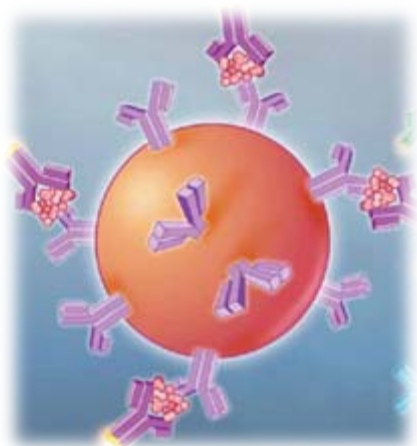


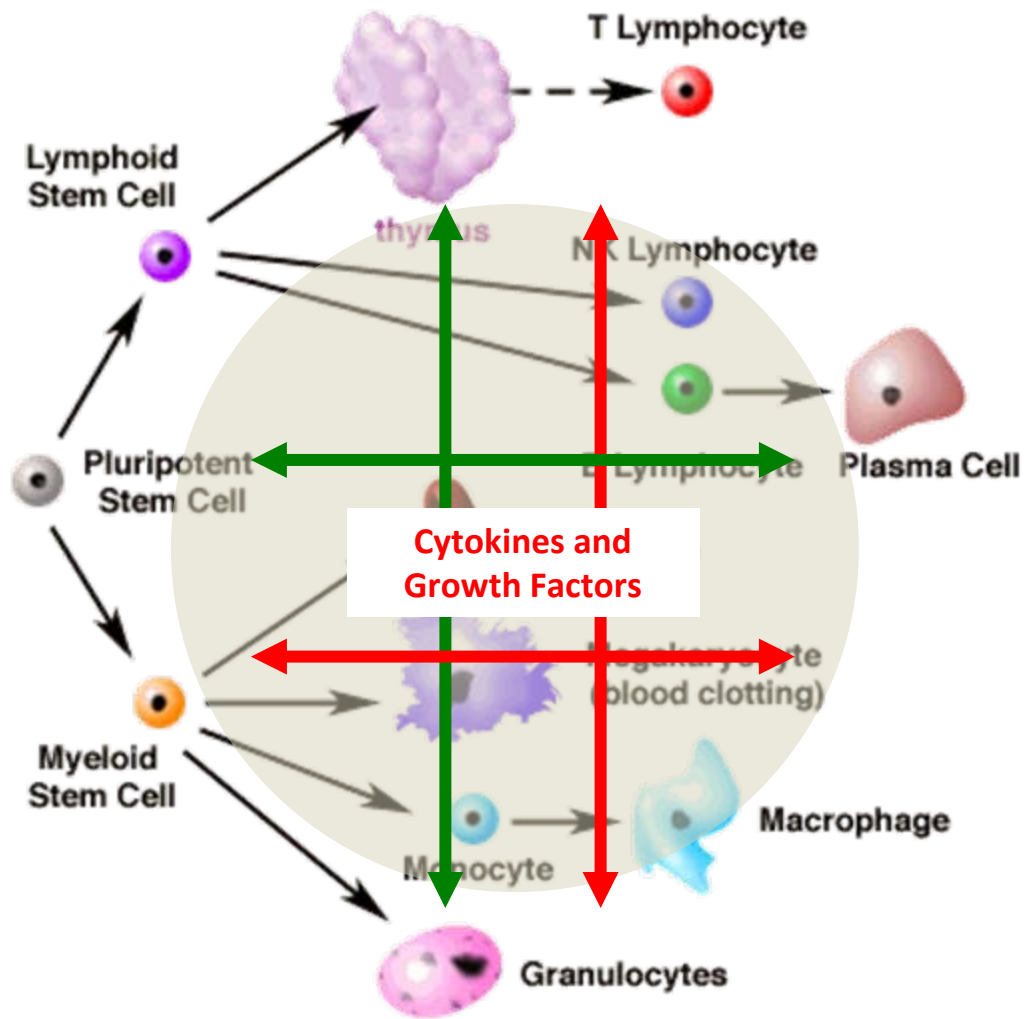
BD™ CBA on the BD Accuri™ C6: Bringing Multiplexed Cytokine Detection to the Benchtop



