How to Use Flow Cytometer and Cell Sorter
(Lecture in English)

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フローサイトメーターは個々の細胞の分子の発現を半定量的に測定できる装置である。装置は解析専用のアナライザーと解析分取が可能なセルソーターの 2 機種があり、分子の発現の同定には蛍光色素で認識された抗体がよく用いられる。更に標識抗体以外の蛍光物質を測定する事も試みられている。 今回は実習として、機器更正用ビーズを用いて、FACS にて検出する。

FACS の基本原理を紹介し、FACS を用いた実験手技については具体例を挙げながら説明します。

Flow cytometer is a device that can semi-quantitatively measure the expression of individual cell molecules. There are two types of devices, an analyzer and a cell sorter. Antibodies recognized by fluorescent dyes are often used to identify the expression of molecules. It has also been attempted to measure fluorescent substances other than labeled antibodies.

As a practical training, we detected with FACS using beads for flow cytometer setup.

＊FACS (fluorescence activated cell sorter) is a trademark of Becton, Dickinson and company.
I will introduce the basic principle of FACS and explain the experimental technique using FACS with concrete examples.

Flow Cytometry Seminar 2026

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Waters™

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Today's contents

Introduction to Flow Cytometry (FCM)

1. Basic of FCM
2. Application for FCM

Flow Cytometry (FCM) is

Technique to analyze the character of cells or particle by measuring the scatter light and fluorescence exposed to a laser running in a stream

Cell Analyzer

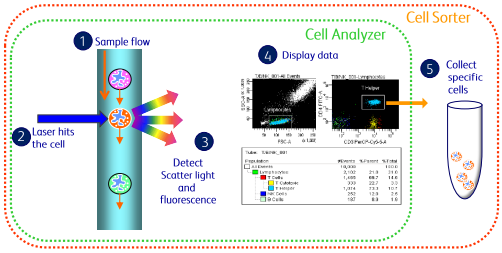
Searching target cells in heterogeneous cell population

Analyzing the character of target cells

Cell Sorter

Sorting target cells

FCM is



History of FCM

- In the late 1960s
  Leonard Herzenberg, PhD, professor at Stanford University developed Fluorescence Activated Cell Sorter (FACS)
- 1973
  Commercialized by US Becton-Dickinson (FACS I)
- New products are released every few years
  Improving laser and data processing system, making it possible to analyze more items much faster

Instrument	Maximum number of laser equipment	Number of fluorescent color	Processing speed (Cells/sec)
Early FACS	1	2	-
Present FACS	6	50 or more	70000

Central Research Laboratory : Shiga University of Medical Science

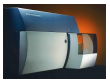
Cell Sorter

BD FACSAria™

FBD ACSAria™ Fusion × 2 Set

Cell Analyzer

BD FACSCalibur™



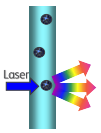
BD FACSCanto™ II

LSRFortessa™ X-20

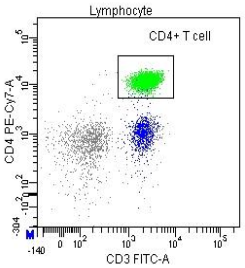
More Colors
&
Higher Performance

Character of FCM

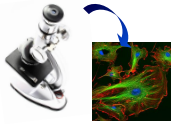
FCM



- ✓ High Throughput
- ✓ Multiparameter
- ✓ Quantitative data
- ✓ Cell sorting
- ✓ Objectivity, Reproducibility
- ✓ Imaging (Imaging Cytometer)



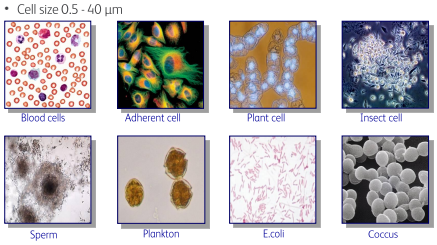
Fluorescence microscope



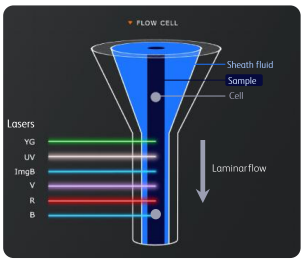
- ✓ Image (3D)
- ✓ Intracellular distribution
- ✓ Time-lapse

