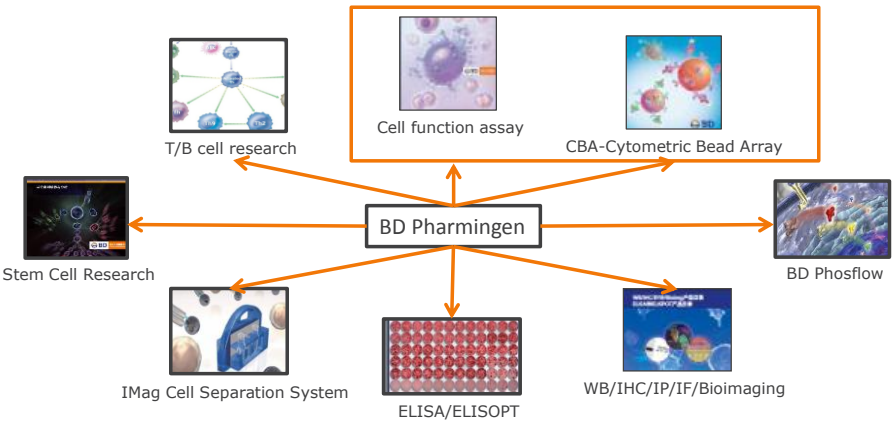
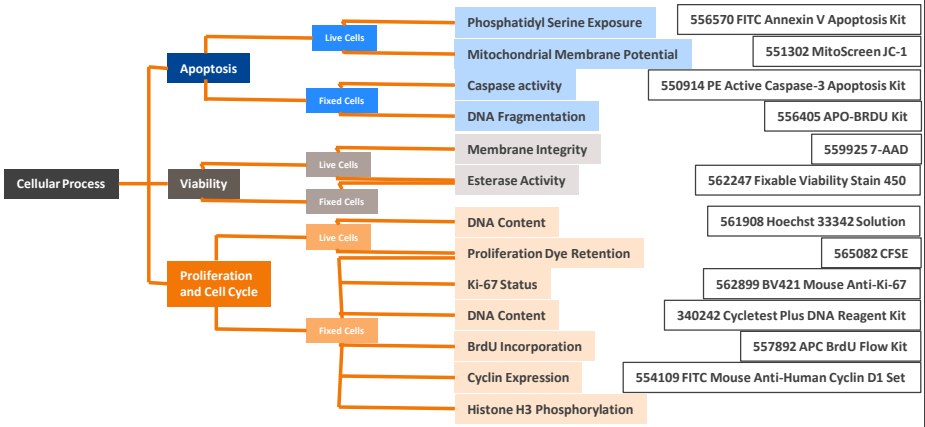
Cell Apoptosis/ Cell Cycle/Proliferation
CBA 微珠免疫分析