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



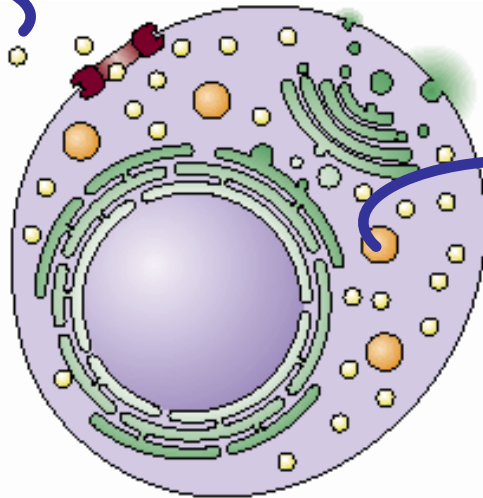
Cellular Communication



Techniques for Measurement of Cytokines

Soluble Proteins

- ELISA
- ELISPOT
- **BD CBA**

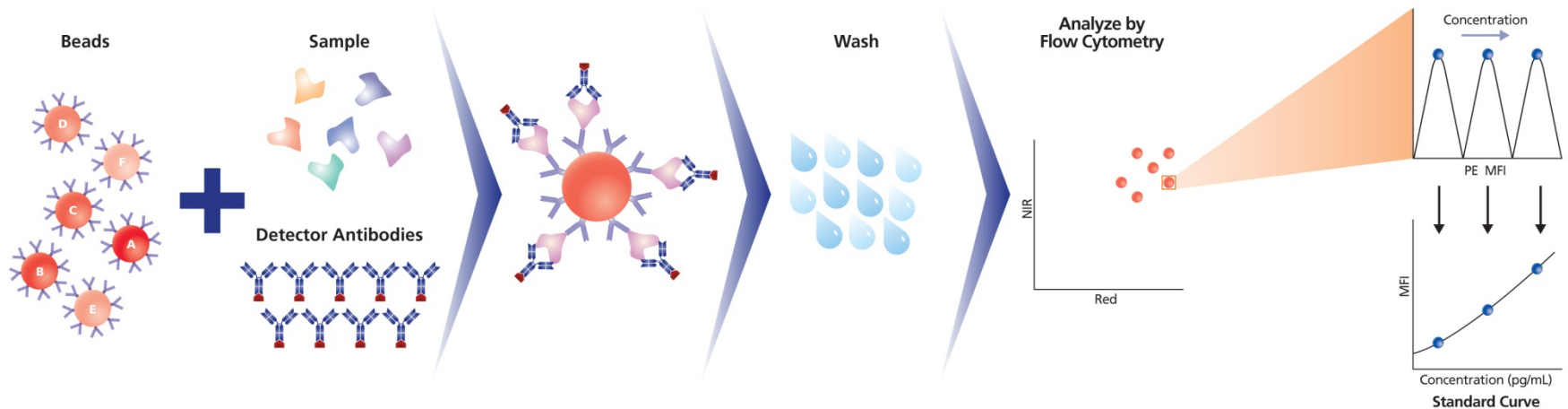


Intracellular Proteins

- 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

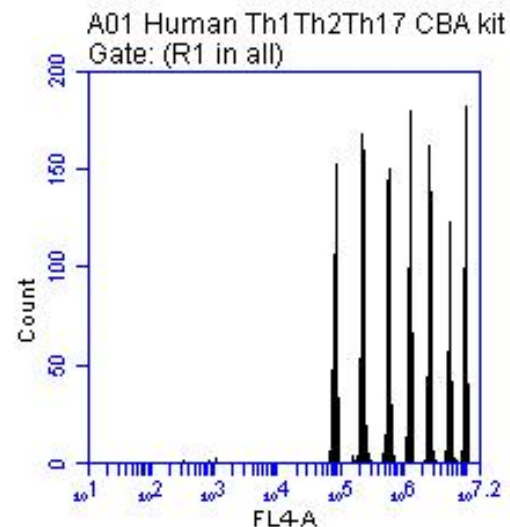
BD CBA Kits

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

Cumulative
 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.

<input type="checkbox"/> 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%		

<input type="checkbox"/> 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%		

<input type="checkbox"/> 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%	

<input type="checkbox"/> 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

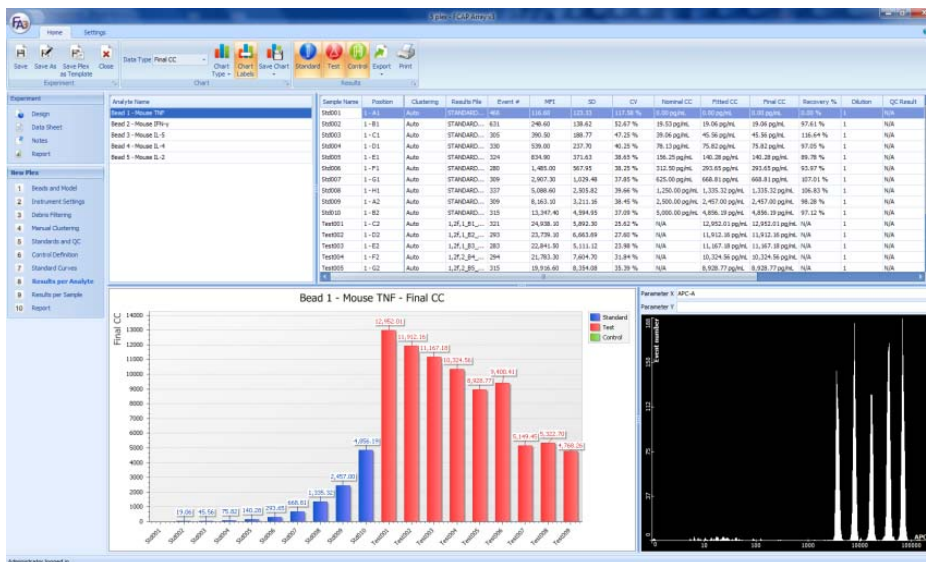
FCAP Array v3.0.1 (Microsoft®
Windows)

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

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 Group			Group Description	
All Beads				
A9	Human GAPDH			560792
C9	Phospho BLNK (Y...			560063
D5	Phospho Btk (Y5...			560004
D8	Phospho c-Jun (...)			560059
C7	Phospho eNOS (...)			560021
C7	Phospho eNOS (...)			560065
C4	Phospho ERK1/2...			560012
C6	Phospho Itk (Y5...			560008
B5	Phospho JNK1/2 ...			560013
A6	Phospho MBK1/2...			560150

