

FCAP Array™ v3.0 Software: A New Tool to Analyze BD™ Cytometric Bead Array (CBA) Data

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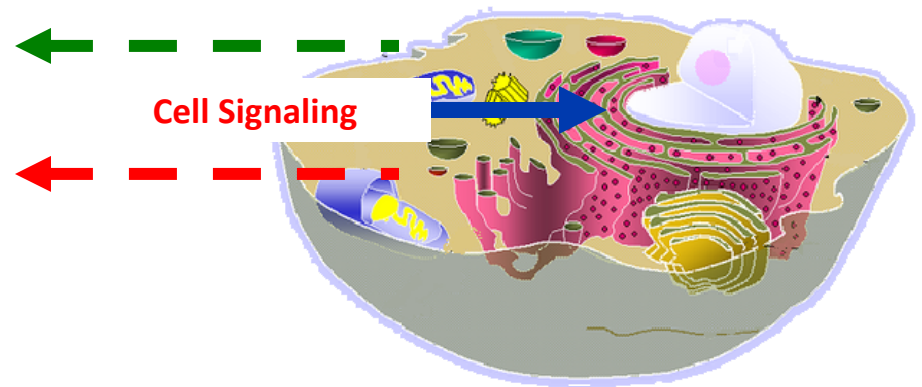
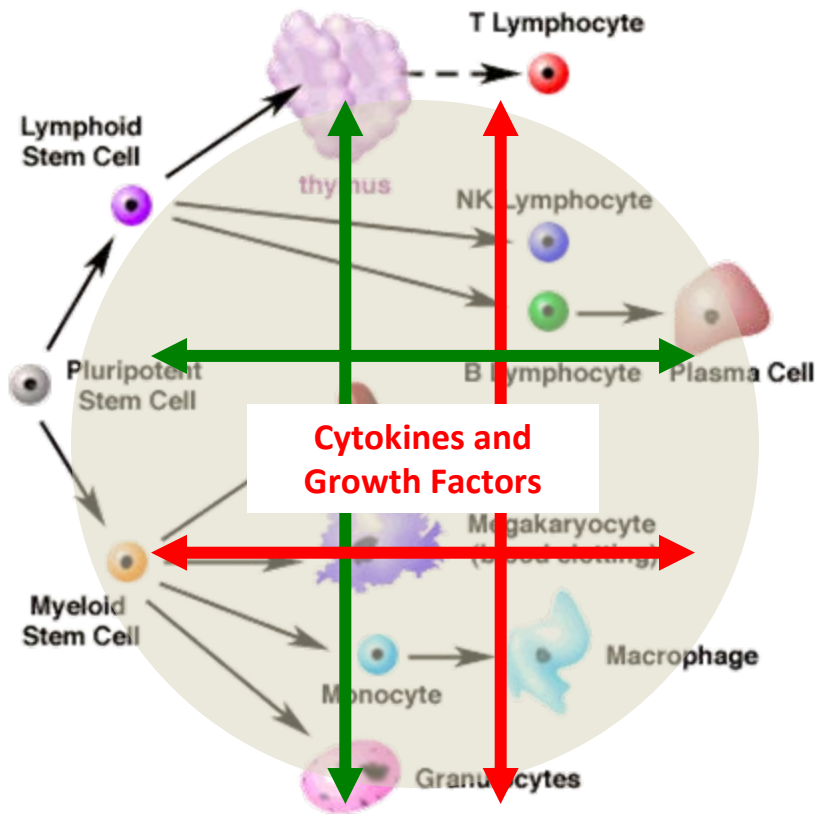
Agenda

- Introduction
- Overview of BD CBA bead based immunoassays
- FCAP Array v3.0 key features
- Live demonstration of data analysis in FCAP Array v3.0



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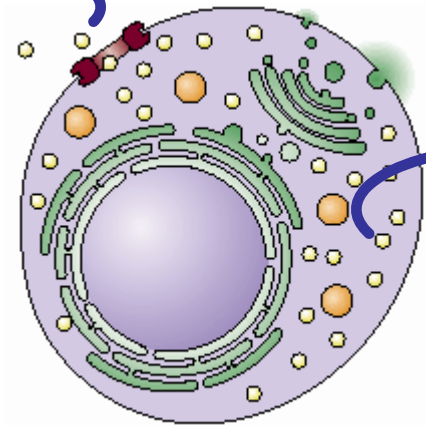
Cellular communication and signaling



