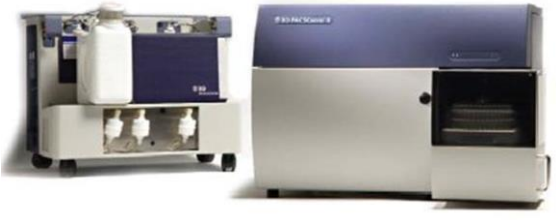
BD FACSCantoII
數據分析與應用操作



Grace Lin 林秀鴻
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Outline

- 數據分析(Diva)
- 多色分析範例與數據管處理
- Cytometric Beads Array(CBA)原理

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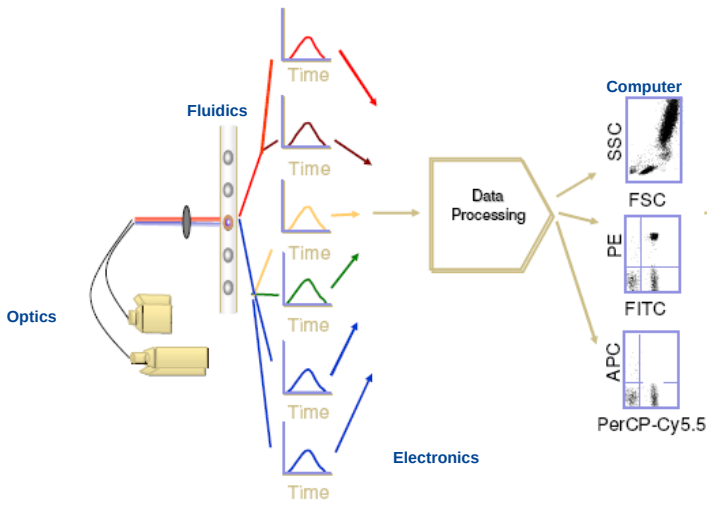
Overview of Flow Cytometry



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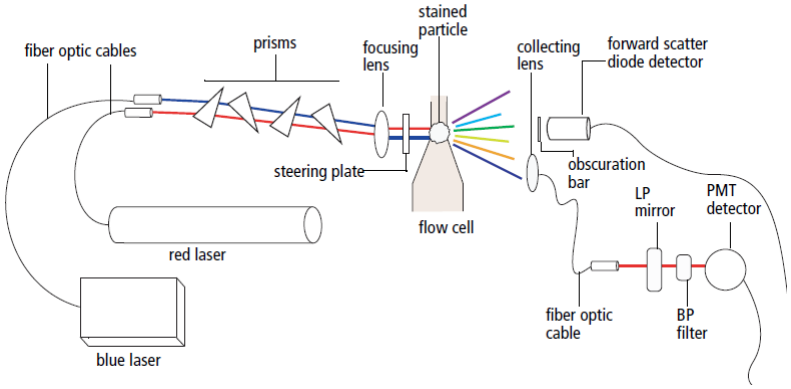
Optics pathway of FACSCanto II

Figure A-3 Light pathway through the BD FACSCanto flow cytometer



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Optics 4-2-2 CantoII

Laser	Wavelength (nm)	Commonly Used Fluorochromes
Coherent® Sapphire™ Solid State	488 (blue)	FITC, PE, PerCP, PerCP-Cy™5.5, PE-Cy™7
JDS Uniphase HeNe Air-Cooled	633 (red)	APC, APC-Cy7, APC-H7
Point Source™ iFLEX 2000™-P-1-405-0.65-30-NP	405 (violet)	Pacific Blue™, AmCyan, V450, V500

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Detector Array (Laser)	PMT Position	LP Mirror	BP Filter or LP Mirror	Intended Dye
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	655	670	PerCP-Cy5.5 or PerCP
	C	610	Blank optical holder	—
	D	556	585/42	PE
	E	502	530/30	FITC
	F	Blank optical holder	488/10 and pinhole	SSC
	G	Blank optical holder	Blank optical holder	—
	H	—	Blank optical holder	—
Trigon (633-nm red laser)	A	735	780/60	APC-Cy7 or APC-H7
	B	685	Blank optical holder	—
	C	—	660/20	APC
Trigon (405-nm violet laser)	A	502	510/50	AmCyan or V500
	B	Blank optical holder	450/50	Pacific Blue™ or V450
	C	—	Blank optical holder	—

Make sure your panel design or fluorescent dye is match to detector

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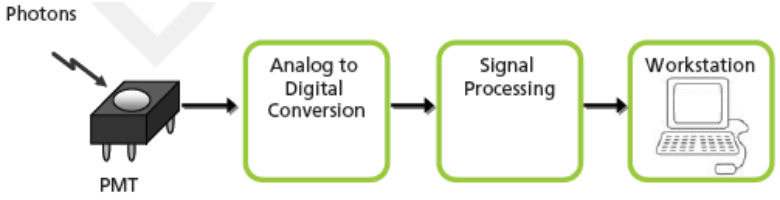
Electronics



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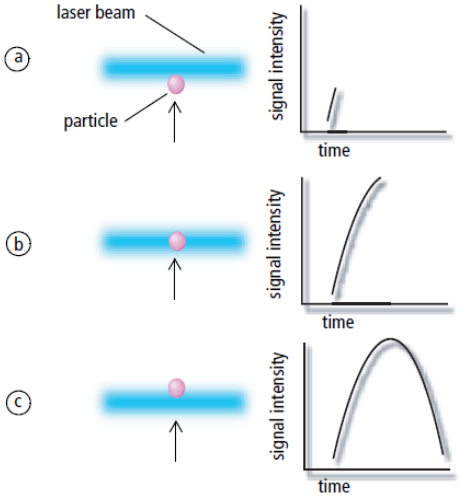
Voltage Pulse generation



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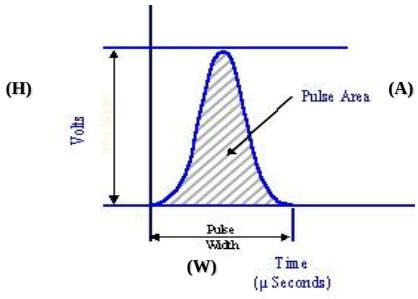
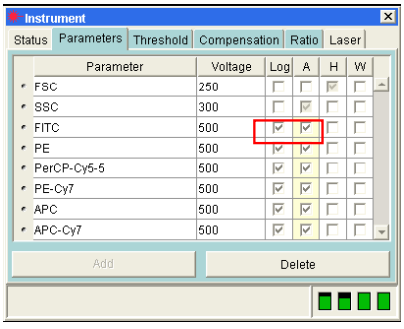
Quantification of a Voltage Pulse





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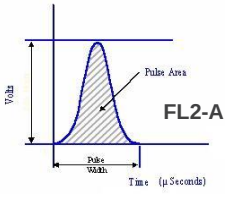
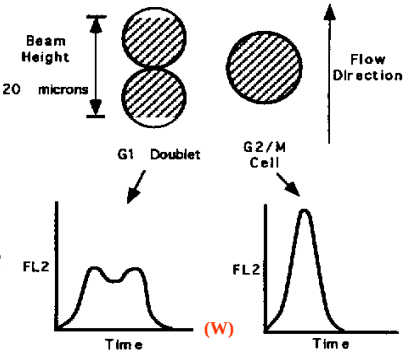
單顆細胞圈選原理



FL2-W

FL2-W 為細胞經過雷射所需飛行時間, 飛行時間與**FL2-A**的關係不是線性者(**FL2-W**訊號異常高)則為多聯體細胞不可圈選。



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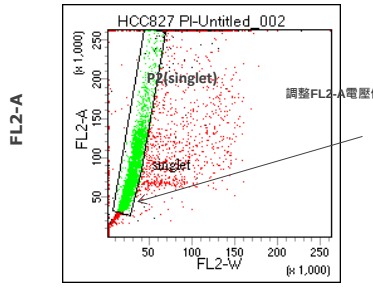
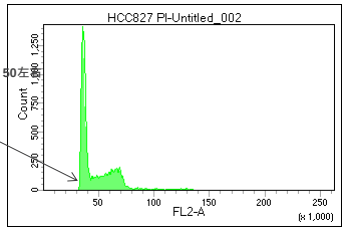
Singlet gating with W and A



FL2-W

Figure 1.
FL2-Width 對 **FL2-Area** 散點圖顯示單顆細胞圈選, 以排除多聯體細胞
。

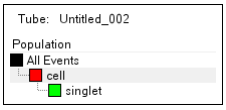
調整FL2-A電壓值讓第一個波峰落在FL2-A 50左右



FL2-A

Figure 2.
排除多聯體後, 留下單顆細胞的**DNA**含量直方圖

直方圖設定show P2(singlet)



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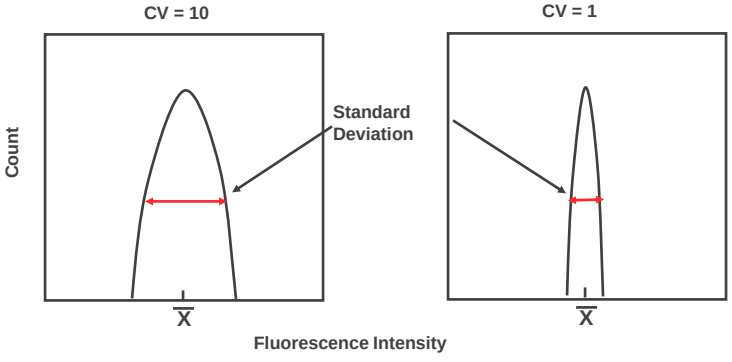
Coefficient of Variation (CV)

$$\%CV = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$



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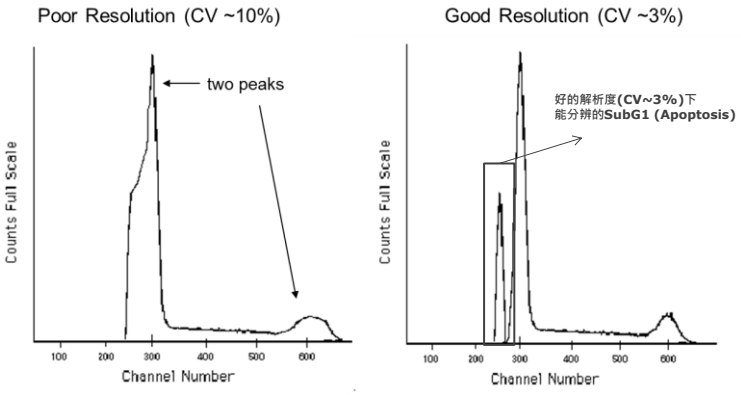
CV affect data quality



高流速會造成數據的CV變高，較差的CV會影響數據的品質，造成不同時期細胞的比例誤差大於真正的差異性，做細胞週期實驗時必須用低流速以獲得較佳的CV值

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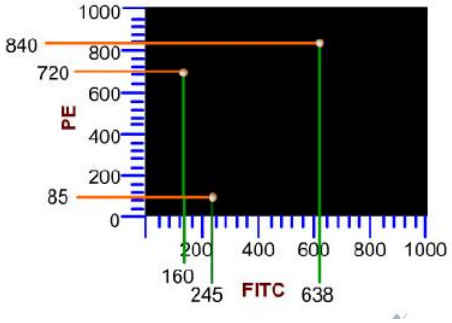
List-Mode Data

	FSC	SSC	FITC	PE
event 1	30	60	638	840
event 2	100	160	245	85
event 3	300	650	160	720



Resolution of Diva =2^{18}

26-262143(4 log) 2-262143 (5 log)

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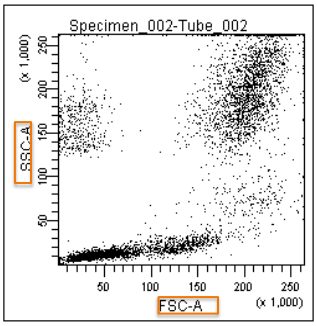
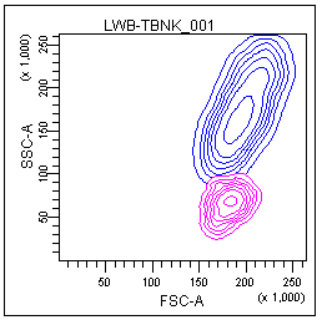


Dot Plot

Each dot is an event
Could observe 2 parameter simultaneously



Contour Plot

Provide a third dimension by joining x- and y-coordinates with similar event counts

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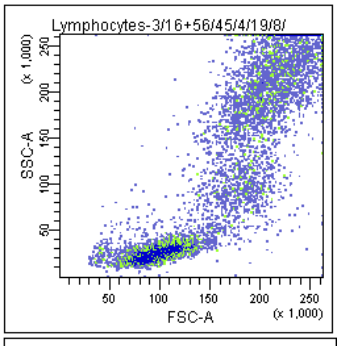
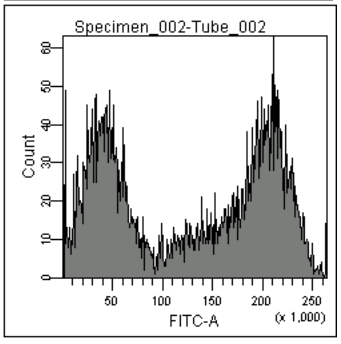


Density Plot

Density plots are similar to dot plots, except colors are used to represent the accumulation of events