Grace Lin 林秀鴻
Senior Product Specialist,
BD Biosciences
Grace.lin@bd.com

BD Pharmingen Total Solution



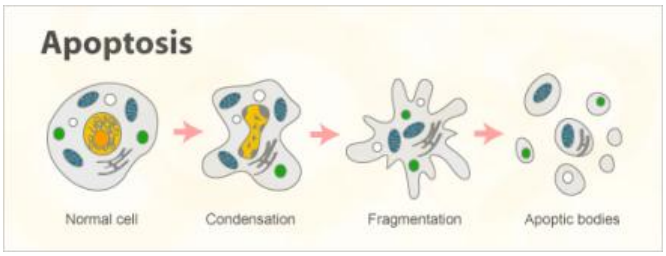
Cell Function Assay Landscape



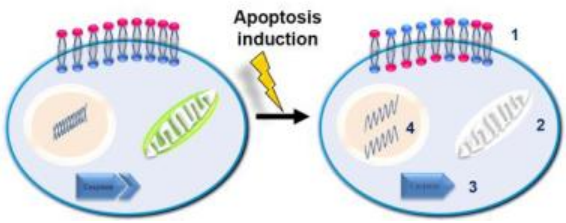
Apoptosis

Apoptosis

細胞凋亡，又稱為程式性細胞死亡 (Programmed Cell Death) 是機體主動的、高度有序地清除無用細胞的過程。

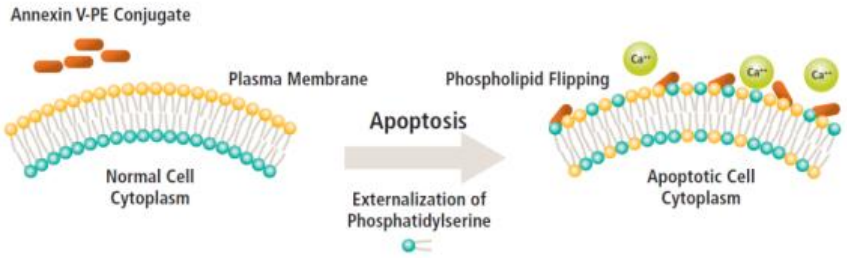


Apoptosis

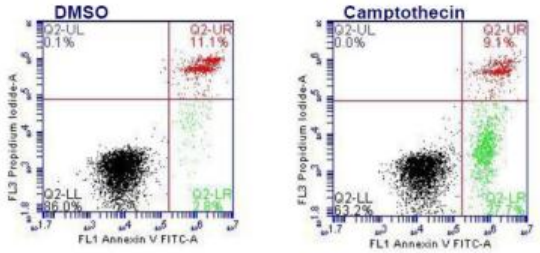


1. Phosphotidyl Serine exposed
2. Mitochondrial potential decreases
3. Caspases activated
4. DNA fragmentation

Apoptosis: Annexin V mechanism



Annexin V/PI detect apoptosis



BD Pharmingen™ FITC Annexin V Apoptosis Detection Kit I (Cat.No. 556547) on Accuri C6

Live cells:
Annexin V Negative
PI Negative

Early Apoptotic:
Annexin V Positive
PI Negative

Late apoptotic/dead:
Annexin V Positive
PI Positive

Protocol for Annexin V/PI

- Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of $1X10^6$ cells/ml
- Transfer 100ul of the solution to a 5ml culture tube
- Add 5ul of FITC Annexin V and 5ul PI
- Gently vortex the cells and incubate for 15min at RT in the dark
- Add 400ul of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1hr.

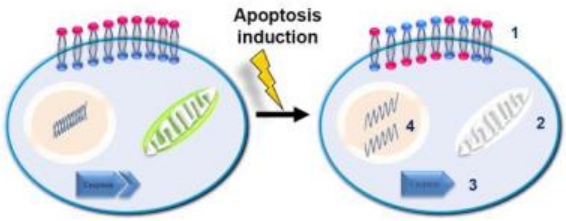
Apoptosis kit:

556547	Annexin V FITC Apoptosis Detection Kit I
559763	Annexin V PE Apoptosis Detection Kit I

Tips:

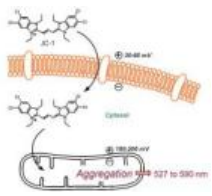
- Optimized for suspension cells
- Compatible across most species
- Compatible for cell surface staining as long as the calcium concentration is maintained
- Using Binding buffer during the whole assay
- Check trypsin/EDTA for adherent cells
- Calculate compensation using single stained apoptotic cells
- Tricky to determine FITC-%PE compensation value sometime
- Negative group and biological control group
- PE Annexin V and APC Annexin V for GFP transfected cell lines