Summary of Techniques for Measurement of Cytokines

Soluble Proteins

- ELISA
- ELISPOT
- **BD CBA**



Intracellular Proteins

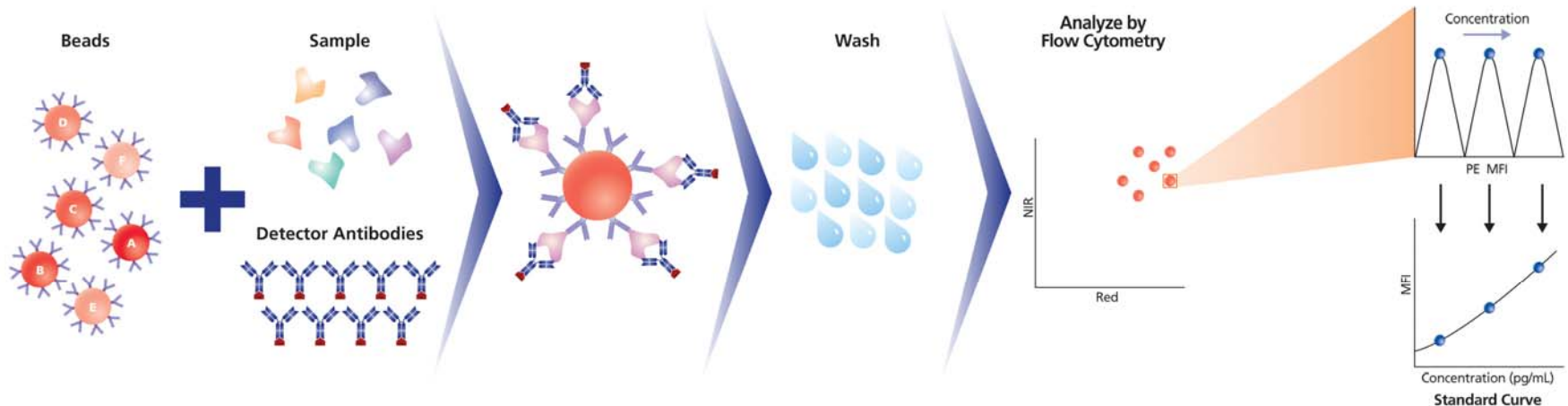
- Flow Cytometry
- WB
- IHC
- **BD CBA**



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BD CBA Assay Overview

- Analytes are bound by specific capture antibodies conjugated to beads with distinct fluorescent properties
 - Based on an antibody pair, the same principle as a sandwich ELISA
- The reporter in the assay is a PE-labeled detection antibody
- Analyte concentrations are estimated by comparison with a standard curve



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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



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Comparison with ELISA

BD CBA	ELISA
Uses culture supernatant or serum/plasma	Uses culture supernatant or serum/plasma
High statistical relevance	Must run samples at least in duplicate
Can detect multiple cytokines simultaneously	One cytokine per ELISA plate
Fewer sample dilutions due to broad dynamic range	Requires multiple sample dilutions
Requires a flow cytometer and analysis software	Relatively easy to perform and fairly inexpensive



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The BD CBA Workflow

Stain

BD CBA Kits
BD CBA Flex Sets

Acquire

BD FACSVerser™
BD LSRFortessa™
BD LSR™ II
BD FACSCanto™ II
BD FACSAria™ III
BD FACSArray™
BD FACSCalibur™

Analyze

FCAP Array v3 (Windows®)
FCAP Array v1 (Mac®)



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BD CBA Reagent Configurations