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			

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

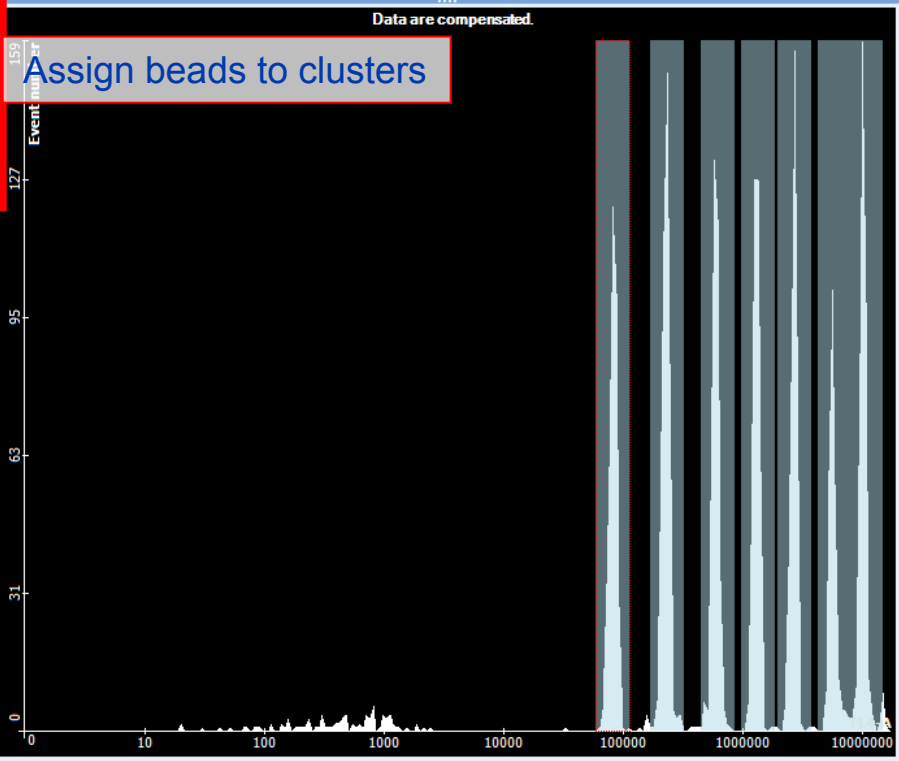
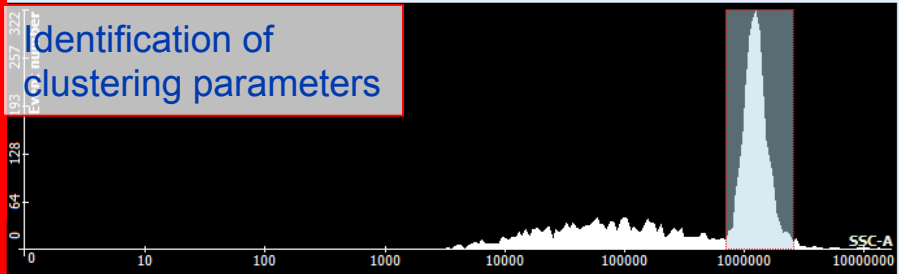
Scatter Peaks: 1