FCM analysis targets

- Single cells in suspension
- Cell size 0.5 - 40 μm



Detection of FCM



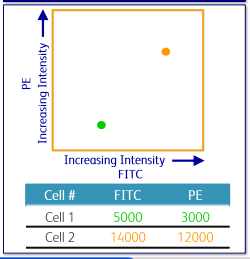
Laminar Flow formation / Laser / Detection

Formation of narrow and laminar flow, enables cells to run one by one and passes through the laser

- Detects scatter and fluorescence
- Process few thousand cells/sec

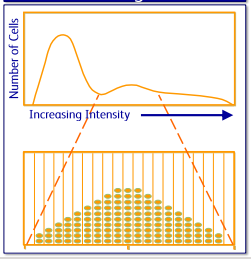
Data Display： Dot Plot and Histogram

Dot Plot

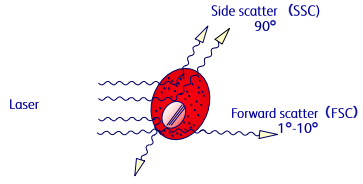


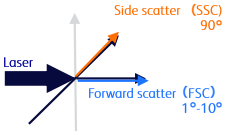
Cell #	FITC	PE
Cell 1	5000	3000
Cell 2	14000	12000

Histogram



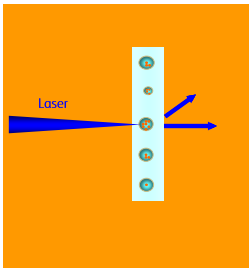
Character of scatter light

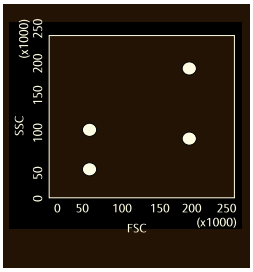




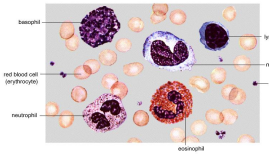
- ●Forward Scatter (FSC)
  Indicates the size of cells, surface area
- ●Side Scatter (SSC)
  Indicates the complexity of internal structure, cellular granule

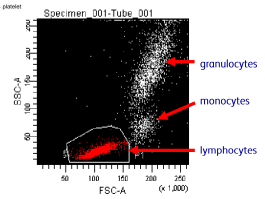
Scatter light signal



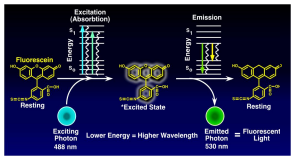


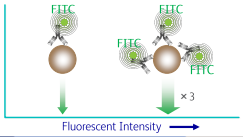
Scatter light analysis of blood cells

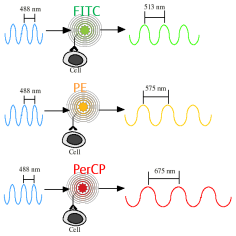




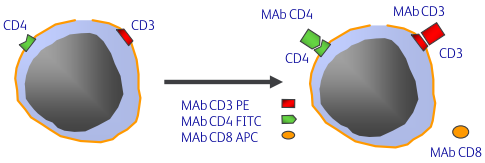
Character of fluorescence





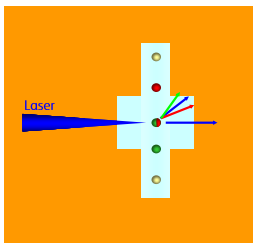


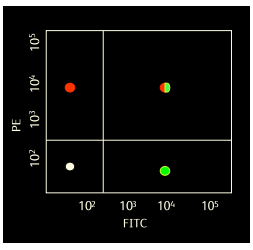
Fluorescent staining with monoclonal antibody



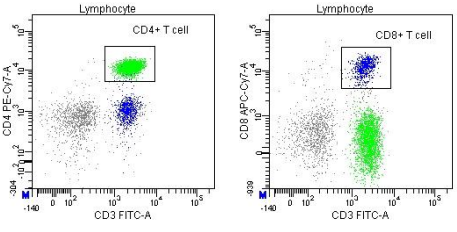
CD # = cluster of differentiation
MAb = monoclonal antibody