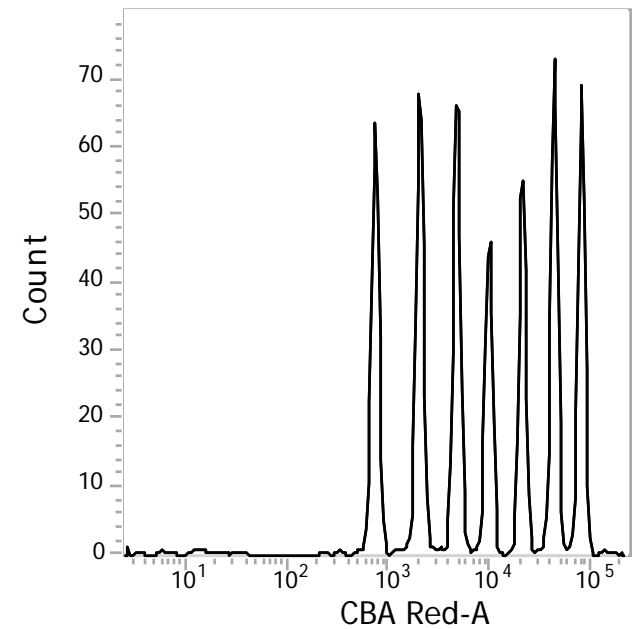
- BD CBA Kits
 - Preconfigured panels of 3 to 7 analytes
 - Th1/Th2
 - Th1/Th2/Th17
 - Inflammatory Cytokines
 - Anaphylatoxins
 - Includes all reagents required to run the assay
 - 80 tests per kit
 - 2-color assay
 - Red dye in beads
 - PE reporter
- BD CBA Flex Sets
 - Build your own multiplex
 - Human Soluble Protein
 - Mouse or Rat Soluble Protein
 - Enhanced Sensitivity (>274 fg/mL)
 - Cell Signaling
 - Human Immunoglobulin
 - Flex Set = Capture Beads, Detection Reagent, Standard (2 curves)
 - 100 tests
 - Master Buffer Kit = all buffers needed for assay
 - 100 or 500 test sizes
 - 3-color assay
 - Two red dyes in beads
 - PE reporter



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Instrument Compatibility: BD CBA Kits

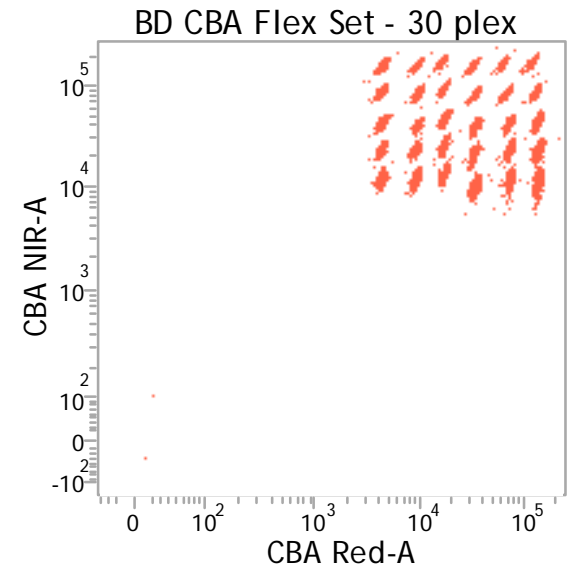
Instrument	Reporter Parameter	Clustering Parameters
BD FACSVerser flow cytometer	PE	CBA Red
BD FACSArrary bioanalyzer	Yellow	Red
BD FACSCanto™ II flow cytometer	PE	APC
BD™ LSR II flow cytometer	PE	APC
BD FACSAria™ II cell sorter	PE	APC
BD FACSCalibur™ flow cytometer (single laser)	FL2	FL3
BD FACSCalibur™ flow cytometer (dual laser)	FL2	FL4



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Instrument Compatibility: BD CBA Flex Sets

Instrument	Reporter Parameter	Clustering Parameters
BD FACSVerser™ flow cytometer	PE	CBA Red and CBA NIR
BD FACSArry bioanalyzer	Yellow	Red and NIR
BD FACSCanto™ II flow cytometer	PE	APC and APC-Cy™7
BD™ LSR II flow cytometer	PE	APC and APC-Cy7
BD FACSAria™ II cell sorter	PE	APC and APC-Cy7
BD FACSCalibur™ flow cytometer	FL2	FL4 and FL3



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Tools to Simplify Data Acquisition

- bdbiosciences.com/cbasetup
- Download templates and instructions
 - Instrument setup
 - Data acquisition
- Assay instructions: either in BD CBA kit or flex set manuals

