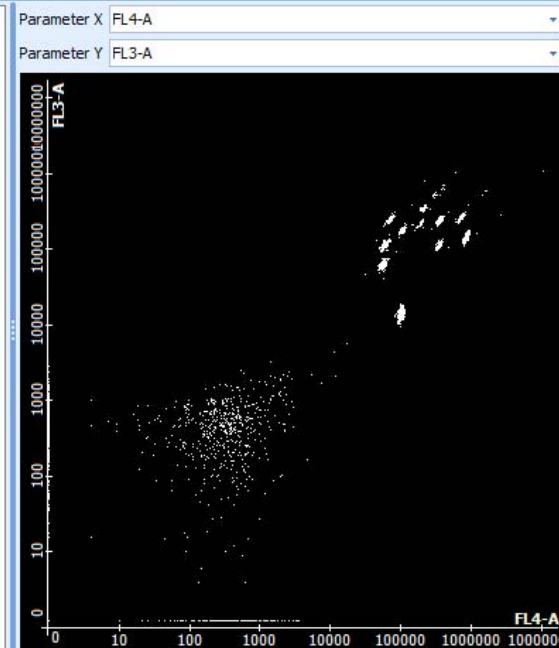
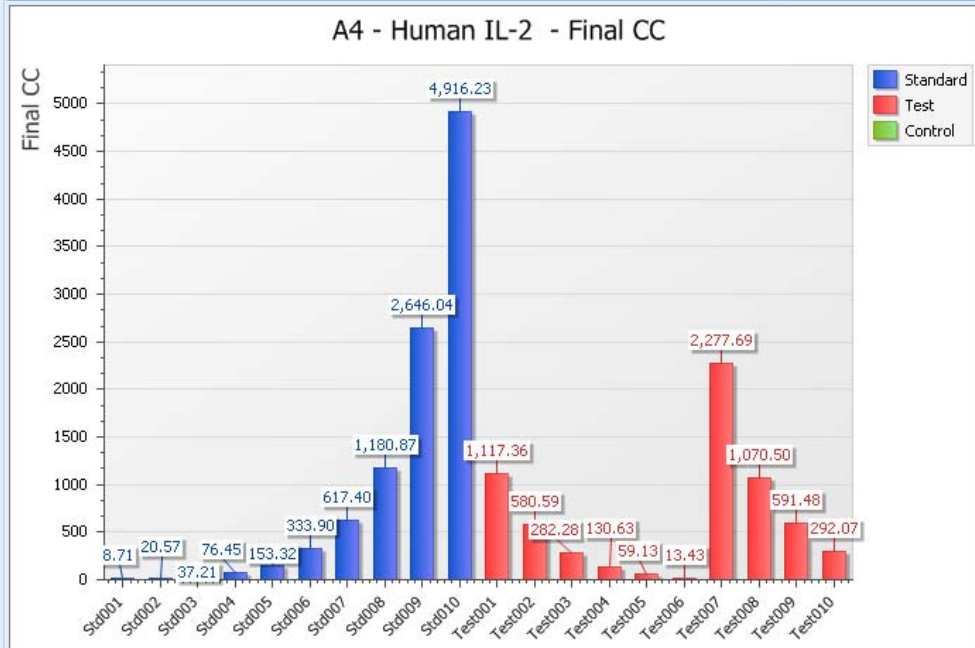
Assign beads to clusters