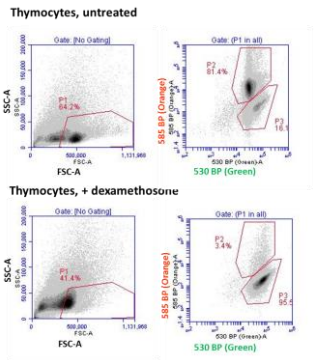
Apoptosis



1. Phosphotidyl Serine exposed
2. **Mitochondrial potential decreases**
3. Caspases activated
4. DNA fragmentation

Apoptosis: Mitochondrial membrane potential

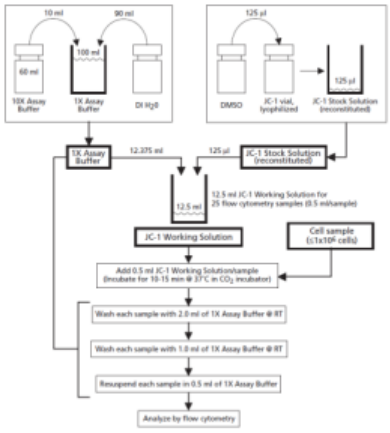




BD Pharmingen™ BD™ MitoScreen (JC-1) (Cat.No. 551302) on Accuri C6

Protocol for JC-1

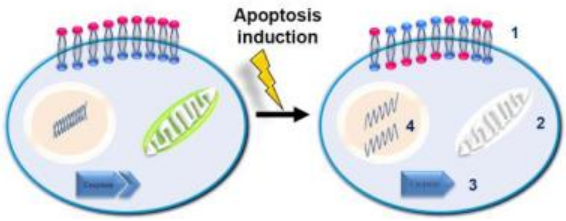
BD Pharmingen™ BD™ MitoScreen (JC-1) contains 4 amber vails of lyophilized JC-1 and 60ml 10X assay buffer



Tips:

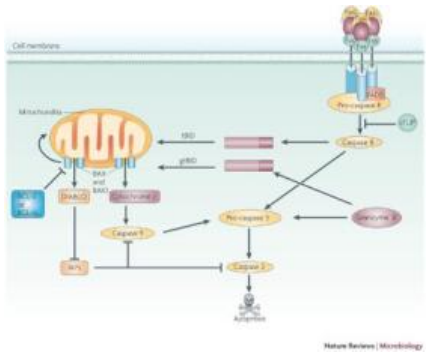
- Warm 10X Assay buffer at 37℃ to completely dissolve salt crystals
- Keep diluted 1X Assay buffer at 37℃ prior to use
- JC1 should be used as fresh as possible, avoid frozen and thaw repeatedly
- After dilute JC1 stock with 1X assay buffer，vortex working solution thoroughly
- After vortexing, JC1 aggregates may be present, but should not interfere with flow cytometric analysis.

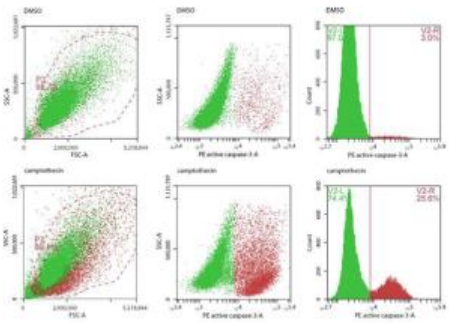
Apoptosis



1. Phosphotidyl Serine exposed
2. Mitochondrial potential decreases
3. **Caspases activated**
4. DNA fragmentation

Apoptosis: Active Caspase-3





BD Pharmingen™ PE Active Caspase-3 Apoptosis Kit (Cat.No. 550914) on Accuri C6

550914	BD Pharmingen™ PE Active Caspase-3 Apoptosis Kit
560901	BD Pharmingen™ FITC Rabbit Anti- Active Caspase-3

Protocol for active caspase-3 detection

- Wash cells twice with cold 1X PBS, then resuspend cells in BD Cytofix/Cytoperm solution at a concentration of $1X10^6$ cells/0.5ml
- Incubate cells for 20min on ice
- Pellet cells, aspirate, and discard BD Cytofix/Cytoperm solution, wash twice with BD Perm/Wash buffer (1X) at a volume of 0.5ml buffer/$1X10^6$ cells at room temperature
- Resuspend each $1X10^6$ cells in 100ul BD Perm/Wash buffer (1X) and 20ul antibody and incubate for 30min at room temperature
- Wash each test in 1.0ml BD Perm/Wash buffer (1X), then resuspend the test in 0.5ml BD Perm/Wash buffer (1X) and analyze by flow cytometry