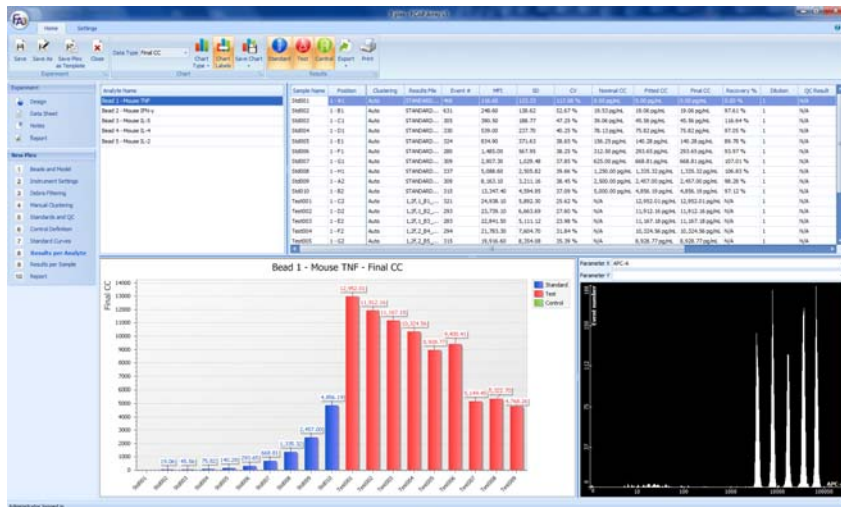


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FCAP Array v3.0 Analysis Software



- Compatible with FCS 2.0 or 3.0 files from any flow cytometer
- Integrated workflow for the BD FACSVerse flow cytometer
- Windows® 7, Vista®, or XP compatible



Design View

The screenshot displays the 'Design View' of the CBA Standard Assay UD software. The main workspace is a 12x8 grid representing a 96-well plate. The top toolbar includes icons for 'Save', 'Save As', 'Save Flex as Template', 'Close', 'Standard', 'Test', 'Control', 'Background', 'Undefined', 'New Sample', 'Number of Replicates', 'Number of Samples', 'Dilution Factor', 'Active Plex', 'Add Plate', 'Clear All File Associations', 'Clear All Plates', 'Vertical Alignment', 'Horizontal Alignment', 'Print', 'Add New', 'Delete', 'Rename', and 'Add New from Template'. The left sidebar shows a navigation menu with 'Design', 'Data Sheet', 'Notes', and 'Report'. The right sidebar contains a 'File Assignment' window with a 'File Explorer' showing a directory structure and a 'Sample List' table. Below the file list is a 'Sample Properties' window with tabs for 'Properties' and 'File Header'.

Name	Date
Tube_001_STD1.fcs	2/17/2011 6:25:35 PM
Tube_001_STD2.fcs	2/17/2011 6:36:35 PM
Tube_001_STD4.fcs	2/17/2011 6:40:19 PM
Tube_001_STD6.fcs	2/17/2011 6:42:24 PM
Tube_001_STD7.fcs	2/17/2011 6:43:12 PM
Tube_001_STD8.fcs	2/17/2011 6:44:12 PM
Tube_001_STD10.fcs	2/17/2011 6:50:44 PM
Tube_001_STD9.fcs	2/17/2011 6:54:10 PM
Tube_001_STD5.fcs	2/17/2011 7:00:50 PM
Tube_001_STD3.fcs	2/17/2011 7:03:04 PM
Tube_001_S001.fcs	2/17/2011 7:21:02 PM
Tube_001_S002.fcs	2/17/2011 7:22:53 PM

- Identify location of data files
- Assign to standards and samples



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Beads and Model

Administrator logged in

Selected Beads						
Bead Name	Lot Number	Catalog Number	Name	Analyte		
				Model	2nd Reporter	
C4		558328	Human Angiogenin	Quantitative	No	
C5		558327	Human Basic FGF	Quantitative	No	
D7		560418	Human CD14	Quantitative	No	
A4		560269	Human CD54	Quantitative	No	
D9		560419	Human CD62E	Quantitative	No	
D8		560420	Human CD62L	Quantitative	No	
D7		560426	Human CD62P	Quantitative	No	
D6		560427	Human CD106	Quantitative	No	
B6		560276	Human CD121a	Quantitative	No	
B7		560281	Human CD121b	Quantitative	No	
C7		560305	Human CD154/CD40L	Quantitative	No	