Fluorescent signal

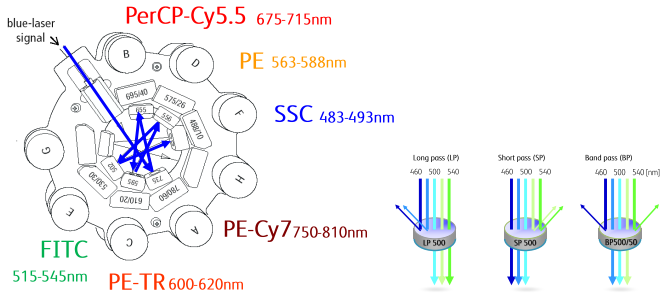




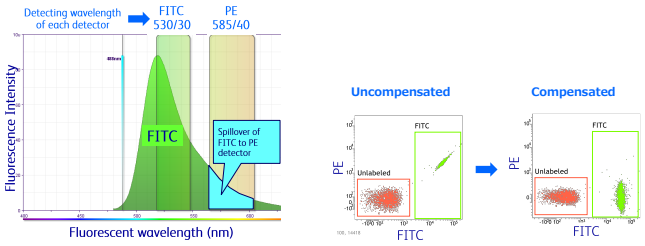
Fluorescent signal analysis



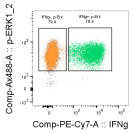
Fluorescence detector of FCM

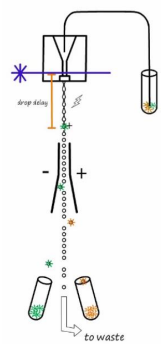


Compensation: Separating fluorochromes with spillover



Sorting Mechanism





1. Detect cells by scatter and fluorescence signals
2. Identify target cells using gating criteria
3. Generate droplets containing individual cells
4. Charge target droplets at droplet breakoff
5. Deflect charged droplets into collection tubes using an electric field
6. Collect purified cell populations for downstream analysis

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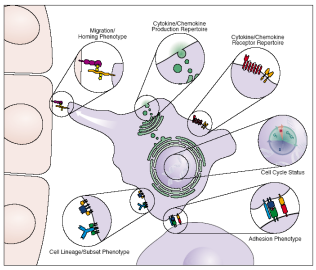
Introduction to Flow Cytometry (FCM)

1. Basic of FCM
2. Application for FCM

Applications for FCM

① Cell surface antigen
② Intracellular antigen
③ Fluorescent protein
④ Cell cycle
⑤ Cell proliferation
⑥ Apoptosis
⑦ Cytokine



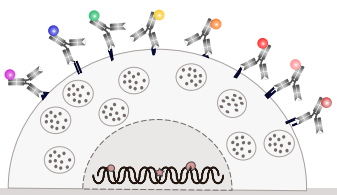
① Analysis of cell surface antigen

Major cell surface antigen

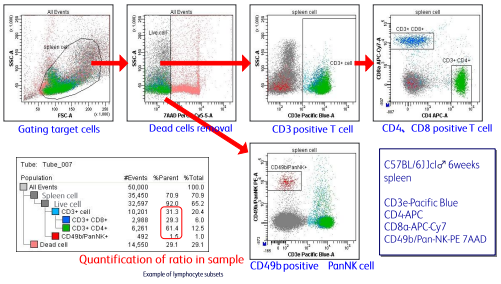
- ✓ Cellular membrane composing protein
- ✓ receptors
- ✓ Adhesion molecules
- ✓ Transporters

Cluster of Differentiation (CD classify)

International classification of monoclonal antibodies used for cell surface antigen



Analysis of cell surface antigen

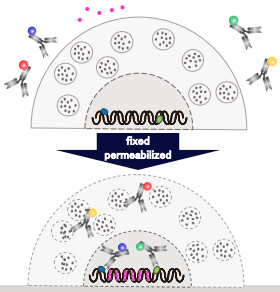


② Analysis of intracellular antigen

- Intracellular antigens
  - Cytokine
  - Transcription factor
  - cytoskeleton

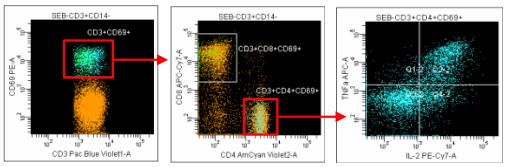
Cells fixed and permeabilized makes antibody and reagents possible to access intracellular

- Fixation reagent
  - Ethanol
  - Methanol
  - Paraformaldehyde
- Permeabilization reagent
  - Saponin
  - Triton-X
- Nucleic acid staining reagent
  - PI
  - 7AAD