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



Standards and QC

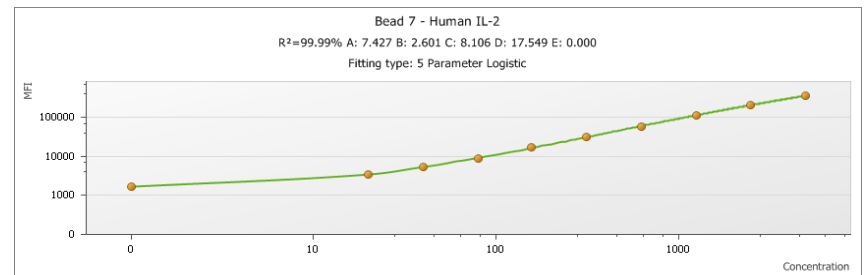
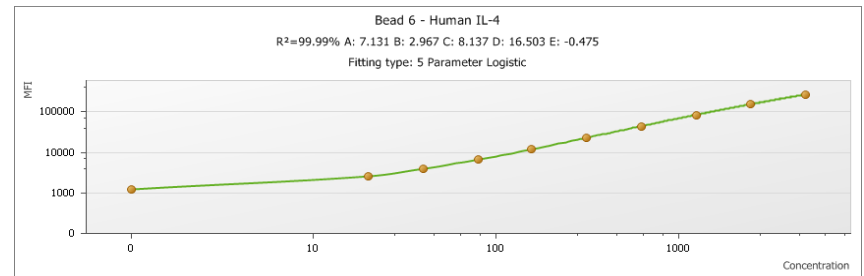
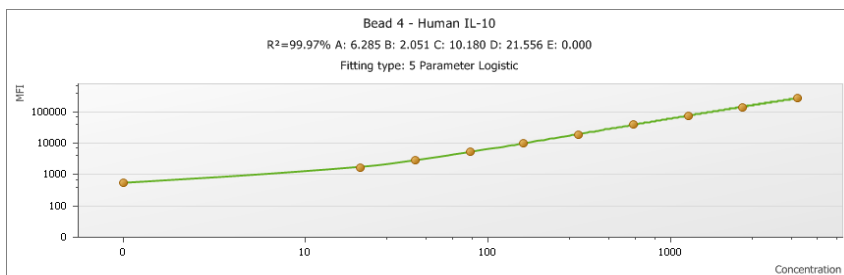
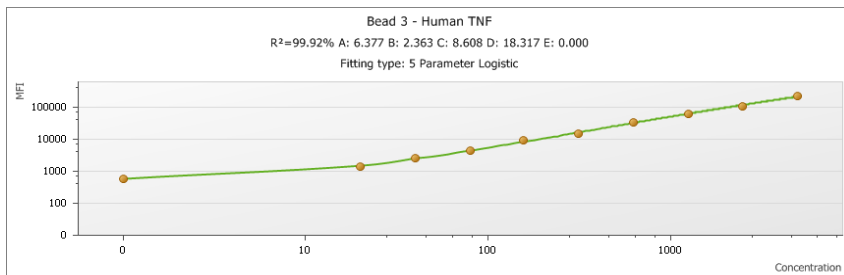
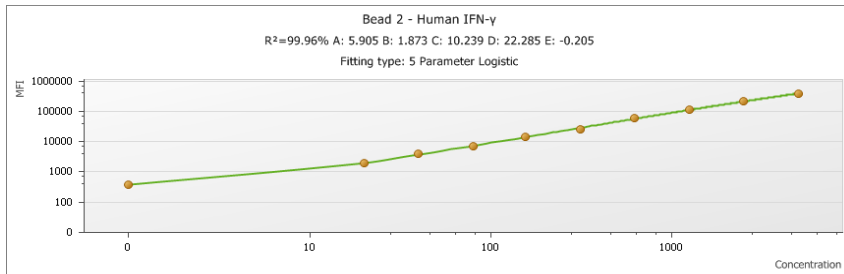
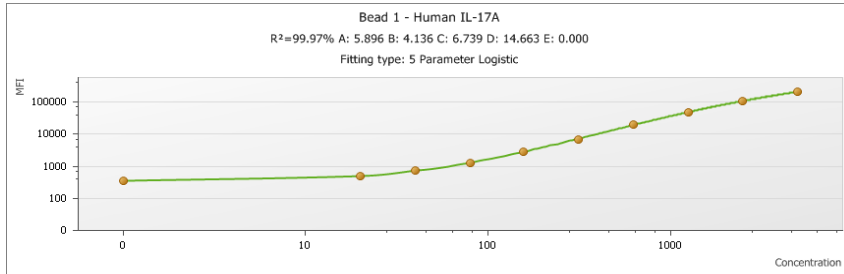


Standard dilution calculator and units

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

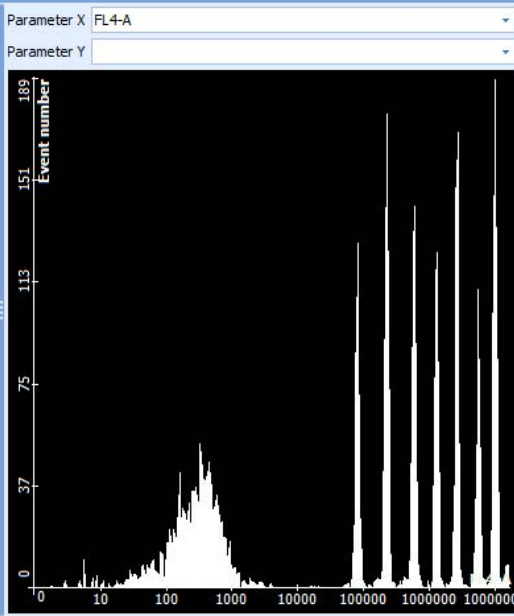
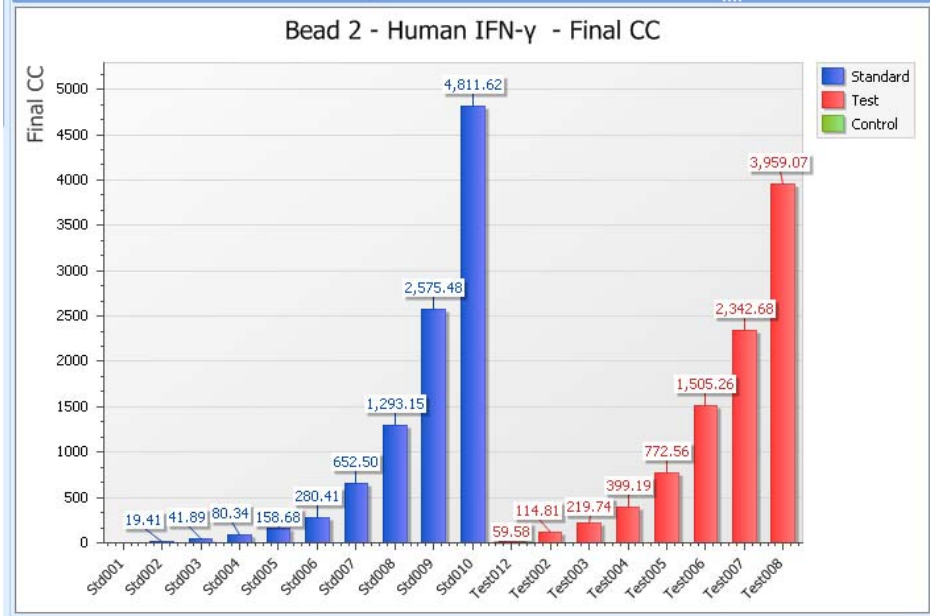
Standard Curves