Bead Group		Group Description
All Beads		
A9	Human GAPDH	560792
C9	Phospho BLNK (Y84)	560063
D5	Phospho Blk (Y551)	560004
D8	Phospho c-Jun (S63)	560059
C7	Phospho eNOS (S1...	560021
C7	Phospho eNOS (T4...	560065
C4	Phospho ERK1/2 (T...	560012
C6	Phospho Ikk (Y511)	560008
B5	Phospho JNK1/2 (T...	560013
A6	Phospho MEK1/2 (S...	560150
B6	Phospho p38 (T180...	560010

Bead Group		Group Description
All Beads	All Beads	
<input type="checkbox"/>	Human Soluble Protein	BD CBA Flex Sets
<input type="checkbox"/>	Mouse Th1/Th2 Cytokine Kit - 551287	BD CBA Kit
<input type="checkbox"/>	Cell Signaling	BD CBA Flex Sets
<input type="checkbox"/>	Human Enhanced Sensitivity (ES)	BD CBA Flex Sets
<input type="checkbox"/>	Human Immunoglobulin	BD CBA Flex Sets
<input type="checkbox"/>	Mouse Soluble Protein	BD CBA Flex Sets
<input type="checkbox"/>	Rat Soluble Protein	BD CBA Flex Sets
<input type="checkbox"/>	Human Anaphylatoxin Kit - 561418	BD CBA Kit
<input type="checkbox"/>	Human Chemokine Kit - 552990	BD CBA Kit
<input type="checkbox"/>	Human Th1/Th2 Cytokine Kit - 550749	BD CBA Kit
<input type="checkbox"/>	Human Th1/Th2 Cytokine Kit II - 551809	BD CBA Kit
<input type="checkbox"/>	Human Th1/Th2/Th17 Cytokine Kit - 560484	BD CBA Kit
<input type="checkbox"/>	Mouse Inflammation Kit - 552364	BD CBA Kit
<input type="checkbox"/>	Mouse Th1/Th2/Th17 Cytokine Kit - 560485	BD CBA Kit
<input type="checkbox"/>	Non-human Primate Th1/Th2 Cytokine Kit - ...	BD CBA Kit
<input type="checkbox"/>	Mouse Immunoglobulin Isotyping Kit - 550026	BD CBA Kit
<input type="checkbox"/>	Mouse Enhanced Sensitivity (ES)	BD CBA Flex Sets
<input type="checkbox"/>	Human Inflammatory Cytokine Kit - 551811	BD CBA Kit

#Beads: 11

- Select the beads corresponding to analytes in your experiment from the bead library
- Download a bead library file from bdbiosciences.com/cbasetup



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Instrument Settings

The screenshot displays the 'CBA Standard Assay UD - FCAP Array v3' software interface. The 'Settings' tab is active, showing the following configuration:

- Selected File:** C:\Users\10062526\Desktop\cbs keyword files\Tube_001_STD1.fcs
- Instrument Data:** Liberty - Liberty Machine 1
- Scatter Parameter:** SSC-A
- Scatter Peaks:** 1
- Clustering Parameters:** APC-A (Scatter), APC-Cy7-A (Reporter)
- Reporter Parameter 1:** PE-A
- Reporter Parameter 2:** (empty)

The 'Selected Beads' table is as follows:

Bead	Analyte
C4	Human Angiogenesis
C5	Human Basic FGF
D7	Human CD14
A4	Human CD54
D9	Human CD62E
D8	Human CD62L
D7	Human CD62P
D6	Human CD106
B6	Human CD121a
B7	Human CD121b
C7	Human CD154/CD40L

The interface also includes a histogram of 'Event number' vs 'SSC-A' and a scatter plot of 'APC-Cy7-A' vs 'APC-A' with data points labeled C4, C5, D7, A4, D9, D8, D7, D6, B6, B7, and C7.