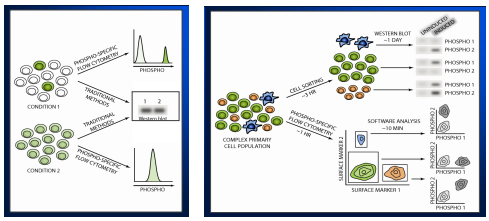
Cytokine producing cells analysis

- Staining with cell surface antigen, enables phenotype analysis of cytokine producing cells
- Possible to detect cytokine production even from rare cells

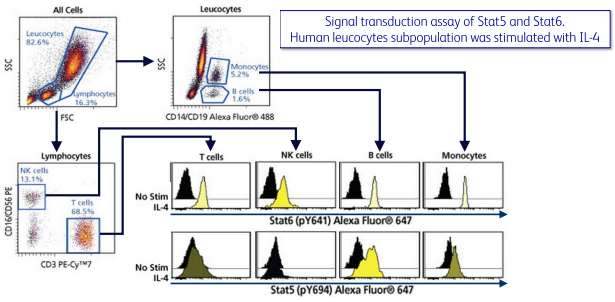


Protein phosphorylation analysis in single cell

- Protein phosphorylation in single cell level
- Combining the staining of cell membrane markers, allows protein phosphorylation analysis of specific cells in heterogeneous cell population
- Easy, short time and less cell sample compared to Western Blot analysis

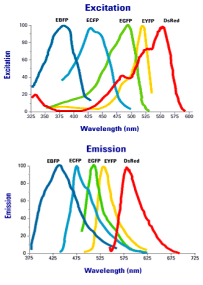


Protein phosphorylation analysis in single cell

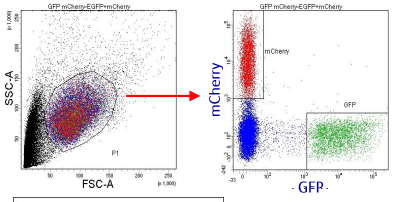


③ Expression of fluorescent protein

- Purpose
  - Transgene expression
  - Transplanted cells survival
  - Interaction between transgene
- Fluorescent proteins
  - GFP series fluorescent protein
    - GFP, YFP, CFP, RFP, BFP etc.
  - Fruits series fluorescent protein
    - mCherry, mPlum, mStrawberry, mBanana etc.
  - CoralHue series fluorescent protein
    - Midoriishi-Cyan, Kusabira-Orange, Azami-Green, Keima-Red, Kaede, Doronpa-Green etc.



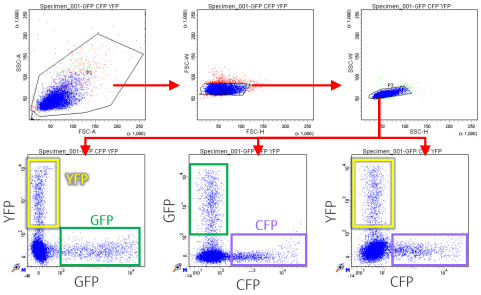
GFP / mCherry Expressing cell analysis



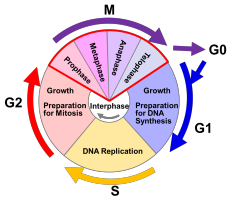
Tube: EGFP+mCherry			
Population	#Events	%Parent	%Total
All Events	38,464	####	100.0
P1	19,035	49.5	49.5
GFP	3,271	17.2	8.5
mCherry	4,295	22.6	11.2

To measure mCherry, FCM equipped with YG Laser is desirable

GFP / YFP / CFP Expressing cell analysis



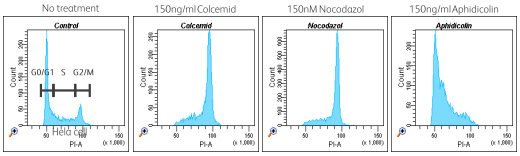
④ Cell cycle analysis



Cell cycle	Marker/reagent	mechanism
All	PI、7-AAD	interacts with double stranded nucleic acid
S phase	BrdU/anti-BrdU Ab	BrdU replaces Thymidine(T) on DNA synthesis, BrdU is detected with Ab
G0/G1 phase	Anti-Cyclin Ab, anti-Rb Ab markers for cell cycle	Amount changes in the phases
M phase	Anti-Phospho-HistonH3 antibody	changes in phosphorylation