Histogram Plot

Graphical representation of single-parameter data, where the horizontal axis represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count)

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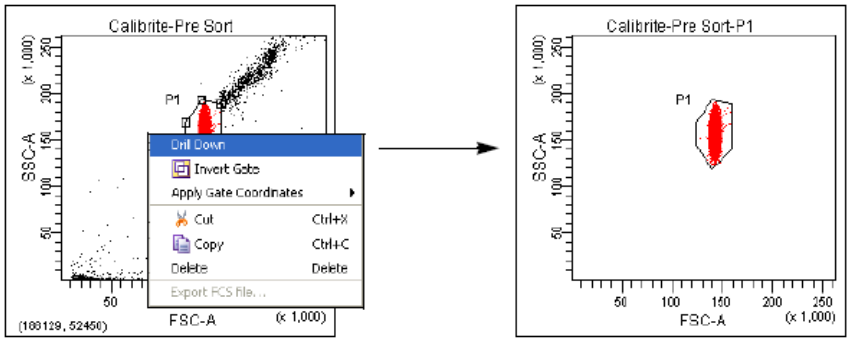
Drill Down 功能:可利用於複雜多色分析
將欲分析的gate選起來, 右鍵>Drill Down
自動產生一新的dot plot 已設定為show 該population
將X軸與Y軸改成欲分析該群的參數即可



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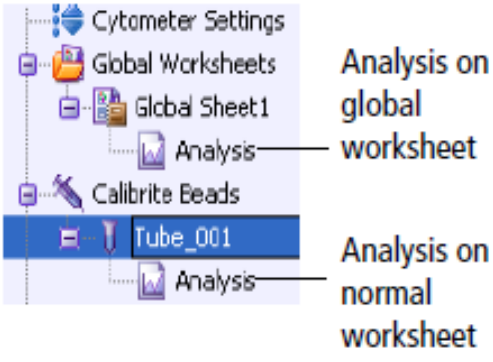
Global worksheet and Normal worksheet



Global worksheet
Show active tube data

Normal Worksheet
Can show different tube data at the same time

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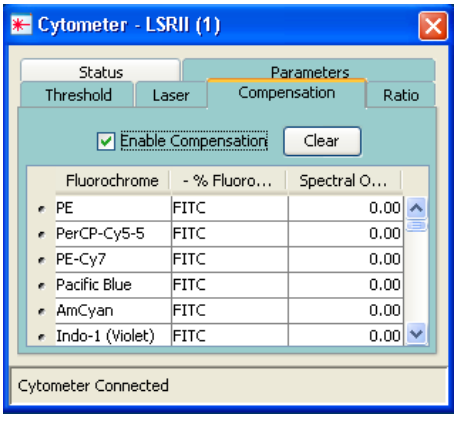
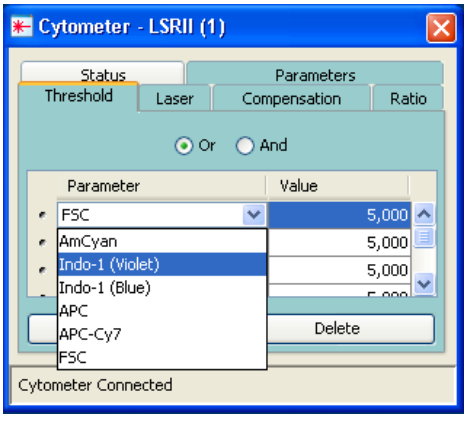
Cytometer Setting>Compensation and Threshold setting





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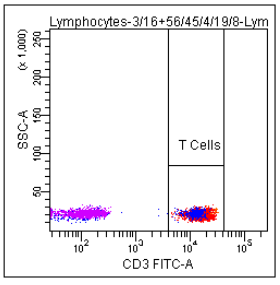
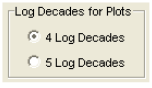
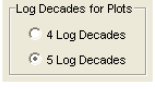
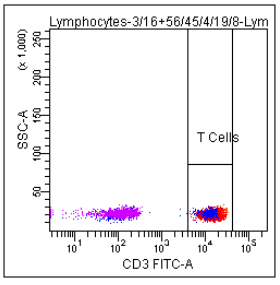
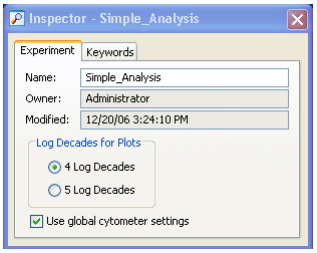
4 log and 5 log display











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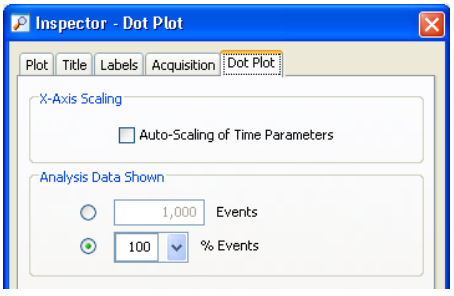
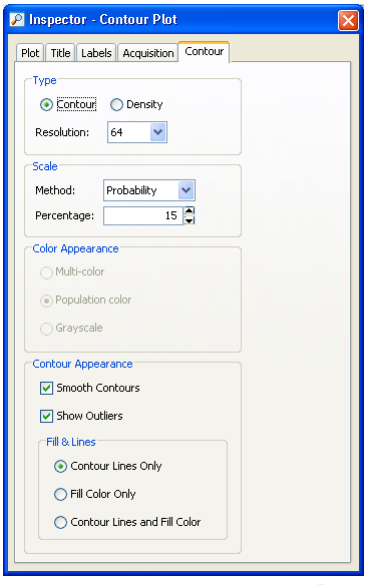
Display setting of plot





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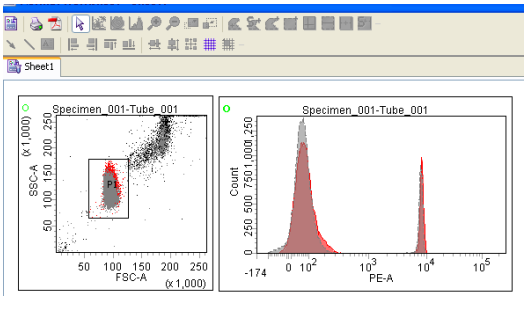
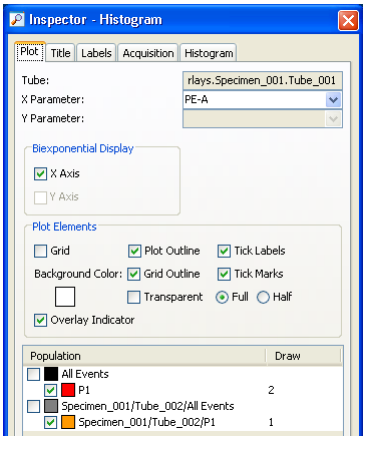
Overlay



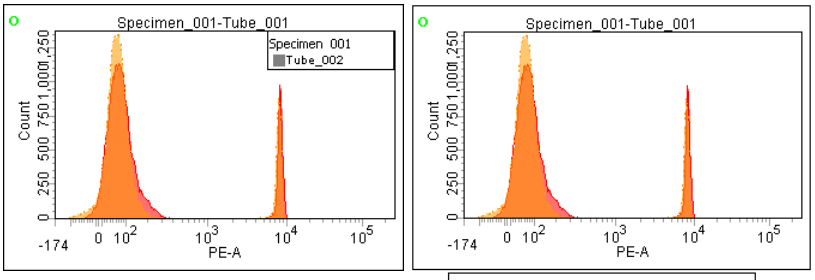


On Normal worksheet
Drag one or multiple tube from Browser over an existing plot

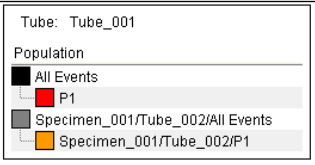
Use Inspector to choose which population should be shown

BD

Overlay

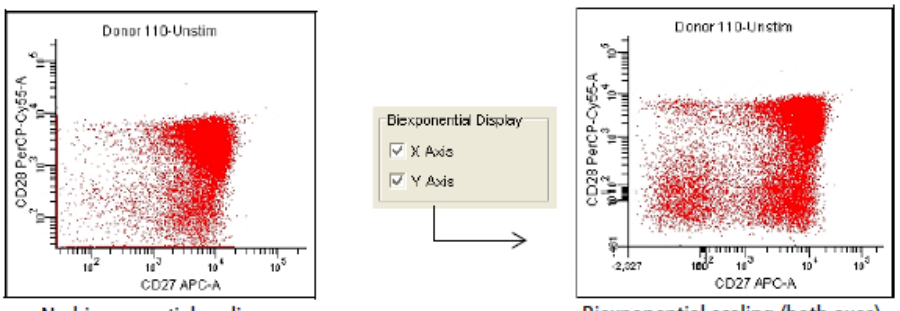


Right-click on overlay plot >Show legend



Use population hierarchy show more information

Biexponential Scaling

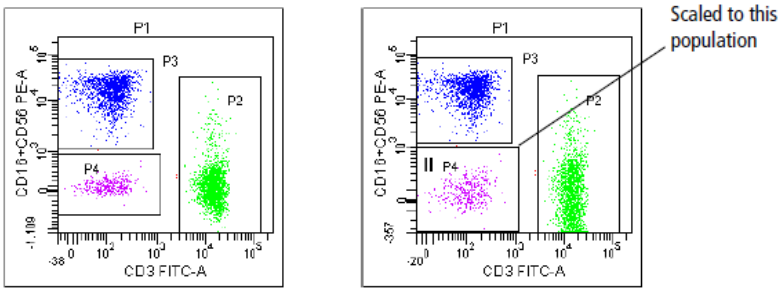


No biexponential scaling

Biexponential scaling (both axes)

Show dim population by present data in linear scale, strong signal remains in log scale

Scaled to assigned population



To return to automatic scaling, right-click the plot and select Scale to Population > All Events.

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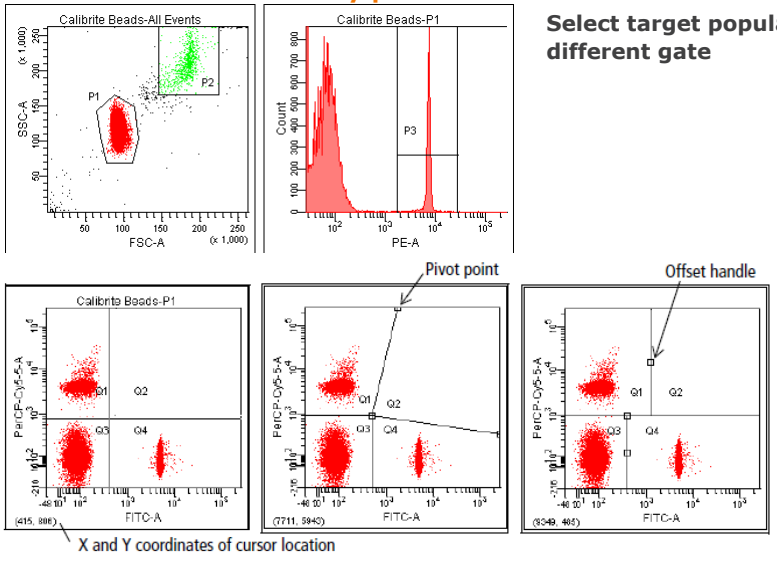
Different Gate Type



Select target population by different gate

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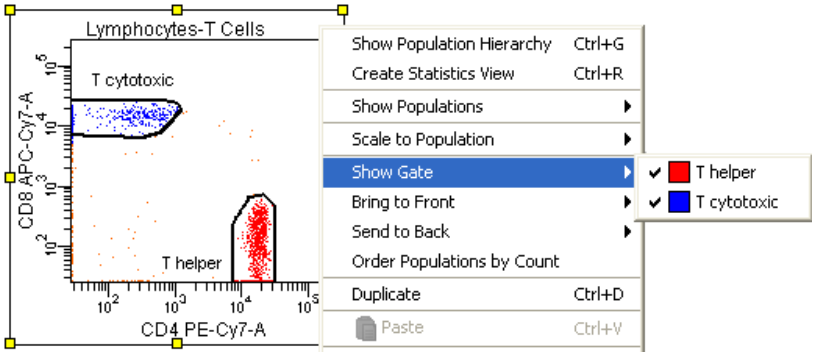
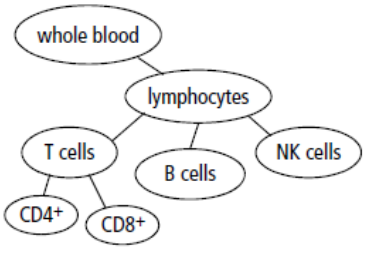
Show or Hide Gate



When the gate is showing, hide menu will show up
When the gate is hiding, show Gate menu will show up

Gate Hierarchy



Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
Lymphocytes	3,373	33.7	33.7
T cells	1,822	54.0	18.2
CD4+	1,282	70.4	12.8
CD8+	339	18.6	3.4
B cells	198	5.9	2.0
NK cells	1,243	36.9	12.4