Results per Analyte

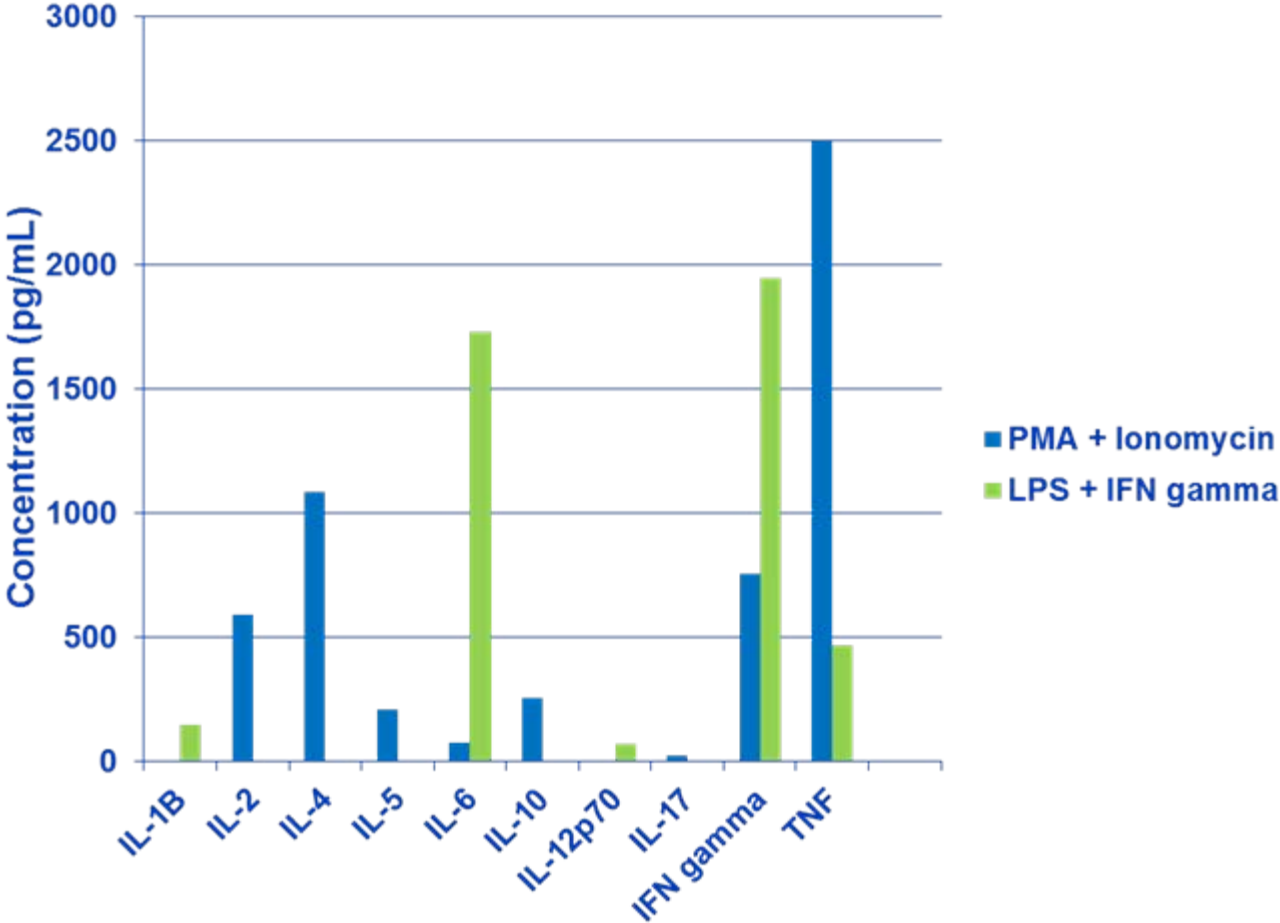


| Analyte Name | Sample Name | Position | Clustering | Results File | Event # | MFI | SD | CV | Nominal CC | Fitted CC |
|------------------------|-------------|----------|------------|--------------|---------|------------|-----------|---------|----------------|----------------|
| C4 - Human TNF | Std001 | 1 - A1 | Manual | A01.fcs | 161 | 3,027.00 | 489.26 | 14.69 % | 0.00 pg/mL | 8.71 pg/mL |
| A7 - Human IL-6 | Std002 | 1 - A2 | Manual | A02.fcs | 192 | 3,207.00 | 502.42 | 15.07 % | 20.00 pg/mL | 20.57 pg/mL |
| A6 - Human IL-5 | Std003 | 1 - A3 | Manual | A03.fcs | 203 | 3,542.00 | 681.25 | 17.26 % | 40.00 pg/mL | 37.21 pg/mL |
| A5 - Human IL-4 | Std004 | 1 - A4 | Manual | A04.fcs | 164 | 4,484.00 | 744.82 | 15.60 % | 80.00 pg/mL | 76.45 pg/mL |
| A4 - Human IL-2 | Std005 | 1 - A5 | Manual | A05.fcs | 155 | 6,632.00 | 956.28 | 15.49 % | 156.00 pg/mL | 153.32 pg/mL |
| A8 - Human IL-7 | Std006 | 1 - A6 | Manual | A06.fcs | 184 | 12,544.00 | 7,895.22 | 47.60 % | 312.50 pg/mL | 333.90 pg/mL |
| B8 - Human IFN-γ | Std007 | 1 - A7 | Manual | B01.fcs | 208 | 23,017.00 | 6,653.17 | 45.44 % | 625.00 pg/mL | 617.40 pg/mL |
| B7 - Human IL-10 | Std008 | 1 - A8 | Manual | B02.fcs | 194 | 44,654.00 | 6,960.99 | 16.77 % | 1,250.00 pg/mL | 1,180.87 pg/mL |
| B4 - Human IL-1β | Std009 | 1 - B1 | Manual | B03.fcs | 174 | 94,621.00 | 21,812.75 | 22.80 % | 2,500.00 pg/mL | 2,646.04 pg/mL |