Results per Analyte



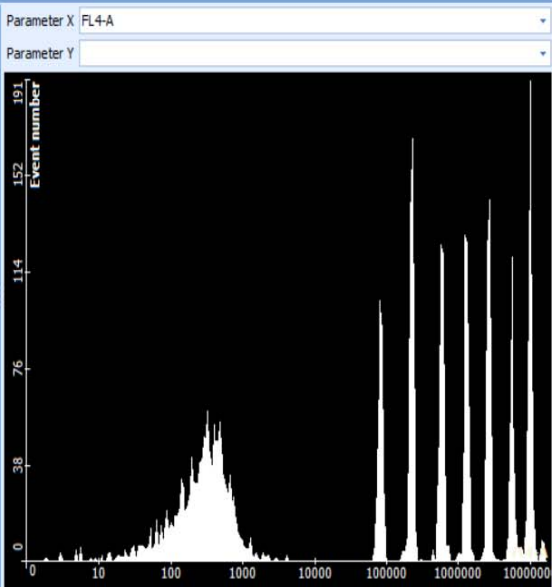
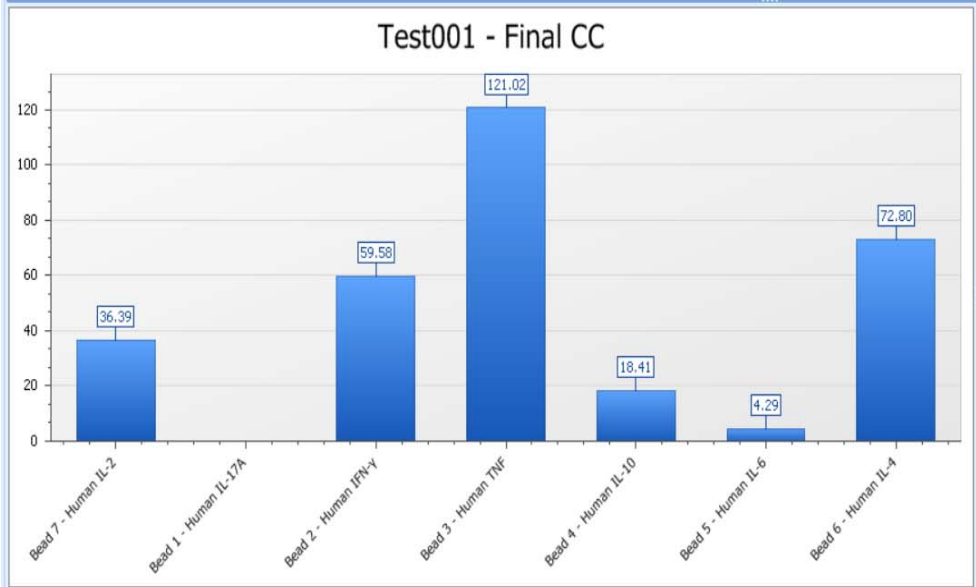
Analyte Name	Sample Name	Position	Clustering	Results File	Event #	MFI	SD	CV	Nominal CC	Fitted CC
Bead 1 - Human IL-17A	Std001	1 - A1	Auto	A01 0 std. fcs	443	370.00	313.57	69.01 %	0.00 pg/mL	0.00 pg/mL
Bead 2 - Human IFN-γ	Std002	1 - A2	Auto	A02 std 1 t...	300	1,976.00	1,226.48	53.31 %	20.00 pg/mL	19.41 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
Print

Quick Print
Print

Options
Page Setup

Header
Page Setup

Scale
Page Setup

Margins
Page Setup

Orientation
Page Setup

Size
Page Setup

Find
Navigation

First Page
Navigation

Previous Page
Navigation

Next Page
Navigation

Last Page
Navigation

Multiple Pages
Zoom

Zoom Out
Zoom

Zoom
Zoom

Zoom In
Zoom

Page Color
Page Background

Watermark
Page Background

Export To
Export

E-Mail As
Export

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

Created by FCAP Amp v3 5/11
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
R²=99.99% A: 7.427 B: 2.601 C: 8.106 D: 17.549 E: 0.000
Fitting type: 5 Parameter Logistic

Name	Event #	MPF	SD	CV% (MPF)	Nominal CC	Fitted CC	Recovery %
Std001	458	1,88900	45349	2581 %	0.00 pg/mL	0.52 pg/mL	0.00 %
Std002	282	3,44400	1,16421	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

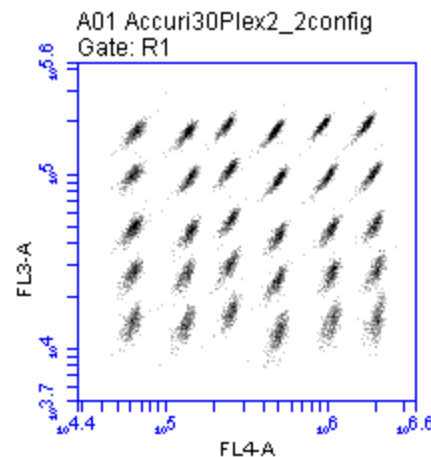
FCAP Array software to measure cytokine concentrations