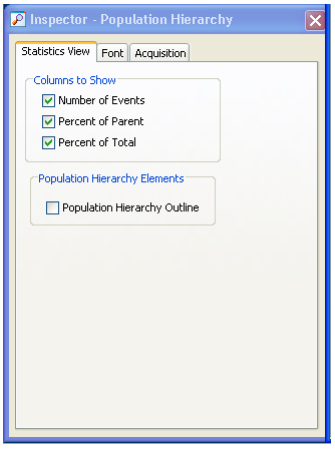
Right-click a tube or plot and select Show Population Hierarchy to understand the logic relationship between different population

Gate Hierarchy

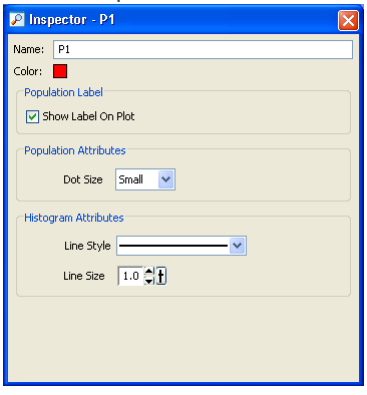
Tube: Tube 1

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	3,612	36.1	36.1
P2	342	9.5	3.4

Population Hierarchy Inspector



Gate Inspector



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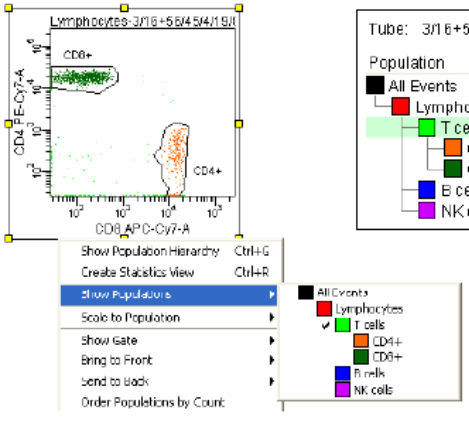
Gate Hierarchy-Creating population subsets



Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
Lymphocytes	3,373	33.7	33.7
T cells	1,822	54.0	18.2
CD4+	1,282	70.4	12.8
CD8+	338	18.6	3.4
B cells	198	5.9	2.0
NK cells	1,243	36.9	12.4

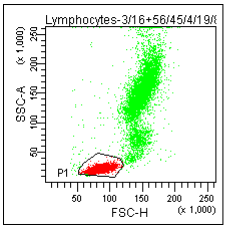
T-cell subsets

Multicolor analysis

Drill Down will create a new plot, while show population change the population the plot show without create new plot

BD

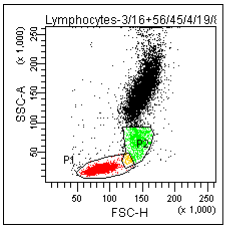
Boolean Logic 布林邏輯



Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
P1	3,373	33.7	33.7
NOT(P1)	6,627	66.3	66.3

Not



Tube: 3/16+56/45/4/19/8

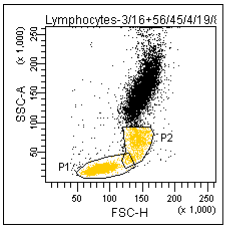
Population	#Events	%Parent	%Total
All Events	10,000		100.0
P1	3,495	34.9	34.9
P2	844	8.4	8.4
P1 AND P2	85	0.9	0.9

And

Ctrl+P1+P1 then right-click

BD

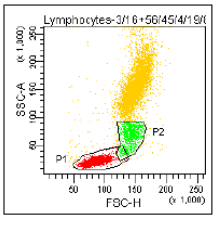
Boolean Logic 布林邏輯



Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
P1	3,495	34.9	34.9
P2	844	8.4	8.4
P1 OR P2	4,254	42.5	42.5

Or



Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
P1	3,495	34.9	34.9
P2	844	8.4	8.4
Rest of All Events	5,746	57.5	57.5

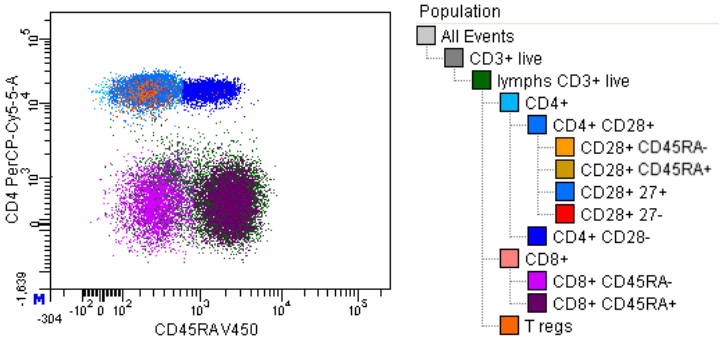
"Rest of" gate

Rest of

Only available when there are subset population already

BD

Multicolor Research



BD

Appropriate Controls

What	Why
Cytometer setup controls • BD CompBeads	Ensure consistent setup and compensation
Gating controls • Isotype • FMO	Obtain reliable gates for problem markers
Biological controls • Unstimulated samples • Healthy donors	Make appropriate biological comparisons and conclusions

BD

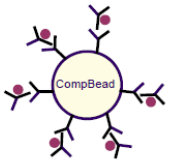
Compensation control

- The fluorescence emission spectrum of compensation controls must match the experiment reagents—this is especially critical with tandem reagents.
- Compensation controls must be bright enough to resolve positive and negative peaks.
- Compensation control negative and positive populations must be from the same cell or particle type. Example: don't use a $CD3^-$ monocyte negative population with a $CD3^+$ lymphocyte positive population.
- Whenever PMT voltages change, rerun compensation.
- Use biexponential display when adjusting compensation.
- Use CompBeads if applicable.

BD

BD CompBeads/CompBeads Plus

A **convenient** way to create **effective** single-color compensation controls

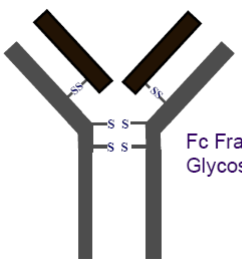


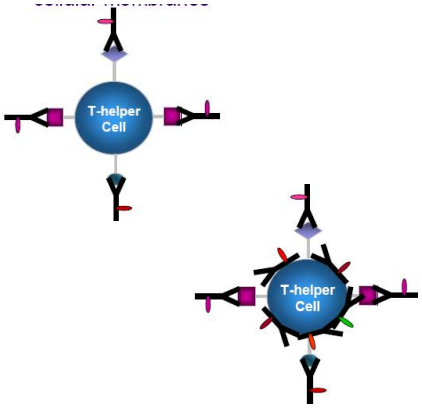
- Use the same antibodies as in the experimental samples.
- Create bright and uniform positive fluorescence peaks.
- Avoid using limited sample.
- Mimic autofluorescence background of lymphocytes/cell lines
- Beads are coated with anti-mouse, anti-rat, and anti-hamster kappa.

BD

Antibody Isotypes

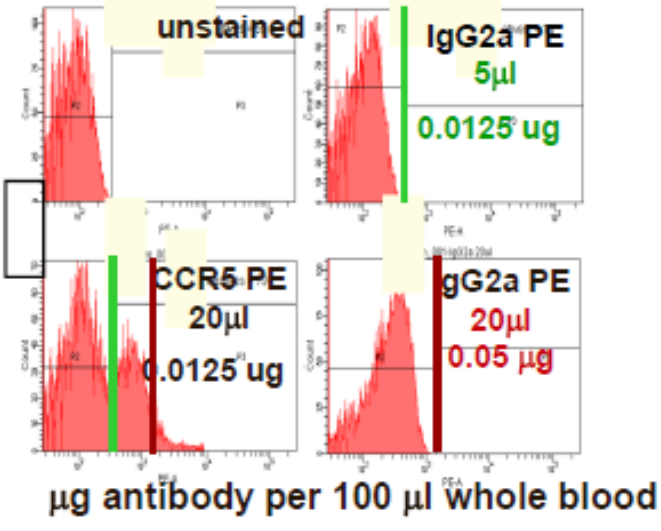
Isotype controls resemble nonspecific binding of antibodies to cellular membranes





BD

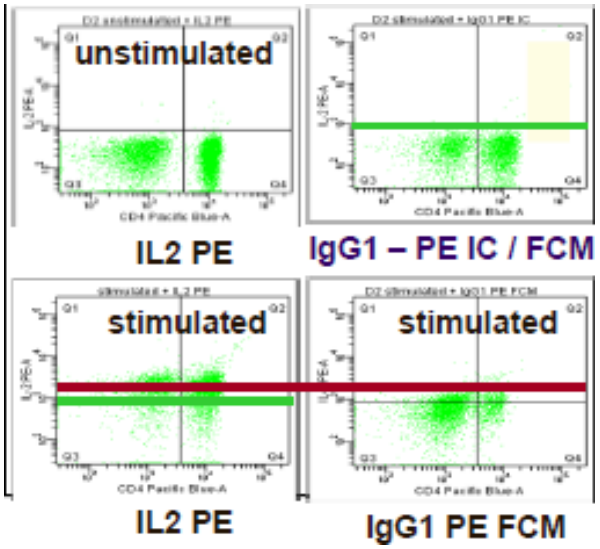
Isotype control_Concentration dependent



µg antibody per 100 µl whole blood

BD

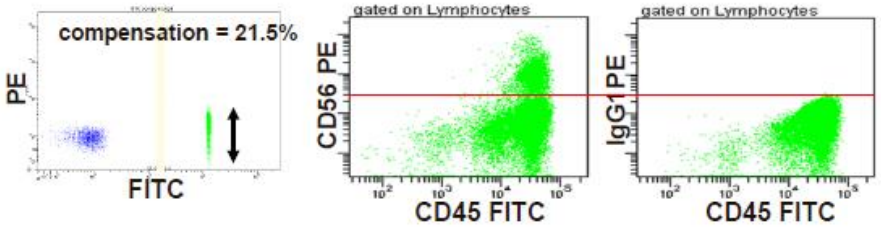
Isotype control Preparation dependent



BD

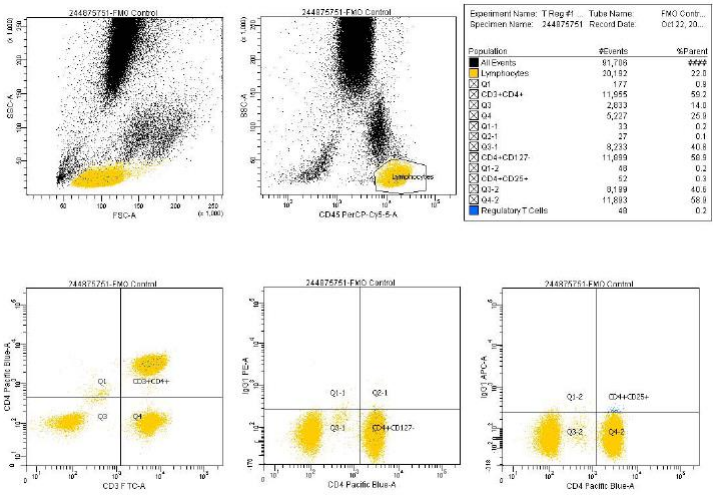
Fluorescence Minus One (FMO)

- **Necessary control if fluorochrome combinations on one cell show reduced resolution between negative / positive population due to strong data spread**

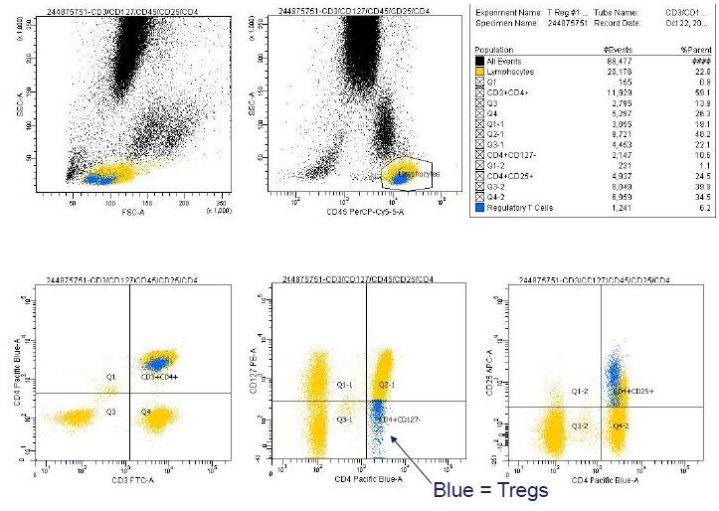


BD

FMO Example -- Treg

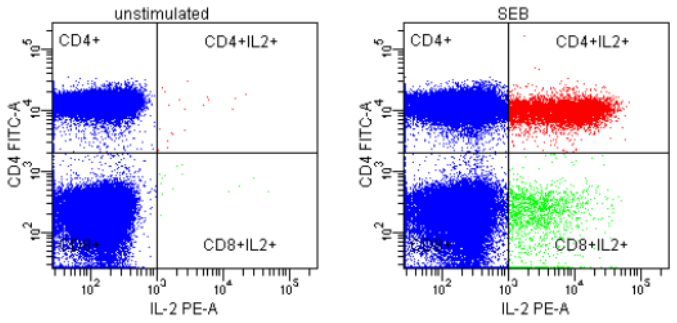


FMO Example -- Treg



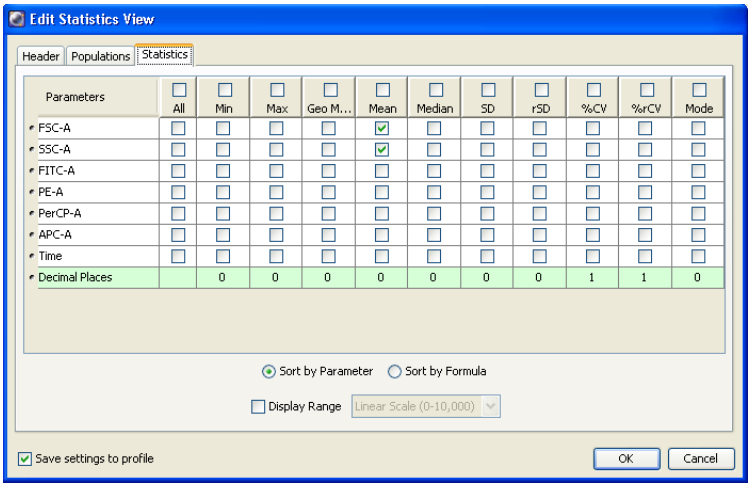
Biological Controls

If possible, an internal control which will not bind to specific antigens is the best!