| E5 - Human IL-12p70 | Std010 | 1 - B2 | Manual | B04.fcs | 151 | 152,272.00 | 34,550.51 | 22.04 % | 5,000.00 pg/mL | 4,916.23 pg/mL |
| | Test001 | 1 - B3 | Manual | B05.fcs | 169 | 42,244.00 | 7,602.03 | 18.78 % | N/A | 1,117.36 pg/mL |
| | Test002 | 1 - B4 | Manual | B06.fcs | 102 | 21,615.00 | 3,755.06 | 22.15 % | N/A | 580.59 pg/mL |
| | Test003 | 1 - B5 | Manual | C01.fcs | 195 | 10,764.00 | 1,805.81 | 15.37 % | N/A | 282.28 pg/mL |
| | Test004 | 1 - B6 | Manual | C02.fcs | 163 | 5,967.00 | 1,165.32 | 17.51 % | N/A | 130.63 pg/mL |



Comparison of Cytokine Concentrations

Analyze



Summary

BD offers a complete solution for soluble analyte measurement with BD CBA reagents, the BD Accuri C6 flow cytometer, and FCAP Array software.

Stain

- BD CBA assays provide a powerful technique for measuring soluble analytes such as cytokines.
- The BD product line includes pre-configured CBA kits, as well as configurable Flex Sets.

Acquire

- The BD Accuri C6 flow cytometer is an affordable, easy-to-use system for data acquisition.
- BD provides software templates that minimize instrument setup.

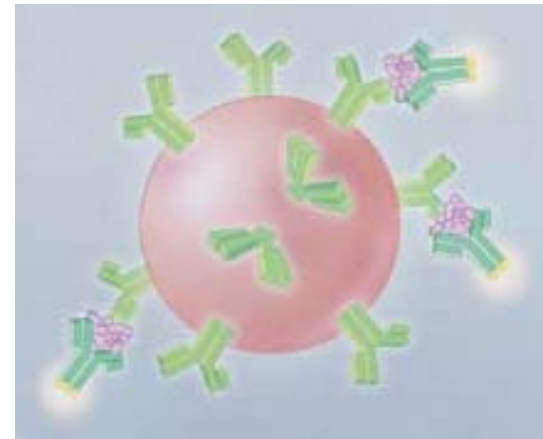
Analyze

- FCAP Array software provides simplified data analysis.
- FCAP Array v3.0.1 is compatible with BD Accuri FCS files.



Thank You!

- Trent Colville
- Stacey Roys
- Jacob Rabenstein



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