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



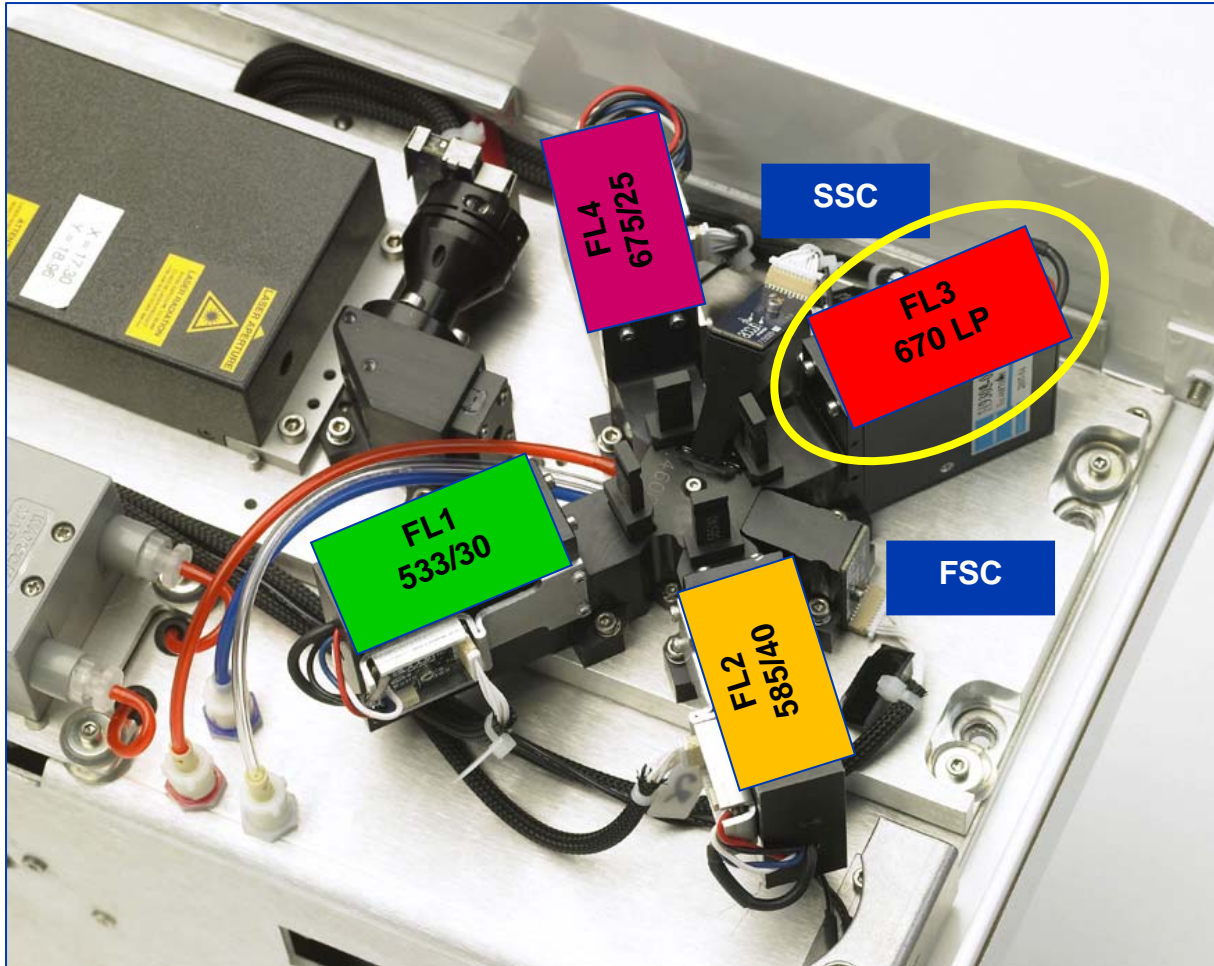
BD CBA Flex Sets

BD Accuri C6



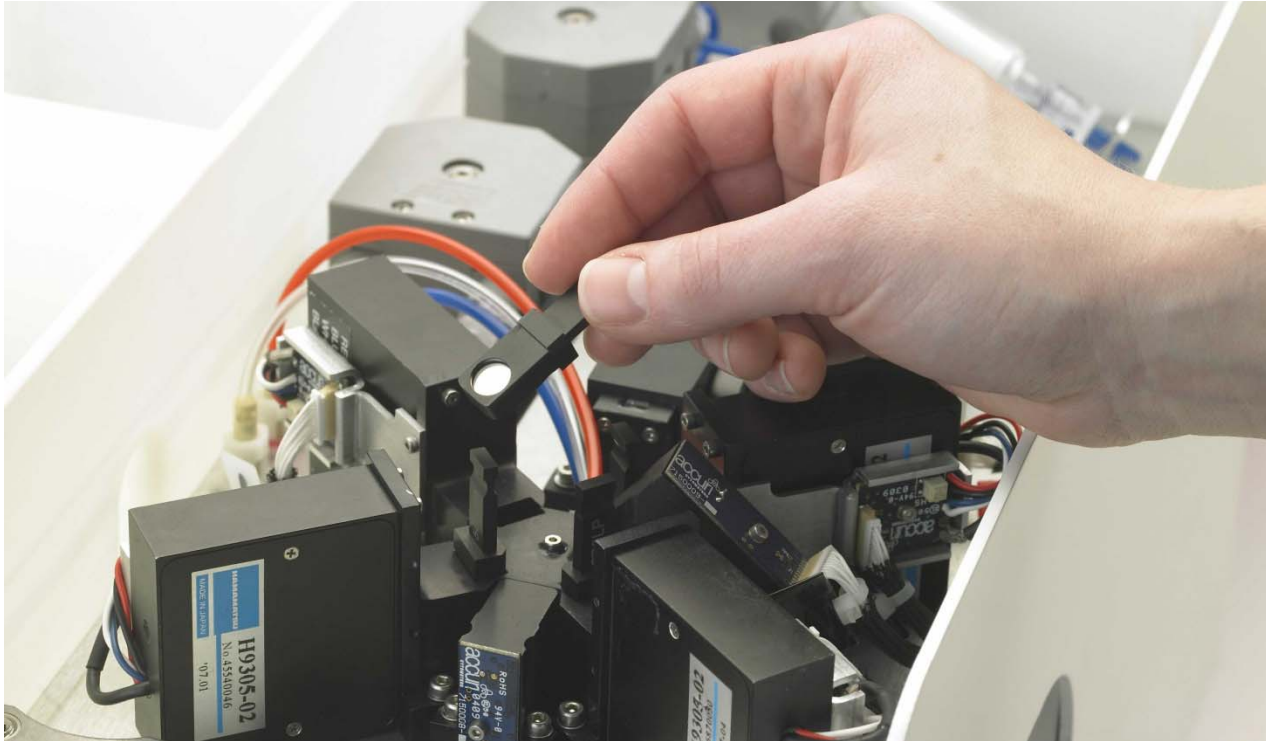
Optical Configuration

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

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

Backflush Unlog

RUN

Set Color Compensation