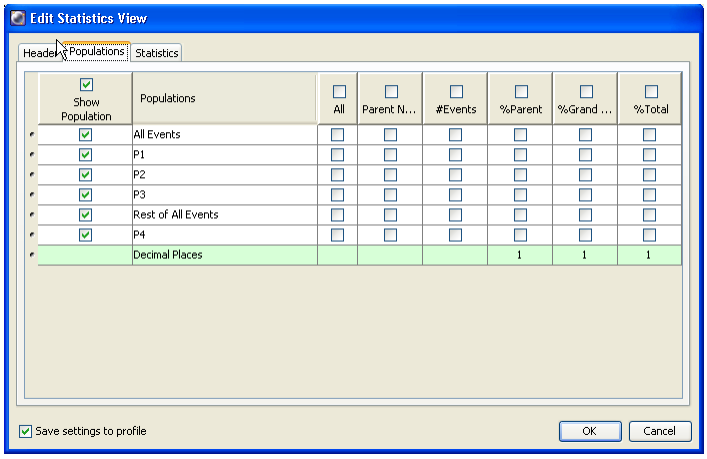
BD

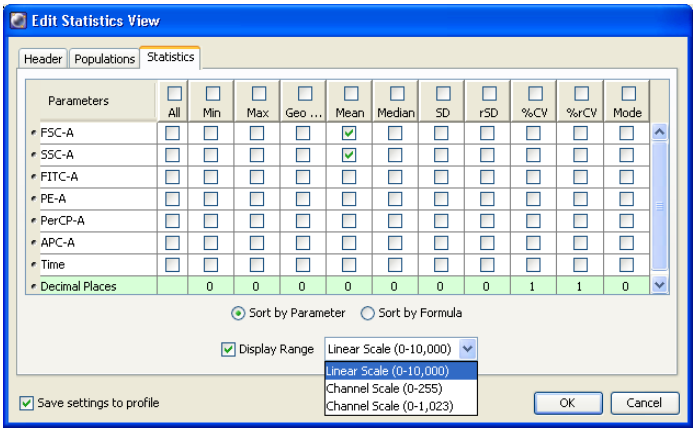
Create Statistics View
Right-click on stats>Edit Statistics View>Statistics



BD

Create Statistics View
Right-click on stats>Edit Statistics View>Population





Experiment Name:	Blank Experiment with Sample Tube
Specimen Name:	Specimen_001
Tube Name:	Tube_001
Record Date:	Oct 31, 2006 3:31:33 PM
$OP:	Administrator
GUID:	e41a8ebe-9ffc-487d-9fbd-4426cab43dbd
Display Range	Channel Scale (0-1,023)

Population	#Events	%Parent	FITC-A Mean
P1	5,035	50	375
P2	4,965	50	887
Delta			512

When Display Range is selected and the statistics view has two populations displayed, delta values are displayed. The delta values show the absolute difference between two statistics (for example, between the P1 mean and P2 mean.)

Mean. 算術平均數 $\bar{X} = \left(\sum_{i=1}^{n} X_i\right)/n$

Average linear value for events in the defined population

Geometric mean. 幾何平均數 $\bar{X}geo = 10^{\left(\sum_{i=1}^{n} \log X_i\right)/n}$

Logarithmic average of the events in the defined population

較不受極端值影響但無法用在含有0或負數的數據

Median. 中位數

Linear value with an equal number of values above and below it.

Min. 最小數

Minimum linear value within a defined population

Max. 最大數

Maximum linear value within a defined population

BD

Standard deviation (SD) 標準差

$$SD = \sqrt{\sum_{i=1}^{n} (X_i - \bar{X})^2 / (n-1)}$$

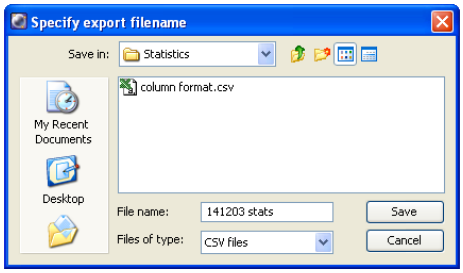
%Coefficient of variation (%CV) 相關係數

The SD divided by the mean within a defined population, expressed as a percentage

$$\text{Percent CV} = (SD/\bar{X}) \times 100$$

BD

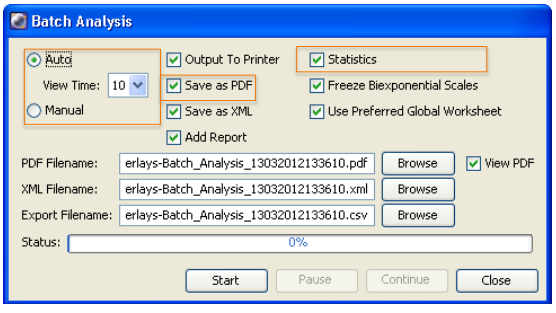
Export Statistics



Select one or more statistics views.
File > Export > Statistics.

locate and select the file, and click **Save.**
When prompted, click **Append.**
Results will be appended to the selected file

Auto-export Statistics



Select item (experiment or specimen or tube)
Right-click>Batch Analysis

FCS file from other application software

- Linear data saved in FCS format from another software application is normalized to the range of BD FACSDiva linear data using the following formula:

$$X_{LIN} = X_{FCS} \times \frac{2^{18}}{\text{resolution}}$$

Where X_{LIN} = linear BD FACSDiva data, X_{FCS} = linear data in FCS file, resolution = resolution in FCS file (256, 1024, or 262,144)

- Log data imported from FCS files is converted to linear data using the following formula:

$$X_{LIN} = 10^{\frac{x_{FCS} \times \#dec}{res}} \times \frac{2^{18}}{10^{\#dec}}$$

Where X_{LIN} = linear BD FACSDiva data, X_{FCS} = log data in FCS file, #dec = number of decades in FCS file (usually four), res = resolution in FCS file (256 or 1024)

BD

利用流式細胞偵測細胞激素 -微珠免疫分析。

BD Senior Product Specialist

Grace Lin 林秀鴻

2016/0314

BD

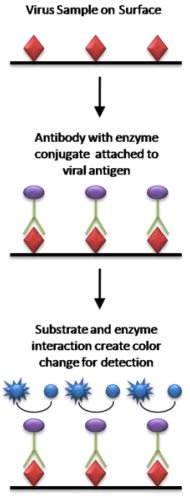
Multiple assay platforms for Protein Quantitation

- ELISA (Enzyme-Linked ImmunoSorbent Assay)
- ELISPOT (Enzyme-Linked ImmunoSpoT assay)
- **Bead-Based Immunoassay**
  - **Cytometric Bead Array**
  - Multiplex
  - …etc.

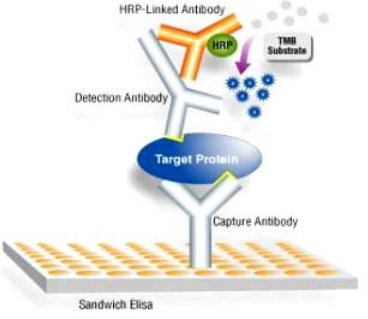
BD

ELISA

Direct



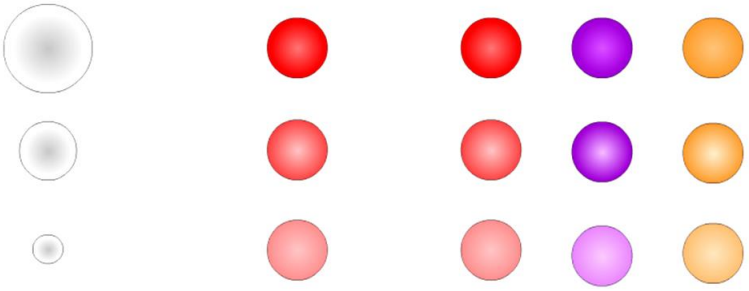
Sandwich



BD

Bead Provide a Flexible Platform

Beads provide an expandable assay platform for use with a flow cytometer



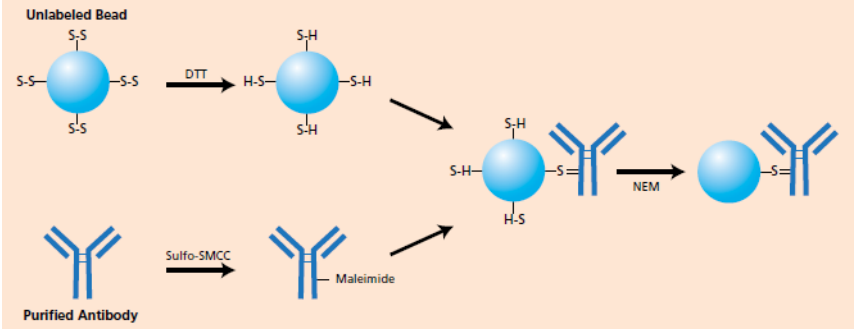
Multiple sizes

Different intensities

Different colors with different intensities

BD

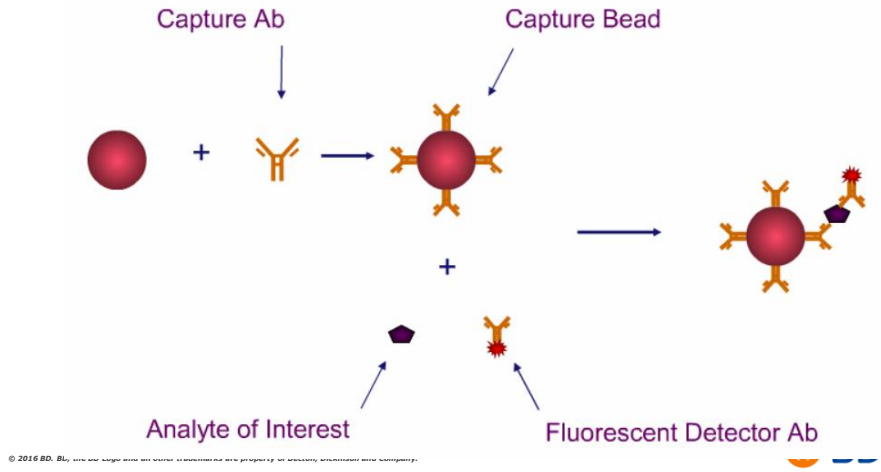
How A Bead-based Immunoassay Works



DTT, Dithiothreitol
Sulfo-SMCC, Sulfosuccinimidyl 4-N-maleimidomethyl cyclohexane 1-carboxylate
NEM, N-Ethylmaleimide

BD

How A Bead-based Immunoassay Works



Features

- Evaluation of multiple analytes in a single sample.
- Utilization of minimal sample volumes to glean data.
- Reproducibility and results comparative with previous experiments.
- Direct comparison with existing assays.
- More rapid evaluation of multiple samples in a single platform.

Ref.
Am J Clin Pathol 2002;118:346-353
Curr Protoc Cytom. 2006 Feb;Chapter 13:Unit13.1
Clinical Immunology Volume 110, Issue 3, March 2004, Pages 252–266

Analytes

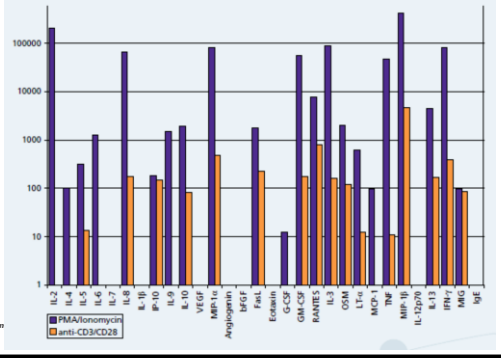
- Inflammatory mediators
- Chemokines
- Immunoglobulin isotypes
- Intracellular signaling molecules
- Apoptotic mediators
- Adhesion molecules
- Antibodies
- …..etc.