550914	BD Pharmingen™ PE Active Caspase-3 Apoptosis Kit
560901	BD Pharmingen™ FITC Rabbit Anti- Active Caspase-3

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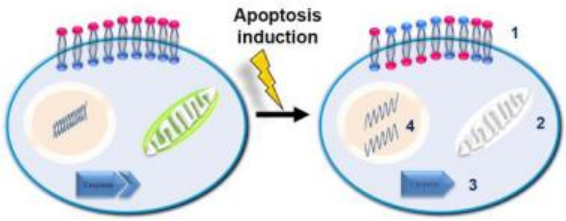
Apoptosis



1. Phosphotidyl Serine exposed
2. Mitochondrial potential decreases
3. Caspases activated
4. **DNA fragmentation**

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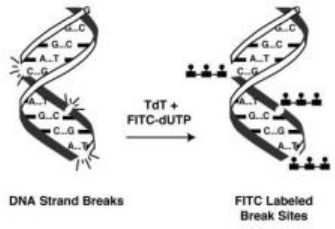
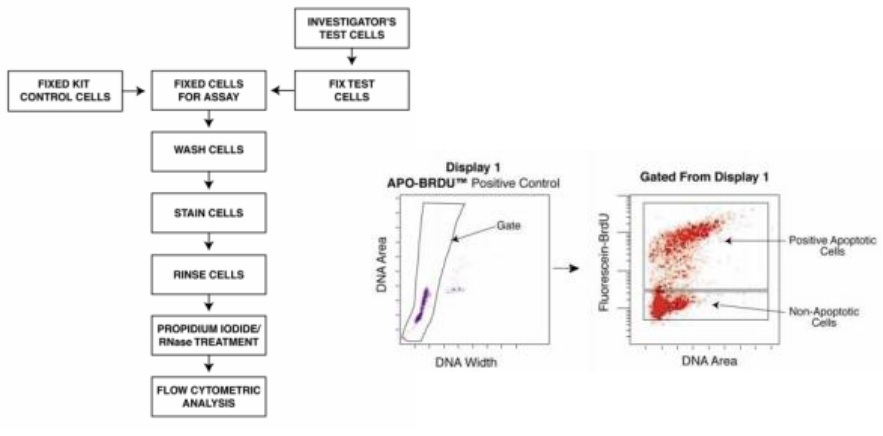
Apoptosis: DNA fragmentation



BD APO-DIRECT Kit Component
Part A:
PI/RNase Staining Buffer
Reaction Buffer
Rinsing Buffer
Wash Buffer
Part B:
FITC-dUTP
Negative Control Cells
Positive Control Cells
TdT Enzyme

Apoptosis: DNA fragmentation



Apoptosis product list

Cat.No.	Description
556547	Annexin V FITC Apoptosis Detection Kit I
559763	Annexin V PE Apoptosis Detection Kit I
551302	BD™ MitoScreen (JC-1)
550914	BD Pharmingen™ PE Active Caspase-3 Apoptosis Kit
560901	BD Pharmingen™ FITC Rabbit Anti- Active Caspase-3
556405	BD Pharmingen™ APO-BRDU™ Kit
556381	BD Pharmingen™ APO-DIRECT™ Kit
550378	BD Pharmingen™ Z-DEVD-FMK, Caspase-3 Inhibitor

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BD

Viability

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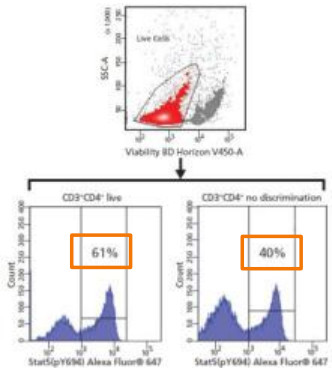
BD