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

Cumulative
 0
 0.00.0
 0
 0
 0

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

Set Color Compensation

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

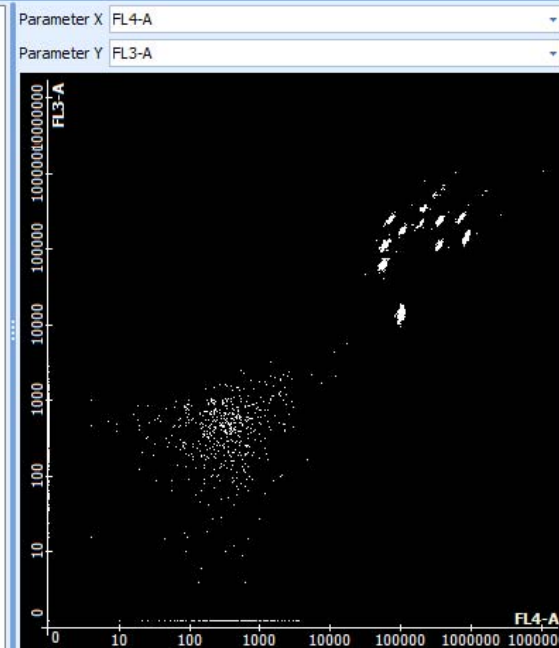
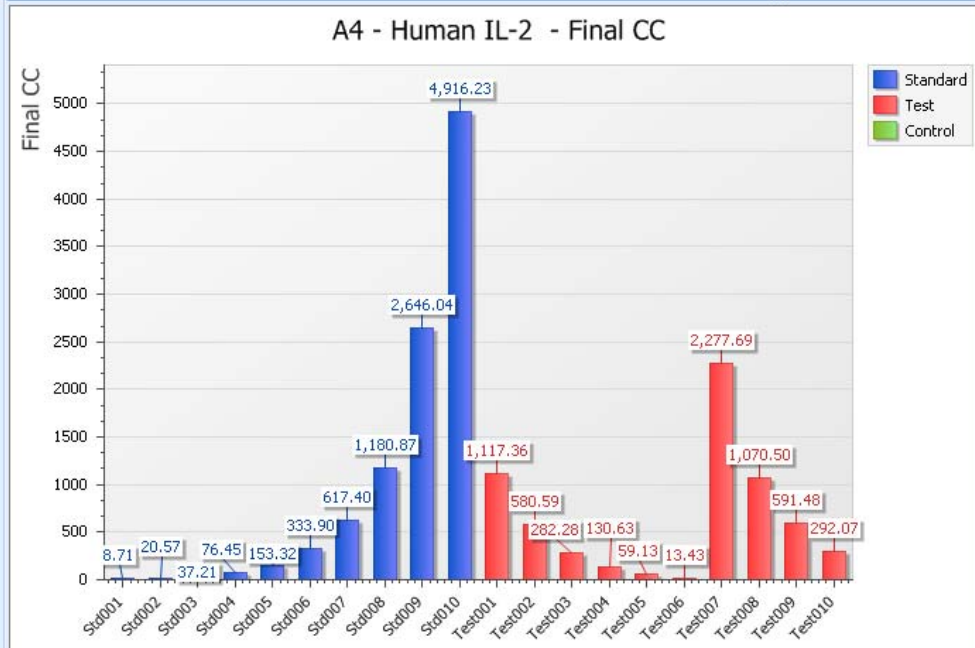
Assign beads to clusters