Ref.
Am J Clin Pathol 2002;118:346-353
Curr Protoc Cytom. 2006 Feb;Chapter 13:Unit13.1
Clinical Immunology Volume 110, Issue 3, March 2004, Pages 252–266

一根釣竿就可以釣起所有想要的魚

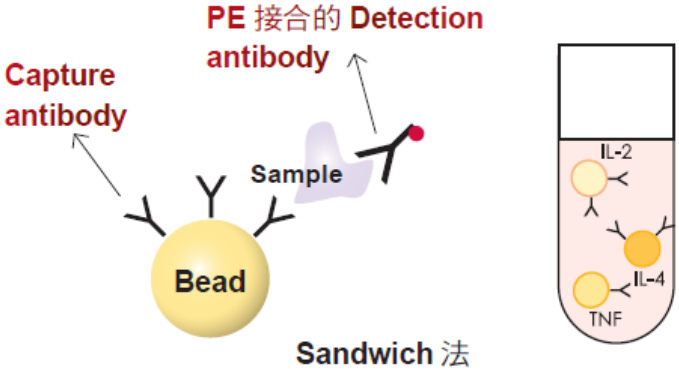
BD™ Cytometric Bead Array (CBA)

- Flow cytometry application
- Bead-Based Immunoassays
- Multiplexed quantitation and detection
- Up to 30 proteins can be analyzed using just 25 to 50 µL of sample.



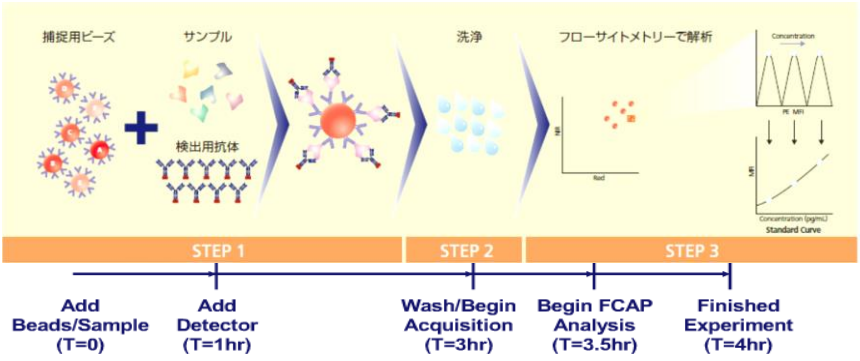
BD™ CBA Flex Set を用いたタンパクレベル解析
ヒト末梢血単核細胞（PBMC）を異なる2 つの条件下で刺激し、上清を回収後 BD™ CBA Flex Set assay（ 30 plex ）で測定した・

Assay

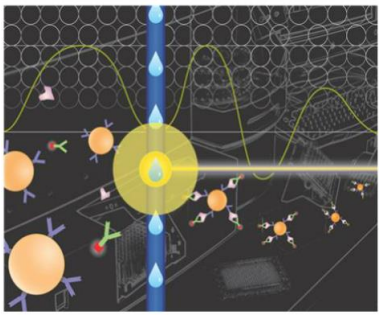


BD

Assay Procedure



BD



BD CBA : designed for easy and efficient multiplexing

- Analyzed multiple analytes simultaneously.
- Reduced sample volume requirement.
- Reduced hands-on time by parallel analysis of samples.
- Wide dynamic range of fluorescence detection. (requires fewer sample dilutions)
- Offer automated sample acquisition and increased throughput.

BD

Product Format

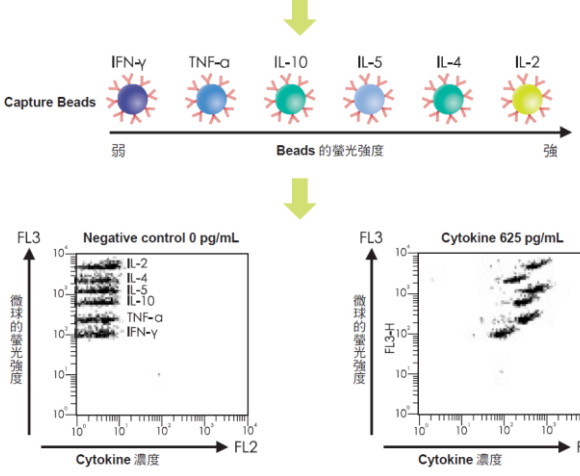
BD CBA Flex Sets	提供開放式的彈性實驗設計，可自由搭配想要偵測的試劑。
BD CBA Kits	將常會一起測試的 **CBA** 試劑合併成套裝組合，例如，**Th1/Th2 cytokine kit**、**Inflammation Kit**，可節省時間。

BD

CBA Kit

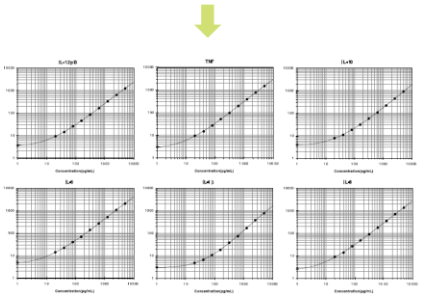
將常常同時偵測的種類，合併成套裝組合

以 **Human BD CBA Th1/Th2 Cytokine Kit** 實驗為例說明實驗流程 (用 **BD Calibur** 分析)。



Capture beads 抓到想要測定的 cytokine 後，再跟帶有 PE 螢光抗體的 Detection reagent 以 Sandwich 方式作用。當測定的 cytokine 濃度越高，FL2 的螢光強度越強，Beads 群落會越往右偏移。

BD



所得結果再以FCAP Array 軟體分析之後，標準品曲線如上。

FCAP Array Parameters for BD CBA Kits

Instrument	Reporter Parameter	Clustering Parameters
BD FACSVerse flow cytometer	PE	CBA Red
BD Accuri C6 flow cytometer	FL2	FL4
BD FACSArray™ bioanalyzer	Yellow	Red
BD FACSCanto™ II flow cytometer	PE	APC
BD LSRFortessa™ flow cytometer	PE	APC
BD FACSAria™ III cell sorter	PE	APC
BD FACSCalibur™ flow cytometer (single laser)	FL2	FL3
BD FACSCalibur™ flow cytometer (dual laser)	FL2	FL4

BD

Instrument to instrument results 24 hr PMA/Ionomycin actiovation sample

System	IL-2	IL-3	IL-4	IL-5	IL-6	IL-8	IL-10
BD FACSArray	137,264	57,731	88	350	1,149	49,045	1,589
BD FACSCanto	124,467	53,513	121	338	1,149	45,992	1,444
BD LSR II	129,311	53,992	87	355	1,085	46,651	1,565
BD FACSCalibur	143,708	62,134	87	345	1,122	48,222	1,328
Average	133,687	56,842	96	347	1,126	47,478	1,481
Standard Deviation	8,512	4,000	17	7	30	1,402	120
% CV	6.4%	7.0%	17.6%	2.1%	2.7%	3.0%	8.1%

System	IL-13	TNF	IFN-γ	IP-10	RANTES	MIP-1α	MIP-1β	GM-CSF
BD FACSArray	3,973	30,785	55,797	158	6,365	51,217	264,846	31,108
BD FACSCanto	3,452	29,138	53,342	191	6,379	49,121	253,883	29,752
BD LSR II	3,607	28,750	52,488	200	6,168	47,642	261,450	30,948
BD FACSCalibur	3,122	30,237	61,637	178	7,111	47,567	287,118	31,607
Average	3,539	29,728	55,816	182	6,506	48,887	266,824	30,853
Standard Deviation	353	945	4,126	18	415	1,711	14,284	786
% CV	10.0%	3.2%	7.4%	9.9%	6.4%	3.5%	5.4%	2.5%

Results expressed in pg/ml

BD
BD Biosciences

產品資訊

Human CBA Kit

Cat. No.	Decription	Contet	Size
561418	Human Anaphylatoxin Kit	C4a, C3a, C5a	80 tests
552990	Human Chemokine Kit	IL-8, RANTES, MIG, MCP-1, IP-10	80 tests
551811	Human Inflammation Kit	IL-8, IL-1 β, IL-6, IL-10, TNF, IL-12p70	80 tests
550749	Human Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IL-10, TNF, IFN- γ	80 tests
551809	Human Th1/Th2 Cytokine Kit II	IL-2, IL-4, IL-6, IL-10, TNF, IFN- γ	80 tests
560484	Human Th1/Th2/Th17 Kit	IL-2, IL-4, IL-6, IL-10, TNF, IFN- γ, IL-17A	80 tests
643939	BCR-ABL Protein Kit	BCR-ABL fusion proteins	50 tests

Non-Human Primate CBA Kit

Cat. No.	Decription	Contet	Size
557800	Non-human Primate Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IL-6,TNF, IFN- γ	80 tests

Mouse CBA Kit

Cat. No.	Decription	Contet	Size
550026	Mouse Immunoglobulin Isotyping Kit	mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, IgE	100 tests
552364	Mouse Inflammation Kit	IL-6, IL-10, MCP-1, IFN- γ, TNF, IL-12p70	80 tests
551287	Mouse Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IFN- γ,TNF	80 tests
560485	Mouse Th1/Th2/Th17 Kit	IL-2, IL-4, IL-6, IFN- γ TNF, IL-17A, IL-10	80 tests

BD

Product Format

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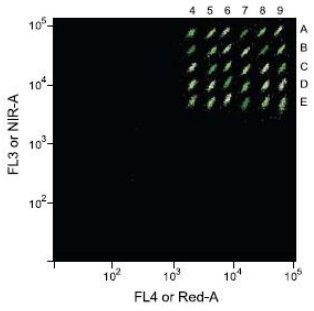
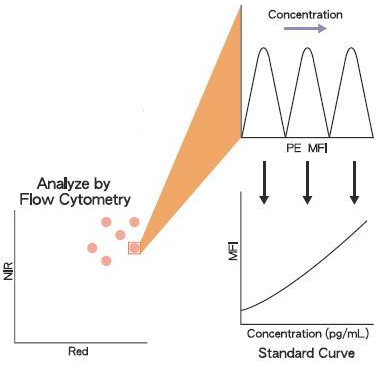
BD

CBA FLEX SET





BD

CBA FLEX SET

Instrument	Reporter Parameters	Clustering Parameters
BD FACSVerse™ flow cytometer	PE	CBA Red and CBA NIR
BD Accuri™ C6 flow cytometer*	FL2	FL4 (675/25) and FL3 (780/60)
BD FACSArray™ bioanalyzer	Yellow	Red and NIR
BD FACSCanto™ II flow cytometer	PE	APC and APC-Cy™7
BD LSRFortessa™ flow cytometer	PE	APC and APC-Cy7
BD FACSAria™ III cell sorter	PE	APC and APC-Cy7
BD FACSCalibur™ flow cytometer	FL2	FL4 and FL3

BD

CBA FLEX SET Product Line

- **Soluble Protein Flex Set**
  - Human Soluble Protein Flex Set
  - Mouse Soluble Protein Flex Set
  - Rat Soluble Protein Flex Set
- **Human TGF-β1 Single Plex Flex Set**
- **Human Immunoglobulin Flex Set** *(For Ab Isotyping)*
- **Enhanced Sensitivity Flex Set** *(0.274–200 pg/mL)*
  - Human Enhanced Sensitivity Flex Set
  - Mouse Enhanced Sensitivity Flex Set
- **Cell Signaling Flex Set**
- **Functional Beads**

BD

Flex Set 建議搭配組合：Human

Human Cytokine Panel

Description	Cat. No.	Bead Location
IFN-γ	558269	E7
IL-1α	560153	D6
IL-1β	558279	B4
IL-2	558270	A4
IL-4	558272	A5
IL-5	558278	A6
IL-6	558276	A7
IL-7	558334	A8
IL-9	558333	B6
IL-10	558274	B7
IL-11	560228	D5
IL-12p70	558283	E5
IL-13	558450	E6
IL-17A	560383	B5
IL-17F	562151	C6
IL-21	560358	B8
TNFα	558273	D9
TNFβ	560083	D5

Human Chemokine Panel

Description	Cat. No.	Bead Location
Eotaxin (CCL11)	558329	C7
Fractalkine (CX3CL1	560265	C6
IL-8 (CXCL8)	558277	A9
IP-10 (CXCL10)	558280	B5
I-TAC (CXCL11)	560364	A7
MCP-1 (CCL2)	558287	D8
MIG (CXCL9)	558286	E8
MIP-1α(CCL3)	558325	B9
MIP-1β (CCL4)	558288	E4
RANTES (CCL5)	558324	D4

Human Th1/Th2 Cytokine Panel

Description	Cat. No.	Bead Location
GM-CSF	558335	C9
IFN-γ	558269	E7
IL-2	558270	A4
IL-4	558272	A5
IL-5	558278	A6
IL-6	558276	A7
IL-10	558274	B7
IL-12p70	558283	E5
IL-13	558450	E6
TNFα	558273	D9

Human Th1 Cytokine Panel

Description	Cat. No.	Bead Location
IFN-γ	558269	E7
IL-1β	558279	B4
IL-2	558270	A4
IL-6	558276	A7
IL-10	558274	B7
IL-12p70	560228	D5
TNFα	558273	D9

Human Th2 Cytokine Panel

Description	Cat. No.	Bead Location
IL-2	558270	A4
IL-4	558272	A5
IL-5	558278	A6
IL-6	558276	A7
IL-9	558333	B6
IL-10	558274	B7
IL-13	558450	E6
TNFα	558273	D9

Human Th17 Cytokine Panel

Description	Cat. No.	Bead Location
GM-CSF	558335	C9
IFN-γ	558269	E7
IL-1β	558279	B4
IL-2	558270	A4
IL-4	558272	A5
IL-5	558278	A6
IL-6	558276	A7
IL-9	558333	B6
IL-10	558274	B7
IL-12p70	560228	D5
IL-13	558450	E6
IL-17A	560383	B5
IL-17F	562151	C6
IL-21	560358	B8
TNFα	558273	D9
TNFβ	560083	D5
sCD154 (sCD40	560305	C7