④ Cell cycle analysis : PI analysis

- PI staining cell cycle analysis
  CycleTEST™ PLUS DNA Reagent Kit (Cat#340242)



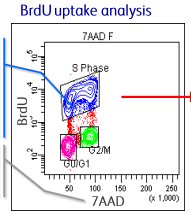
Hela cells cultured 48hr in DMEM with 0.5% FBS were treated with reagents.
After 16hr culture, cell cycle analysis was performed

④ Cell cycle analysis : BrdU / 7AAD analysis

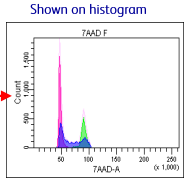
BD Pharmingen™ FITC BrdU Flow Kit (Cat#559619)

- BrdU is uptaked in DNA in S phase cells
- BrdU incorporated cells are detected by anti-BrdU Ab
- Total amount of DNA per nuclei is detected by 7AAD

BrdU uptake analysis



Shown on histogram



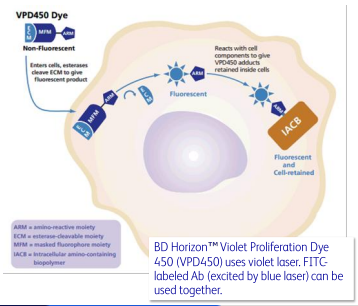
Jurkat cells stained with BrdU Flow kit and 7AAD

⑤ Cell proliferation assay

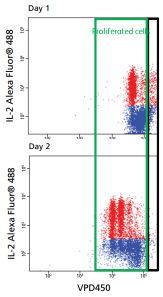
BD Biosciences offers BD Horizon™ Violet Proliferation Dye 450 (VPD450) and BD Horizon™ CFSE for the detection of cell proliferation. Once the dye is inside the cell, esterases cleave off the ester group to convert the dye into a fluorescent product and trap it inside the cell. With each replication event the amount of dye in the cell is decreased, leading to a characteristic pattern.

Number of division	NA	0	1	2	N
Fluorescence intensity	NA	100%	50%	25%	[100/2^N]%
Cells	Unlabeled cell	Label / Labed cell	division		. . .

⑤ Cell proliferation assay: VPD450 assay



BD Horizon™ Violet Proliferation Dye 450 (VPD450) uses violet laser. FITC-labeled Ab (excited by blue laser) can be used together.

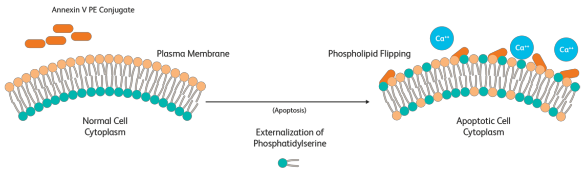


⑥ Apoptosis analysis

Apoptosis stages

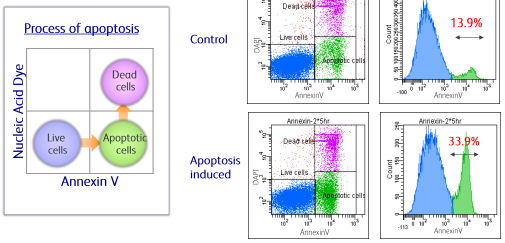
Feature Measured	Assays	Key Features
Plasma Membrane Alterations (Phosphatidylserine Exposure)	Annexin V binding assay • Single conjugates • Annexin V Kits	• Detects early apoptosis markers • Quick and easy • Flow cytometry or immunofluorescence application
Mitochondrial Changes	• BD MitoScreen Kit	• Fast, easy, single cell resolution by flow cytometry or fluorescent microscopy
Caspase Activation	• Caspase Activity Assay Kits and Reagents	• Quick and easy, uses spectrofluorometry
	• Active Caspase -3 immunoassays (ELISA)	• ELISA application
DNA Fragmentation	• APO-BrdU TUNEL Assay • APO-DIRECT TUNEL Assay	• Works adherent cells, single cell resolution in conjunction with cell cycle analysis by flow cytometry

Apoptosis analysis: Annexin V assay for Surface Phosphatidylserine detection



Phosphatidylserine (PS), which is normally located on the cytoplasmic face of the plasma membrane, translocates to the outer leaflet of the plasma membrane during apoptosis and can be detected by flow cytometry and cell imaging through binding to fluorochrome-labeled annexin V when calcium is present.