Results per Analyte

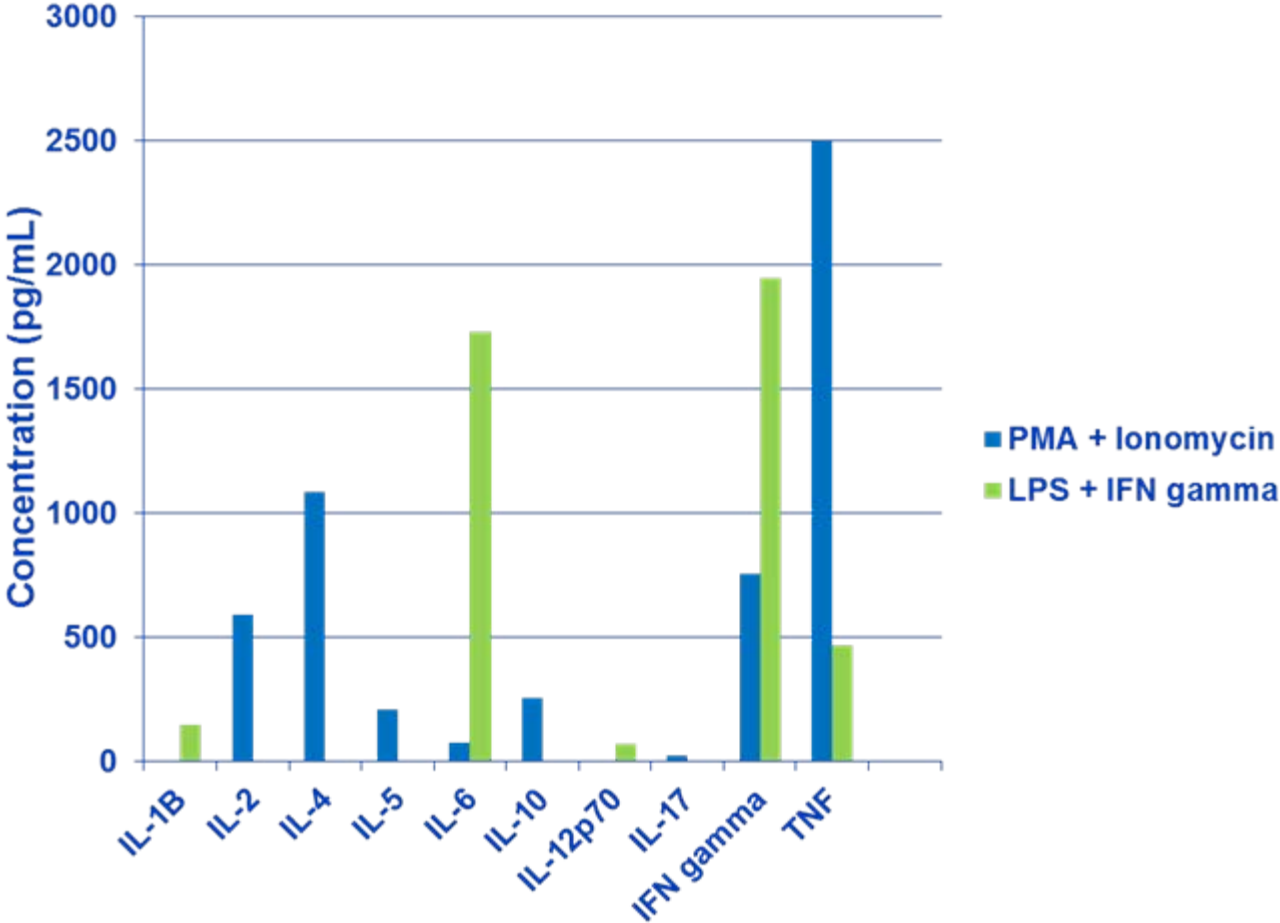


Analyte Name	Sample Name	Position	Clustering	Results File	Event #	MFI	SD	CV	Nominal CC	Fitted CC
C4 - Human TNF	Std001	1 - A1	Manual	A01.fcs	161	3,027.00	489.26	14.69 %	0.00 pg/mL	8.71 pg/mL
A7 - Human IL-6	Std002	1 - A2	Manual	A02.fcs	192	3,207.00	502.42	15.07 %	20.00 pg/mL	20.57 pg/mL
A6 - Human IL-5	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.

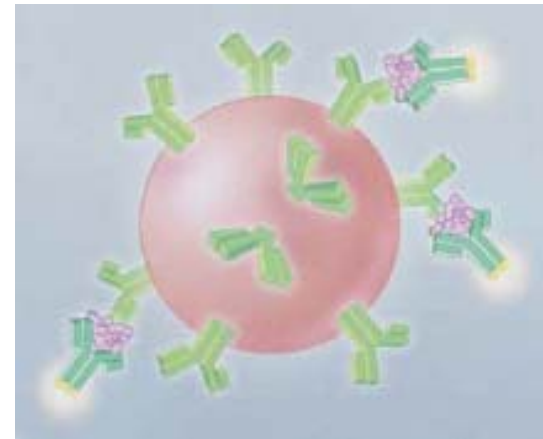
Analyze

- 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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