- Verify scatter, clustering, and reporter parameters
- Assign beads to clusters



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Standards and QC

Administrator logged in

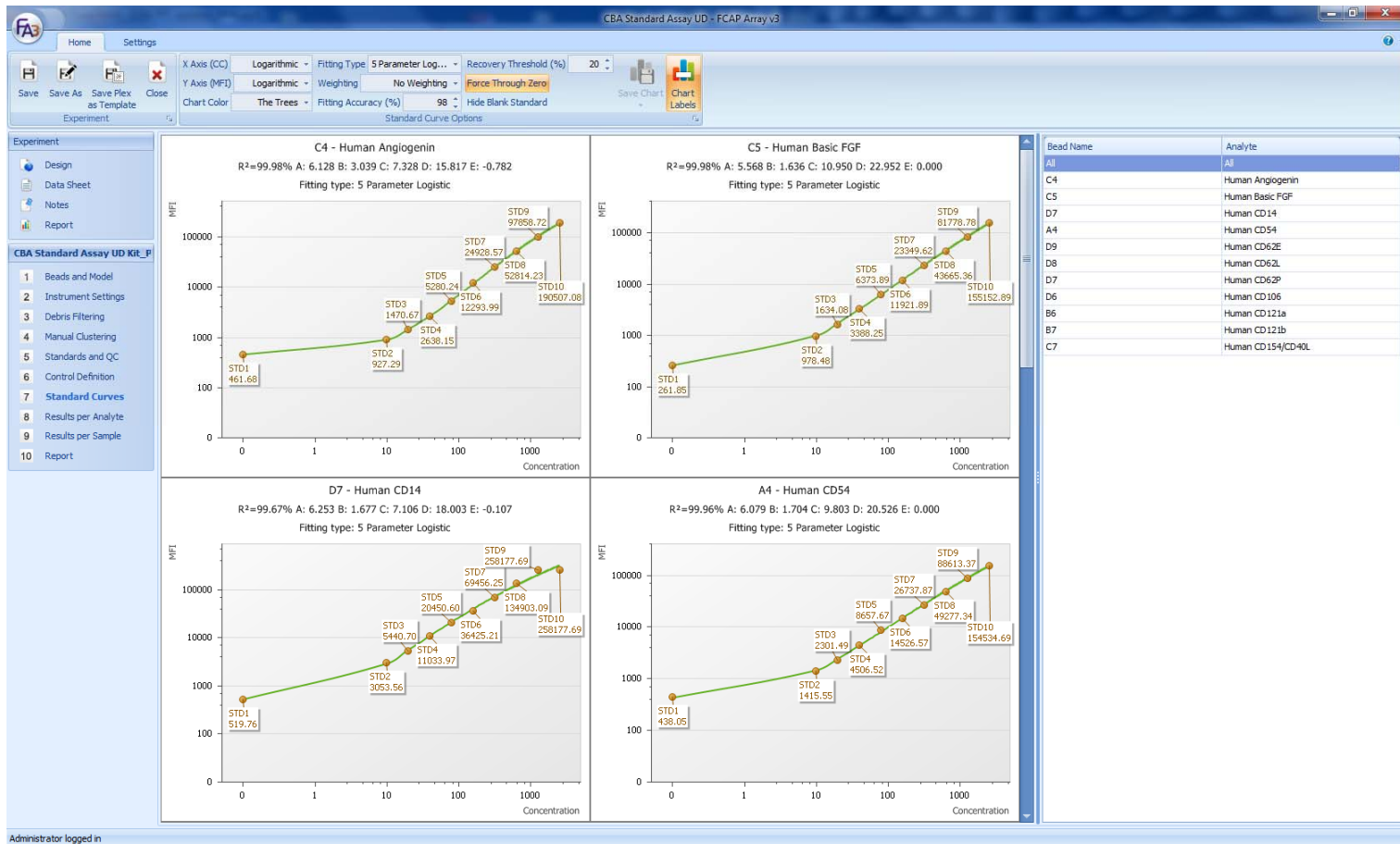
Standard Sample	Concentration
STD1	0.00 pg/mL
STD2	9.77 pg/mL
STD3	19.53 pg/mL
STD4	39.06 pg/mL
STD5	78.13 pg/mL
STD6	156.25 pg/mL
STD7	312.50 pg/mL
STD8	625.00 pg/mL
STD9	1,250.00 pg/mL
STD10	2,500.00 pg/mL

- Assign concentration levels to the standard curves
- Manual entry or dilution profile calculator