Viability staining improves results



Dye	Unfixed cells	Fixed cells	Detector	Laser
DAPI	√	x	BV421	UV/Violet
Via-Probe Green	√	x	FITC	Blue
PI	√	x	PE	Blue/YG
7-AAD	√	x	PerCP-Cy™5.5	Blue/YG
DRAQ7™	√	x	APC	Red
Via-Probe Red	√	x	APC	
FVS450	√	√	BV421	Violet
FVS510	√	√	BV510	Violet
FVS575V	√	√	BV605	Violet
FVS520	√	√	FITC	Blue
FVS570	√	√	PE	Blue/YG
FVS620	√	√	PE-CF594	Blue/YG
FVS660	√	√	APC	Red
FVS700	√	√	AF700	Red
FVS780	√	√	APC-H7	Red

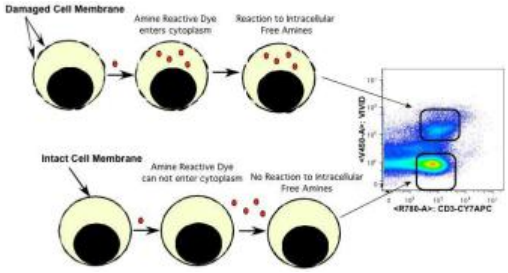
PI & 7-AAD: Cell membrane integrity

Live Dead

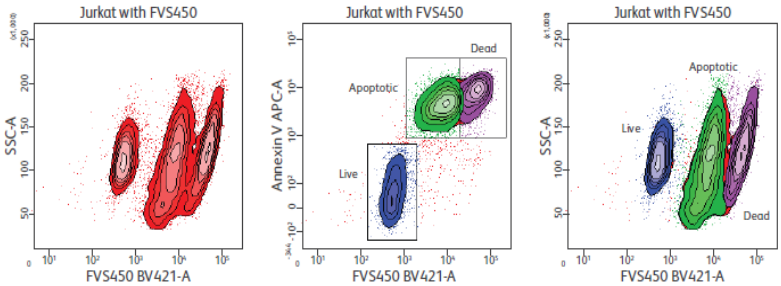
無法針對固定過的細胞分別死活→解決方案:FVS染劑

Fixable viability stain (FVS)-可固定死活染劑

- Amine-reactive membrane impermeable dyes useful for live/dead discrimination
- Labeled cells can be fixed and permeabilized, making the dyes compatible with multiple downstream applications 可與不同下游應用相容



Fixable viability stain and apoptosis



Protocol for FVS staining

- Harvest cells and wash once with azide- and protein-free 1X DPBS
- Resuspend cells at 1-10X10^6 cells/ml in 1X DPBS
- Add 1ul of Fixable Viability Stain 450 stock solution for each 1ml of cell suspension and vortex immediately
- Incubate the mixture for 10-15min at RT protected from light
- Wash cells twice with 2ml of Stain buffer
- Decant the supernatant and gently mix the cell pellet, resuspend the cell in Stain buffer
- Stain, fix and permeabilize cells as desired for down stream applications

Tips for FVS staining

- Incubate mixture at 2-8℃ for 20-30min for mouse cell applications
- Incubate mixture at 37 ℃ for 5-7min for BD Phosflow applications
- The reactivity of free dye is quenched by washing with buffer containing protein (FBS or BSA) prior to staining with fluorescent antibodies

Viability product list

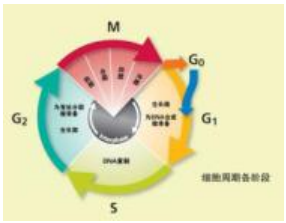
Description	Cat. No.	Laser
BD Pharmingen™ 7-AAD	559925	Blue
BD Pharmingen™ Propidium Iodide Staining Solution	556463	Blue
BD Horizon Fixable Viability Stain 520	564407	Blue
BD Horizon Fixable Viability Stain 660	564405	Red
BD Horizon Fixable Viability Stain 700	564997	Red
BD Horizon Fixable Viability Stain 780	565388	Red