Human Tc Cytokine Panel

Description	Cat. No.	Bead Location
CD178 (Fas	558330	C6
GM-CSF	558335	C9
Granzyme A	560299	D9
Granzyme B	560304	D7
IFN-γ	558269	E7
IL-2	558270	A4
IL-4	558272	A5
IL-5	558278	A6
IL-6	558276	A7
IL-10	558274	B7
IL-13	558450	E6
MIP-1α(CCL3)	558325	B9
MIP-1β (CCL4)	558288	E4
TNFα	560112	C4

Human Soluble Cytokine Receptor Panel

Description	Cat. No.	Bead Location
CD121a (IL-1RI)	560276	B6
CD121b (IL-1RII)	560281	B7
soluble TNF RI	560156	C4
soluble TNF RII	560155	C5

Flex Set 建議搭配組合：Mouse

Mouse Cytokine Panel

Description	Cat. No.	Bead Location
IFN-γ	558296	A4
IL-1α	560157	E4
IL-1β	560232	E5
IL-2	558297	A5
IL-3	558346	A8
IL-4	558298	A7
IL-5	558302	A6
IL-6	558301	B4
IL-9	558348	B5
IL-10	558300	C4
IL-12p70	558303	D7
IL-13	558349	B8
IL-17A	560283	C5
IL-17F	562174	D6
IL-21	560160	B6
TNFα	558299	C8

Mouse Chemokine Panel

Description	Cat. No.	Bead Location
KC	558340	A9
MCP-1 (CCL2)	558342	B7
MIG (CXCL9)	558341	D9
MIP-1α(CCL3)	558449	C7
MIP-1β (CCL3)	558343	C9
RANTES (CCL5)	558345	D8

Mouse Th1/Th2 Cytokine Panel

Description	Cat. No.	Bead Location
GM-CSF	558347	B9
IFN-γ	558296	A4
IL-2	558297	A5
IL-4	558298	A7
IL-5	558302	A6
IL-6	558301	B4
IL-10	558300	C4
IL-12p70	558303	D7
IL-13	558349	B8
TNFα	558299	C8

Mouse Th1 Cytokine Panel

Description	Cat. No.	Bead Location
IFN-γ	558296	A4
IL-1β	560232	E5
IL-2	558297	A5
IL-6	558301	B4
IL-10	558300	C4
IL-12p70	558303	D7
TNFα	558299	C8

Mouse Th2 Cytokine Panel

Description	Cat. No.	Bead Location
IL-2	558297	A5
IL-4	558298	A7
IL-5	558302	A6
IL-6	558301	B4
IL-9	558348	B5
IL-10	558300	C4
IL-13	558349	B8

Mouse Th17 Cytokine Panel

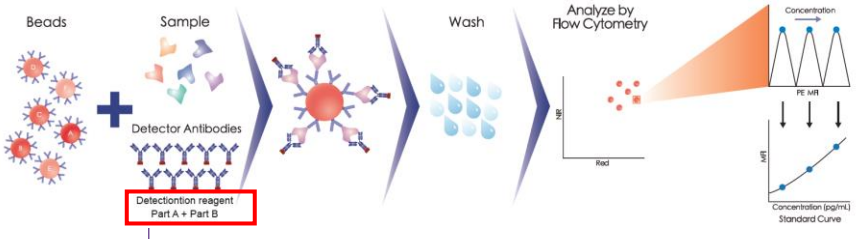
Description	Cat. No.	Bead Location
GM-CSF	558347	B9
IFN-γ	558296	A4
IL-1α	560157	E4
IL-1β	560232	E5
IL-2	558297	A5
IL-4	558298	A7
IL-5	558302	A6
IL-6	558301	B4
IL-9	558348	B5
IL-10	558300	C4
IL-13	558349	B8
IL-17A	560283	C5
IL-17F	562174	D6
IL-21	560160	B6
IL-23 p19/p40	562575	D7
TNFα	558299	C8

Mouse Tc Cytokine Panel

Description	Cat. No.	Bead Location
GM-CSF	558347	B9
IFN-γ	558296	A4
IL-2	558297	A5
IL-4	558298	A7
IL-5	558302	A6
IL-6	558301	B4
IL-10	558300	C4
IL-13	558349	B8
MIP-1α(CCL3)	558449	C7
MIP-1β (CCL3)	558343	C9
RANTES (CCL5)	558345	D8
TNFα	558299	C8

BD

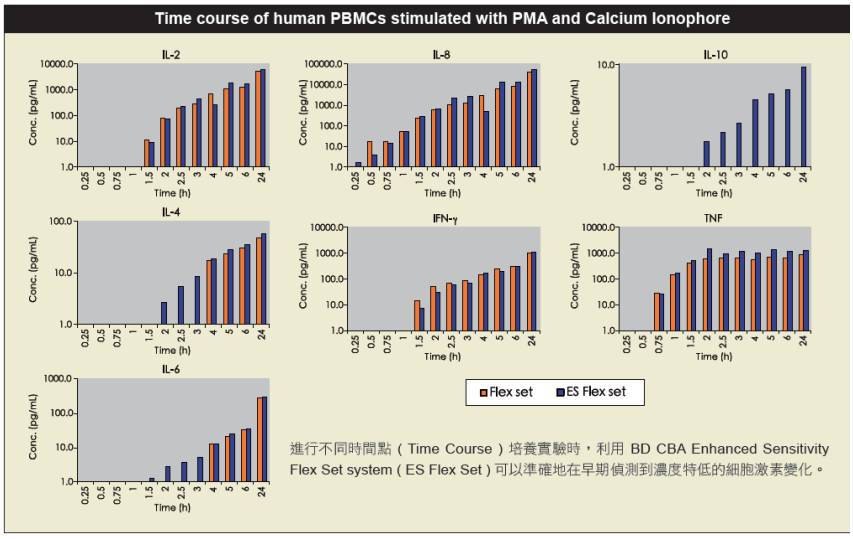
Enhanced Sensitivity Flex Set System



Features

- Quantitation range of **0.274–200 pg/mL**
- Designed for multiplex analysis, providing more data using a single sample
- Open and configurable to maximize flexibility
- Analyzes soluble proteins with just 25 to 50 µL of sample
- Works with flow cytometers equipped with 488-nm and 633-nm lasers

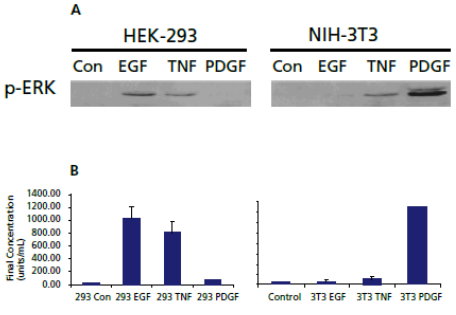
BD



BD

Cell Signaling Flex Set System

- 定性及定量
  - 可同時偵測Cell lysate 中多種磷酸化蛋白
  - 標準品
- 單一、少量的樣本可進行多種分析
- 樣本製備與Western Blot 類似



Analysis of phospho-ERK1/2 protein levels in HEK 293 and NIH 3T3 cells in response to EGF, TNF, and PDGF stimulation.

A shows the results of Western blot analysis.

B shows the results of BD CBA Flex Set analysis.

Data courtesy of Dr. Tony Pawson and Dr. Jay Park, Mount Sinai Hospital, Toronto, Canada.

BD

Cell Signaling Flex Set System

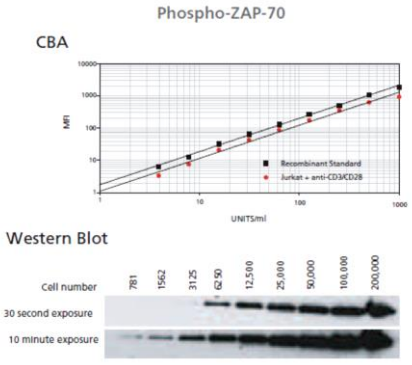
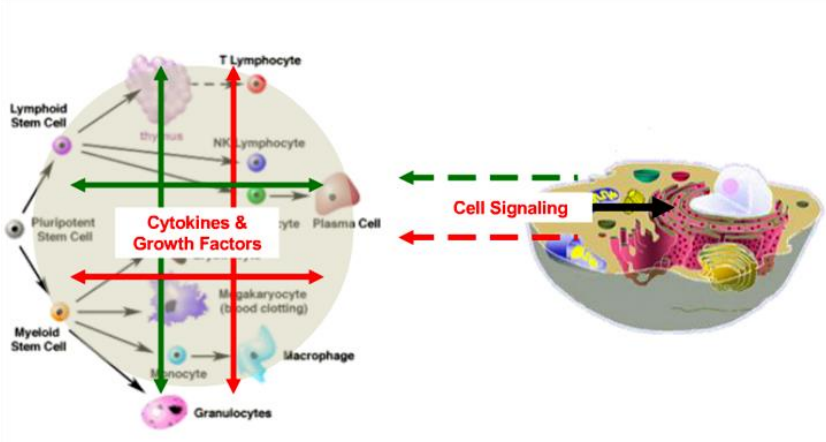


Figure 2. Jurkat cells activated with anti-CD3 and anti-CD28 and crosslinked by anti-mouse Ig for 2 minutes. The reaction was stopped by the addition of SDS (1% final) whereupon the samples were placed in a boiling water bath. A phosphorylated recombinant standard was used to generate a standard curve. The upper panel shows the standard curve versus a titration of the activated Jurkat lysate. An important characteristic of the assay is that these two curves are parallel to each other. This ensures linearity of the readings so that twice as much lysate will have twice as many units/ml. The same concentrations of lysate were also run on an SDS-PAGE gel followed by immunoblotting with an anti-phospho-ZAP-70 antibody. Both a 30 second and a 10 minute exposure are shown. The BD CBA Flex Set assay is at least as sensitive as a Western blot. In this particular example, phosphorylation of ZAP-70 can be detected in a BD CBA Flex Set assay using lysate from less than 1,000 cells.

BD

Cellular Communication and Signaling



BD

Cell Activation

- Isolated PBMC
  - Activated 2 days with anti-CD3/CD28 in the presence of rhIL-2 and rhIL-4
  - Cells rested in culture for 3 days with rhIL-2 and rhIL-4
  - Cells reactivated with either anti-CD3/CD28 or PMA/Ionomycin

BD

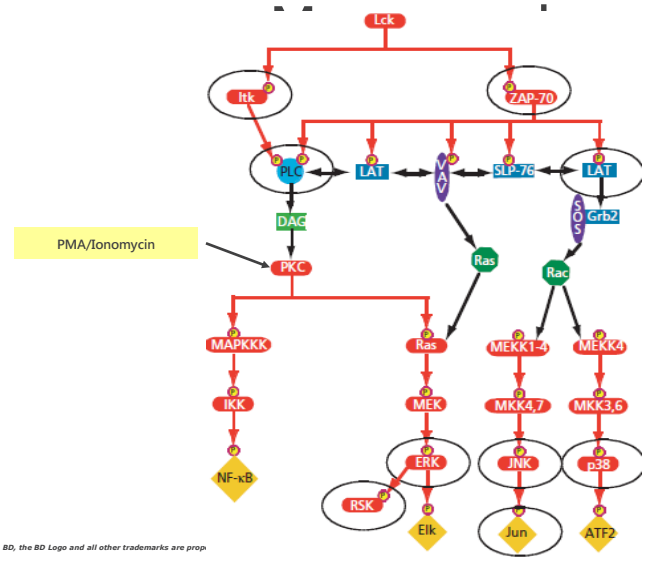
Cell Signaling Multiplex Panels

Bead Positions	4	5	6	7	8	9
A						
B					ZAP70 (Y319)	
C			Itk (Y511)			
D			SLP-76 (Y145)	MEK1 (S222)	MEK2 (S227)	
E						Akt1 (T308)

Bead Positions	4	5	6	7	8	9
A						
B		JNK (T183/Y185)	P38 (T180/Y182)	PLC-γ (Y783)	Akt2 (S474)	
C	ERK1/2 (T202/Y204)					
D				RSK (T573)	c-Jun (S63)	
E						Akt1 (S473)