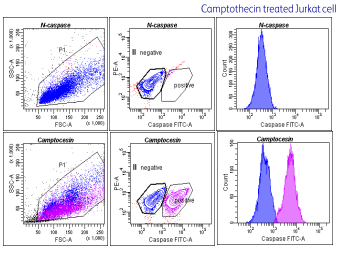
Apoptosis analysis: Annexin V assay



Apoptosis analysis: Active Cleaved Caspases and Cleaved poly-ADP ribose polymerase (PARP) detection

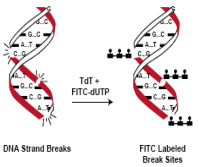
Isotype Control

FITC labeled Anti-Cleaved Caspase-3 Ab

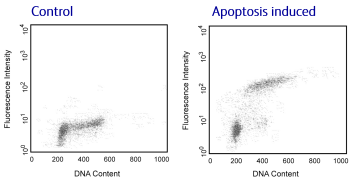


Caspases are activated upon during the earliest stages of apoptosis. Active caspases can then cleave many proteins, including PARP. This leads to the loss of cellular structure and function, and ultimately results in cell death.

Apoptosis analysis: Apo-Direct to detect DNA damage



One of the later steps in apoptosis is DNA fragmentation. A method which is often used to detect fragmented DNA utilizes a reaction catalyzed by exogenous TdT, often referred to as "end-labeling" or "TUNEL" (terminal deoxynucleotidyltransferase dUTP nick end labeling).

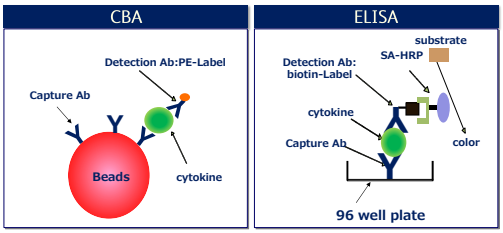


The APO-DIRECT™ assay is a single-step method for labeling DNA breaks with FITC or APC-dUTP.

⑦ Cytokine measurement

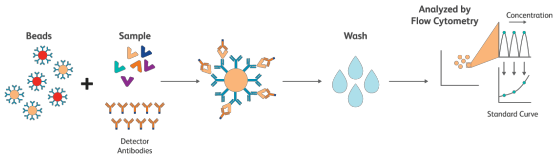
method	object	measurement	instrument
ELISA (OptEIA)	Cytokine concentration in solution	Antibody and enzyme reaction in 96 well plate to color	Plate reader
Intracellular Cytokine	Cells producing cytokine	Stain cytokine with Ab within cells	FCM
ELISPOT	Cells secreting cytokine	Antibody and enzyme reaction in 96 well plate to color	Microscope
Cytometric Beads Array (CBA)	Cytokine concentration in solution	Use special beads to measure cytokine	FCM

Cytometric Beads Array (BD™ CBA)



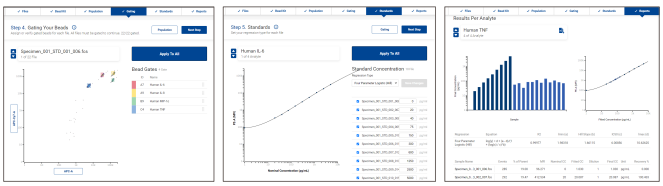
BD™ CBA is a reagent to measure multiple cytokines in a single tube using FCM.

Cytometric Beads Array (BD™ CBA)



	BD™ CBA	ELISA
Multiplexing Capability	Up to 30 proteins /sample	one protein /sample
Simple procedure and Time Efficiency	Wash once	Wash more than 10 times
Sample amount	50 uL or less	About 100 uL
Format	From single tube	96 well plate
Required Instrument	Flow cytometer	Plate reader

Cytometric Beads Array (BD™ CBA)



Capture beads for each different target protein are distinguished by unique fluorescence intensity of beads. The PE intensity of each capture bead population reflects the target protein concentration. Standard curves are plotted using titrated standard protein samples.

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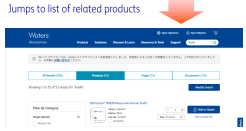

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