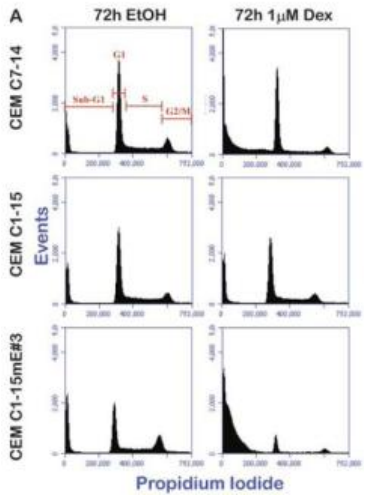
BD

Cell cycle and Proliferation

BD

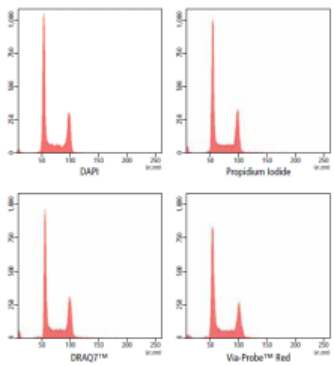
Cell cycle: DNA content





DNA probes

- PI-Propidium Iodide
- EB-Ethidium Bromide
- 7AAD-7-amino-actinomycin D
- AO-Acridine Orange
- Hoechst 33342
- DAPI- 4',6-diamidino-2-phenylindole
- DRAQ7
- Via-Probe Red



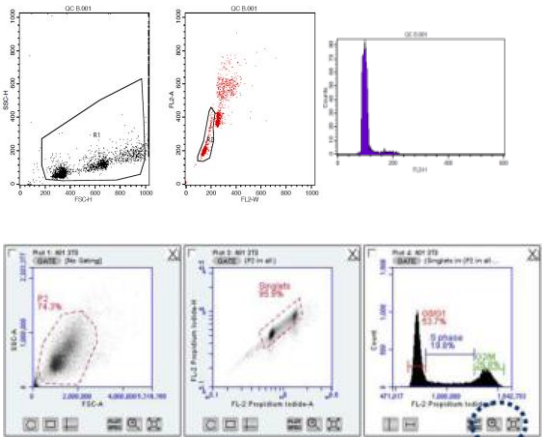
Protocol for EtOH fixed PI/RNase staining

- Harvest cells and prepare single cell suspension in PBS
- Wash cell twice with PBS and resuspend at $1X10^6$ cells/ml
- Aliquot 1ml cells in a 15ml PP V-bottomed tube and add 3ml cold absolute ethanol dropwise while vortexing
- Fix cells for at least 1 hour at 4℃
- Wash cells twice in PBS
- Add 0.5ml/10^6 cells of PI/RNase staining buffer and mix well. Incubate 15min at RT
- Store samples at 4℃ until analyzed by flow cytometry

Protocol for Cycletest Plus DNA Kit

- Centrifuge the cell suspensions at 400g for 5min at RT
- Carefully decant all the supernatant
- Add 250ul of Solution A (trypsin buffer) to each tube and gently mix by tapping the tube by hand. DO NOT VORTEX
- Incubate for 10min at RT
- Add 200ul of Solution B (trypsin inhibitor and RNase buffer) to each tube and gently mix by tapping the tube by hand
- Incubate for 10min at RT
- Add 200ul of cold Solution C (PI stain solution) to each tube and gently mix tapping by hand
- Incubate for 10min in the dark on ice or in the refrigerator
- Filter the sample through 50um nylon mesh and then analyzed by flow cytometry