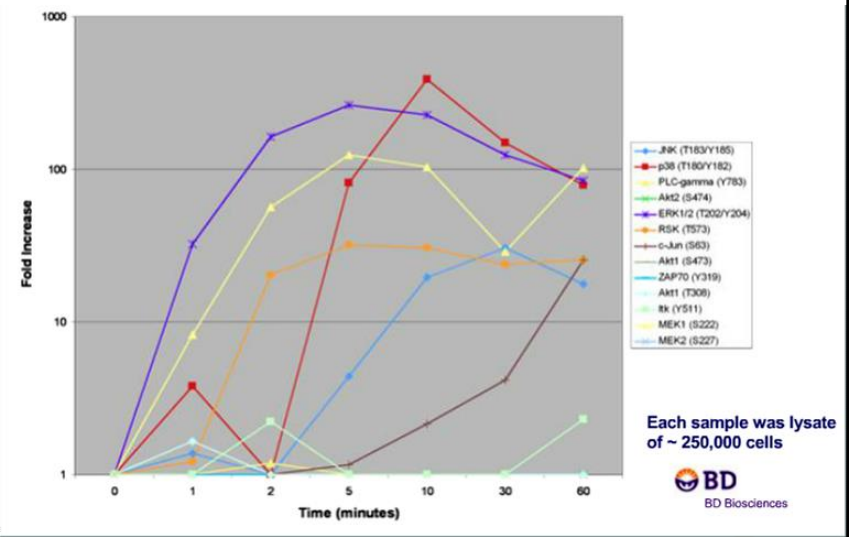
BD

Signaling Pathway

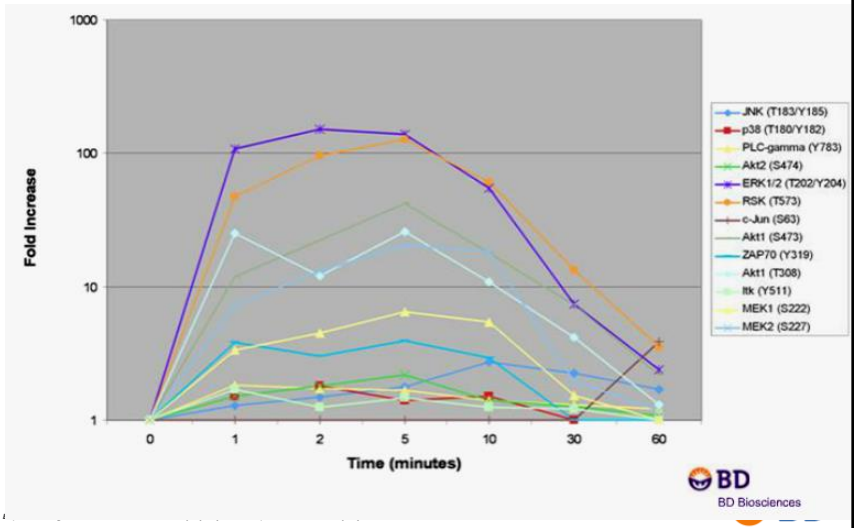


BD

Protein Phosphorylation - PMA / Ionomycin activation kinetics



Protein Phosphorylation - CD3 / CD28 activation kinetics



Difference in cellular response to PMA/Ionomycin and CD3/CD28

- PMA/Ionomycin activation results in higher levels and more sustained phosphorylation of intracellular proteins
- CD3/CD28 activation results in a specific protein phosphorylation profile
  - ERK1/2 phosphorylation but not p38 or JNK
  - Phosphorylation of upstream molecules such as ZAP70 (not activated by PMA/Ionomycin)

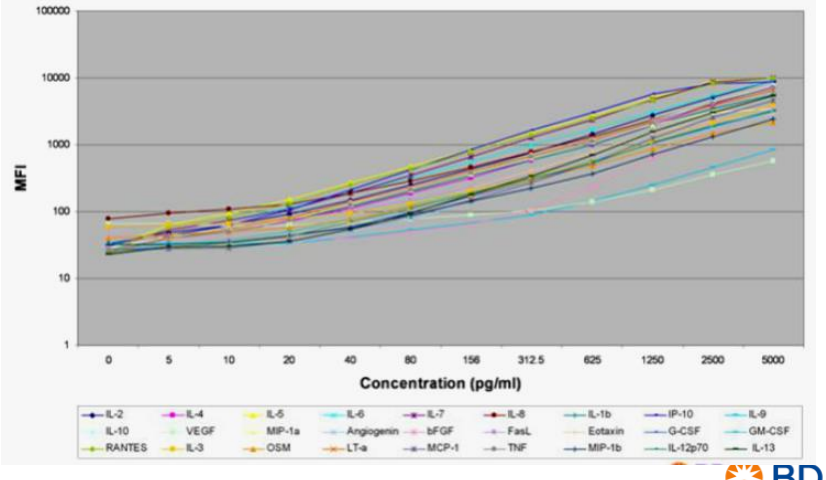
BD

Human soluble protein 30 plex

Bead Position	4	5	6	7	8	9
A	IL-2	IL-4	IL-5	IL-6	IL-7	IL-8
B	IL-1β	IP-10	IL-9	IL-10	VEGF	MIP-1α
C	Angiogenin	basic FGF	FasL	Eotaxin	G-CSF	GM-CSF
D	RANTES	IL-3	OSM	LT-α	MCP-1	TNF
E	MIP-1β	IL-12p70	IL-13	IFN-γ	MIG	IgE

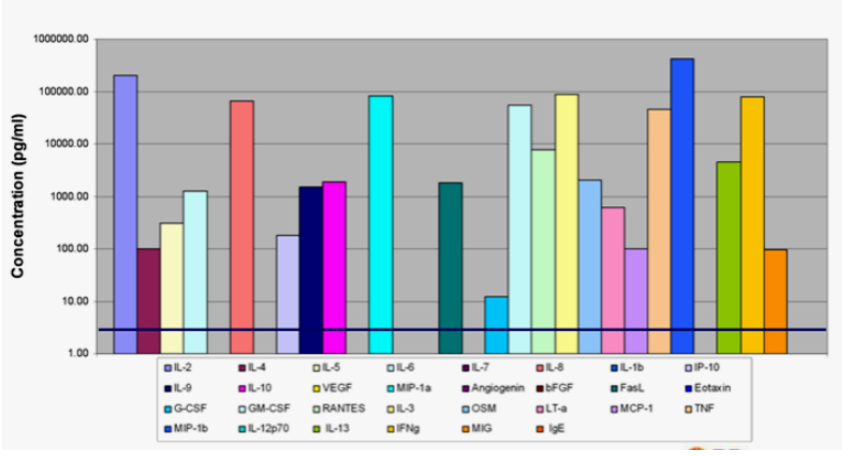
BD

Standard Curve



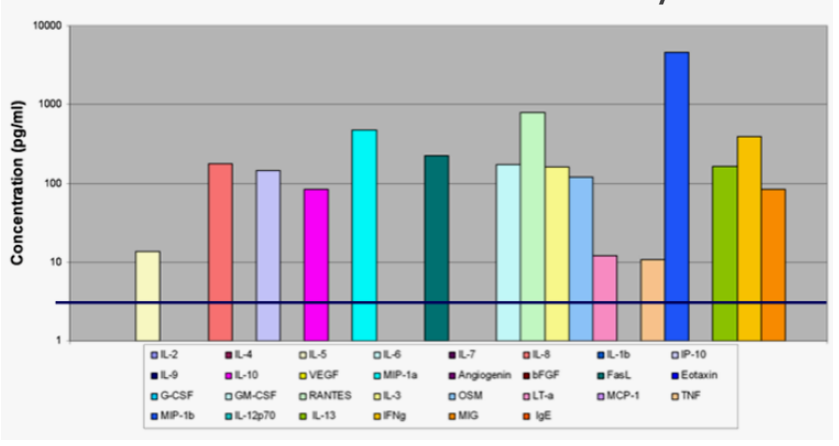
BD

Human Protein level of PBMC actived 24 hours with



BD

Human Protein level of PBMC actived 24 hours with CD3/CD28



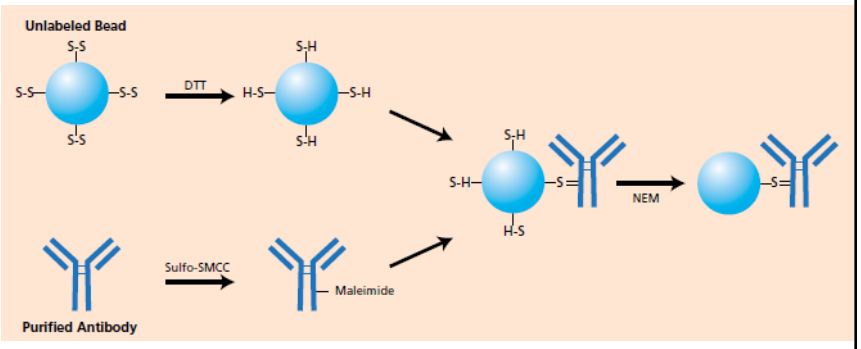
BD

Difference in cellular response to PMA/Ionomycin and CD3/CD28 activation

- PMA/Ionomycin activation results in broader profile of secreted proteins
  - 22 of 30 proteins measured had levels above 10 pg/ml
- CD3/CD28 activation results in a specific secreted protein profile
  - Little or no production of IL-6, IL-9 and MCP-1
  - No IL-2 or IL-4 measured likely due to consumption of protein by activated cells

BD

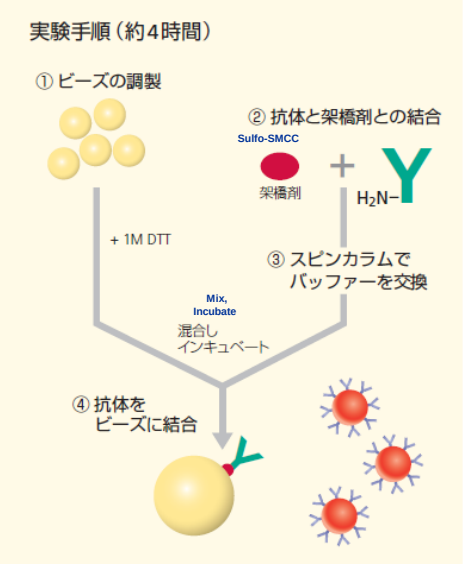
BD™ CBA Functional Bead



DTT, Dithiothreitol
Sulfo-SMCC, Sulfosuccinimidyl 4-N-maleimidomethyl cyclohexane 1-carboxylate
NEM, N-Ethylmaleimide

BD

BD™ CBA Functional Bead



- Step 1: Bead Preparation
- Step 2: Protein Modification
- Step 3: Buffer Exchange to Remove Unreacted Components
- Step 4: Protein Conjugation

BD

Advantages

- Simple conjugation procedure completed in less than 4 hours.
- Requires less than 100 µg of protein at a concentration of 1 mg/mL.
- Ability to conjugate any protein molecule containing a free amino group to the beads.
- Compatible with a wide selection of flow cytometers for ease of analysis.
- Specific reagents available for confirming the success of conjugation reactions.
- Supporting reagents and procedures available for performing instrument setups and assays

BD

FAQ

- **What are the physical properties of the BD CBA beads?**
  - The BD CBA beads are hard-dyed polystyrene-based microspheres with an average diameter of 7.5 µm
- **Which blood anticoagulants can be used for preparation of serum and plasma samples?**
  - EDTA-treated plasma
- **Will hemolysis hinder results in BD CBA assays?**
  - A small amount of hemolysis is unlikely to be a problem because the samples are washed before acquisition
- **Can BD CBA cytokine or chemokine assays be used with tissue lysates?**
  - Milano F, Jorritsma T, Rygiel AM, et al. Expression pattern of immune suppressive cytokines and growth factors in oesophageal adenocarcinoma reveal a tumor immune escape-promoting microenvironment. *Scand J Immunol.* 2008;68:616-623.
  - Bhowmick S, Duseja R, Das S, Appaiahgiri MB, Vrati S, Basu A. Induction of IP-10 (CXCL10) in astrocytes following Japanese encephalitis. *Neurosci Lett.* 2007;414:45-50.
  - Ghoshal A, Das S, Ghosh S, et al. Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. *Glia*. 2007;55:483-496.
  - Tong J, Bandukwala HS, Clay BS, et al. Fas-positive T cells regulate the resolution of airway inflammation in a murine model of asthma. *J Exp Med*. 2006;203:1173-1184.
  - Mansour MK, Yauch LE, Rottman JB, Levitz SM. Protective efficacy of antigenic fractions in mouse models of cryptococcosis. *Infect Immun*. 2004;72:1746-1754.

ELISA v.s. CBA

Comparison

ELISA	方法	BD CBA Flex Set	BD CBA Kit
1 個樣本 1 種	同時測定蛋白種類數目 勝	1 個樣本最多可 30 種	1 個樣本最多可 6 種
化學發光、吸光值；螢光	檢測原理	螢光	螢光
繁雜，需清洗 10 次以上。	實驗操作 勝	簡便，只需清洗一次。	
100 µl 以上	需要樣本量 勝	25-50 µl	
用 96-well plate	測定方式 勝	可用 96-well plate 或 Tube 來進行	
Plate Redaer	測定儀器	2-Laser Flow Cytometry	1-Laser Flow Cytometry

Correlation

- *E. Morgan et al. / Clinical Immunology 110 (2004) 252–266*
- *BLOOD, 15 AUGUST 2005 VOLUME 106, NUMBER 4*
- *Electrophoresis* 2009, 30, 4008–4019
- *Methods, Volume 38, Issue 4, April 2006, Pages 317–323*
- *Cytometry Part B (Clinical Cytometry) 61B:35–39 (2004)*
- *Cytokine, Volume 54, Issue 2, May 2011, Pages 136–143*

…………etc

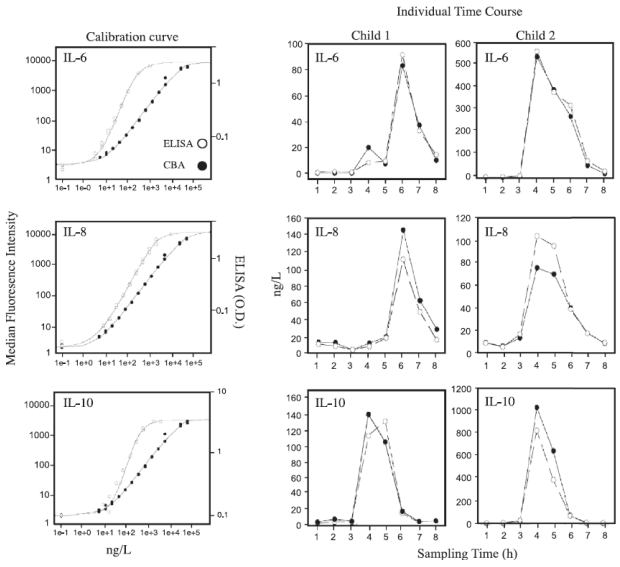


Fig. 4. Serum cytokine levels determined by ELISA and by CBA. The plots show the standard curves (left column). The values for the ELISAs are in absorbance units on the right (y) axis. CBA values are in fluorescence units on the left (y) axis. The individual kinetics of serum cytokines responses (ng/L) in two children undergoing cardiovascular surgery with cardiopulmonary bypass (middle and right columns; Child 1: 11 day old neonate; Child 2: 7.6 year old; *x*- axis: 1 – 2 hr: preoperative, 3 – 5 hr: intraoperative, 6 – 8 hr: postoperative samples). With ELISA (O) all samples where values exceeded the highest standard were diluted and reanalyzed. With CBA (●) all samples were measured without dilution.