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Standard Curves

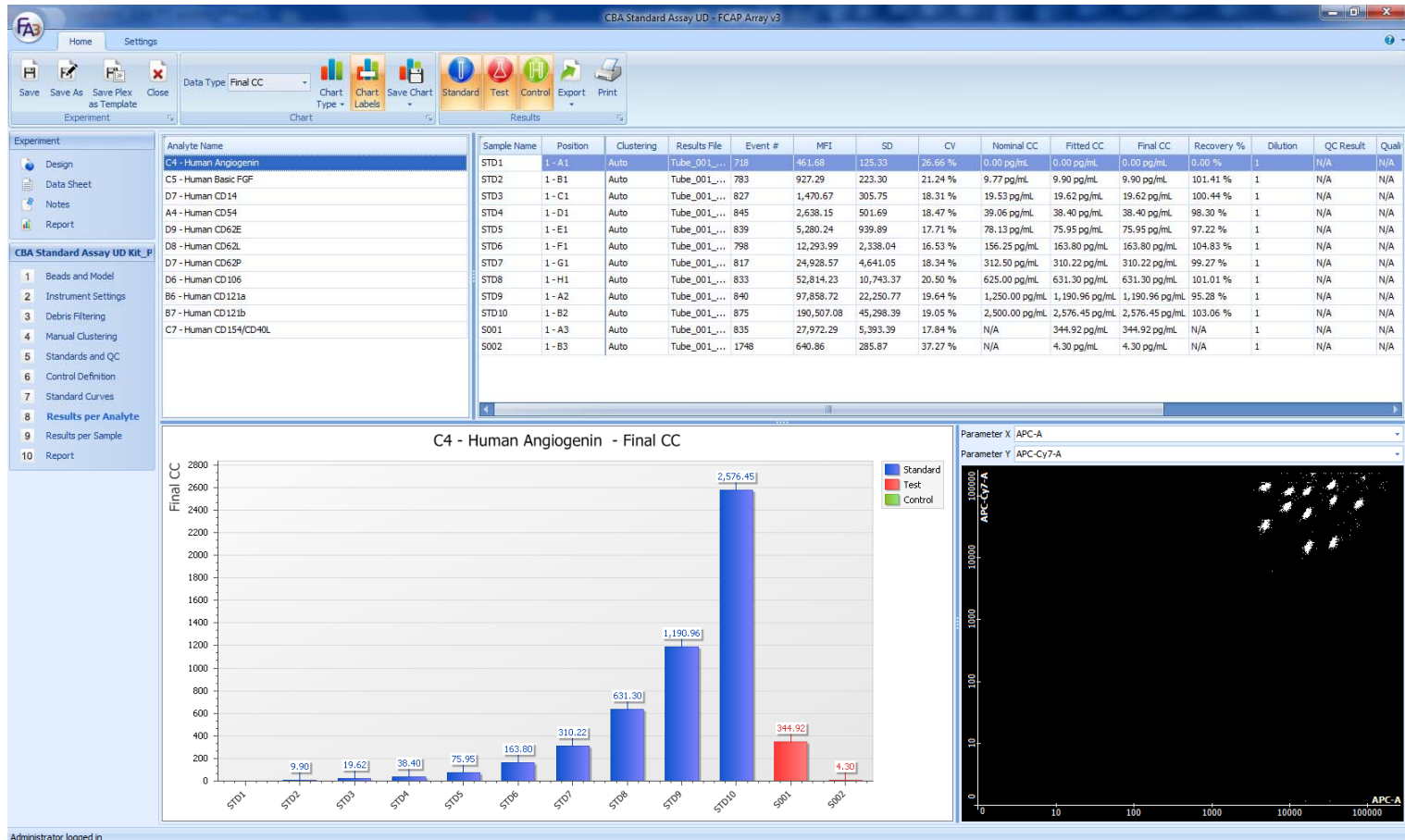


- Review standard curves
- Options: force through zero, axis scale (lin/log), curve type, weighting, fitting accuracy, colors



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Results



- View raw data by analyte or by sample
- Chart options for visualization and export
 - Bar chart, 3D bar chart, polar chart

Additional Features

- Manual clustering available in instances when the automatic algorithm cannot identify all bead clusters
- Debris filtering available for cases when debris is causing the automatic algorithm to fail
- Summary reports available in PDF
- Raw data export to a spreadsheet
- Export standard curve and chart images as JPG, BMP, or PNG



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Summary

- BD CBA reagents are available in two formats
 - BD CBA kits: preconfigured panels of ≤ 7 analytes
 - BD CBA flex sets: mix and match, up to 30 analytes
 - Flex sets also available in Enhanced Sensitivity format
 - Assay range: 274 to 200,000 fg/mL
- Compatibility with BD FACS™ brand flow cytometers
- New FCAP Array v3.0 analysis software with intuitive workflow and enhanced features



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Acknowledgment

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Zoltán Mike, Lead Developer

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