E. Morgan et al. / Clinical Immunology 110 (2004) 252–266

Tools and Techniques for Cytokine Quantitation

Tool/Technology	BD Cytometric Bead Array (CBA)	ELISA	ELISPOT
Molecules detected	Secreted or intracellular	Secreted	Secreted (in situ)
Multiparameter	Yes	No	No
Single-cell/cell-subset information	No	No	Frequencies, no subset information
Antigen-specific	Yes	Yes	Yes
Post-assay viability	Yes	Yes	No
Protein quantitation	Yes	Yes	No
Instrumentation	Flow cytometer	Spectro-photometer	ELISPOT reader

Thanks!

BD

Berthoud et al. Malaria Journal 2011, 10:115

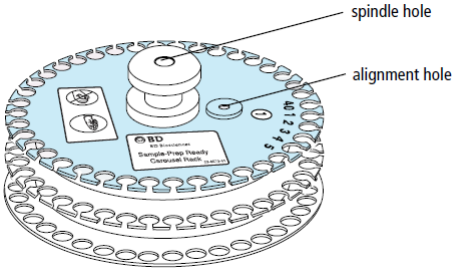
Table 8 Summary of the four assays

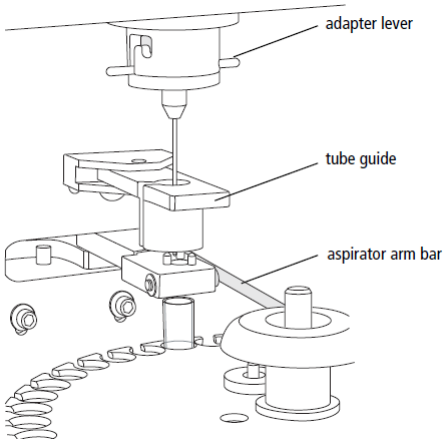
Measure	Bio-Rad	Invitrogen	BD	Bender MedSystems
Cost (1 = highest cost)	2	1	3	4
Time taken for assay completion	2 h 10 min	5 h	4 h	4 h
Time taken for reading samples	30 min	30 min	2 1/2 h	2 1/2 h
Mean correlation (duplicate wells)	0.95	0.82	0.95	0.96
Number of cytokines tested	17	10	11	7
Volume of sample needed	100 µl	100 µl	50 µl	25 µl

The kits were compared in terms of cost, time for assay completion, correlation between duplicate wells, variability, number of cytokines detected and sample volume. Cost was based on quotes received from Spanish distributers (2007).

BD

Canto II Loader

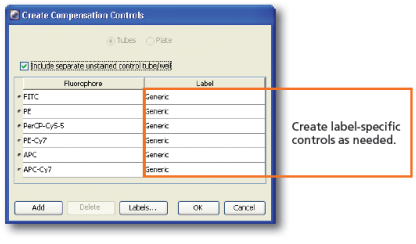




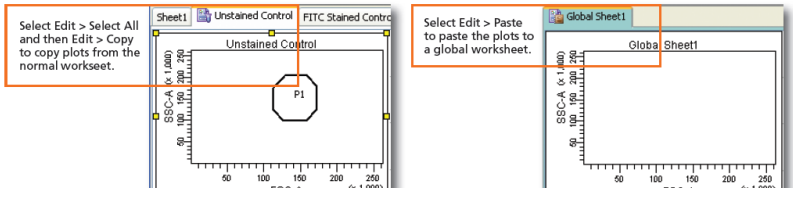
BD

Use Canto II Loader for autocompensation

❹ Select Experiment > Compensation Setup > Create Compensation Controls.

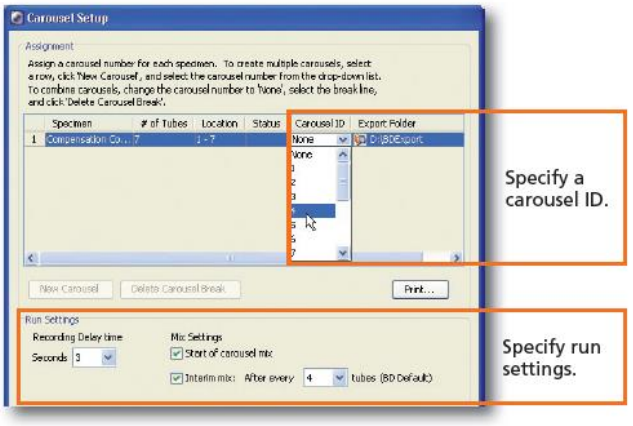


❺ Copy and paste plots from the Unstained Control normal worksheet to a Global Worksheet.



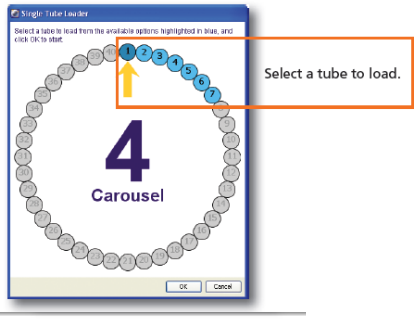
BD

Carousel>Carousel setting



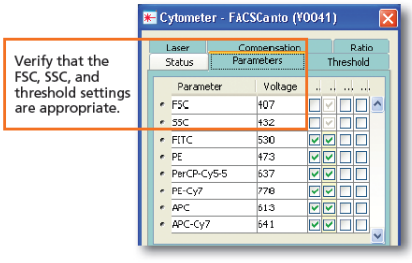
BD

❾ Verify that the cytometer is configured for automatic loading and Click Run Single Tube .



Verify cytometer setting

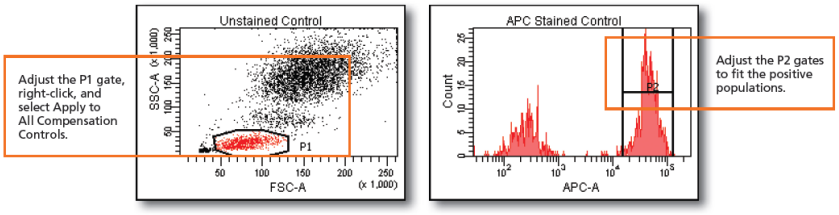
By acquire data, make sure all the parameter voltage setting is correct



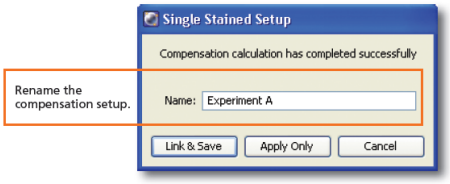
❿ Click Unload and then click Run Carousel .

BD

11. View recorded data in the normal worksheets and gate the positive populations.



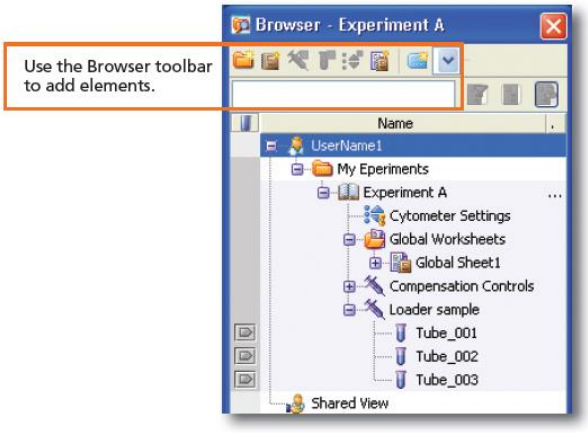
12. Select Experiment > Compensation Setup > Calculate Compensation.



BD

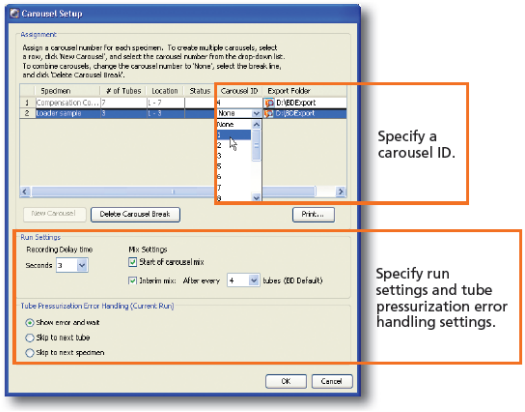
Run Carousel

1. Create Browser elements.

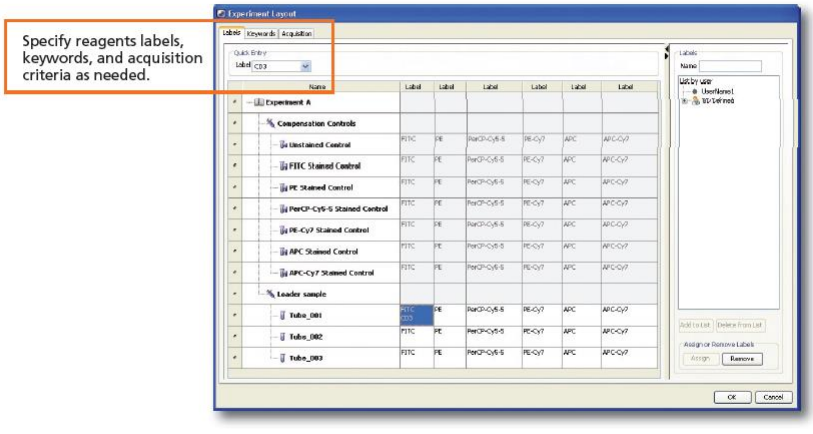


BD

❷ Specify Carousel Setup settings.

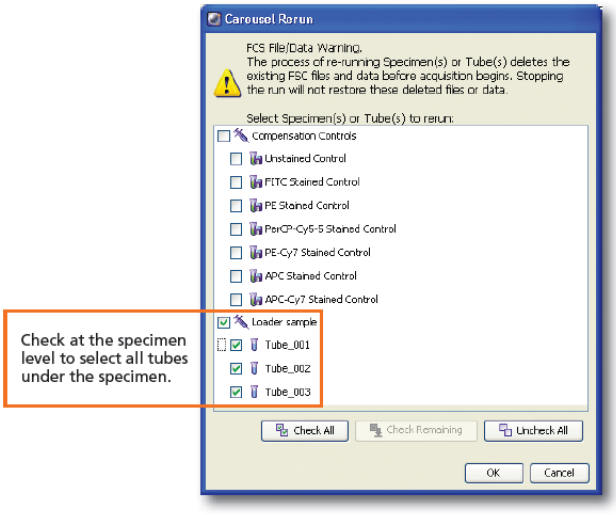


❸ Make entries in the Experiment Layout.



❹ Install the carousel on the Loader and click Run Carousel .

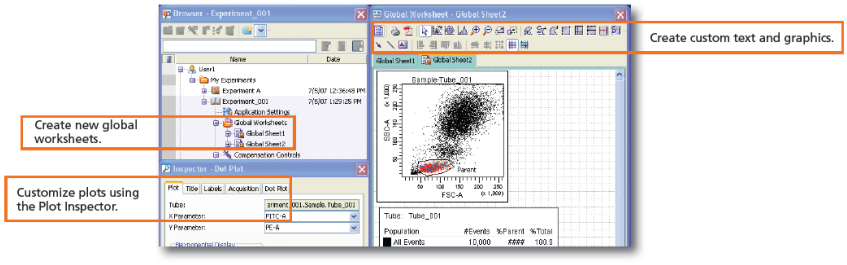
❺ Specify tubes to run.



BD

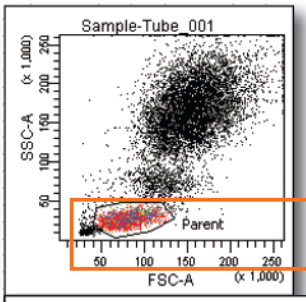
Analyzing Data

❶ Create plots, gates, and statistics needed for analysis.



BD

Perform quality control of the analysis



Verify that gates are set appropriately for all samples.

Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
Parent	1,867	18.7	18.7
Child A	128	6.9	1.3
Child B	219	11.7	2.2

Use the population hierarchy to verify parent/child relationships.

BD