Gating strategy for Cell cycle assay



1. FSC/SSC: cell population
2. PI-A/PI-H(W): double-let discrimination
3. PI-A(H) Histogram: cell cycle distribution

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Tips:

- Fixation process using ethanol is critical for cell cycle assay
- Make sure discriminate doublelets
- Using LOW flow rate to decrease CV
- Record more than 20,000 cells in singlelets gate
- Using ModFit or other software to analyze cell cycle data

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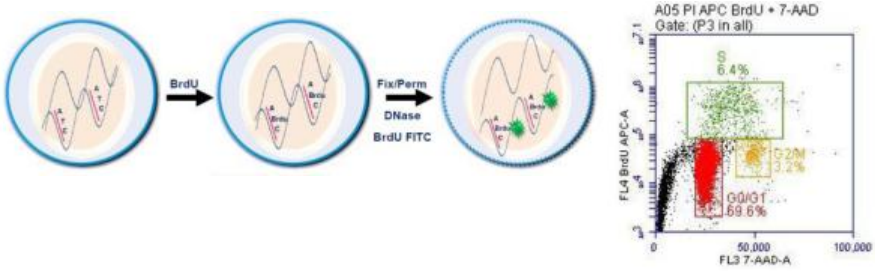
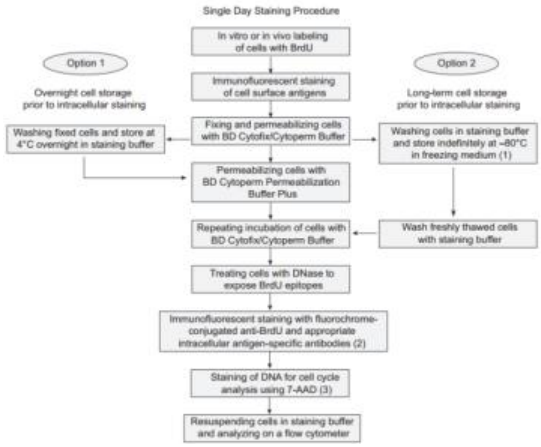
DNA Synthesis: BrdU

- BrdU is incorporated into the DNA of newly synthesized cells (S phase)
- Incorporated BrdU is stained with specific anti-BrdU antibodies
- Staining with a dye that binds total DNA is often coupled with BrdU

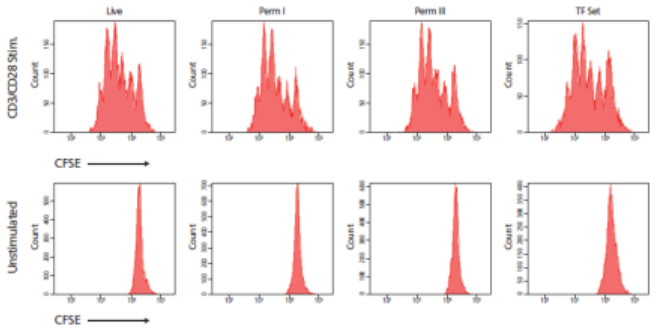


Protocol for BrdU Kit



Proliferation dye: CFSE

- CFSE (carboxyfluorescein succinimidyl ester) is non-fluorescent molecules that diffuse into cells. Once inside cells, they are hydrolyzed by intracellular non-specific esterases and covalenty bind to cellular components to become fluorescent products



Tips:

- Avoid staining in azide-, serum-, or protein-containing buffers during CFSE labeling
- Higher initial staining intensities can be achieved by increasing the initial CFSE concentration, but may lead to increase cell toxicity or cell death

Cell cycle and proliferation product list

Cat.No.	Description
340242	BD Cycletest™ Plus DNA Reagent Kit
550825	BD Pharmingen™ PI/RNase Staining Buffer
561980	BD Pharmingen™ Hoechst 33342 Solution
564907	BD Pharmingen™ DAPI Solution
565082	BD Horizon™ CFSE
557892	BD Pharmingen™ APC BrdU Flow Kit

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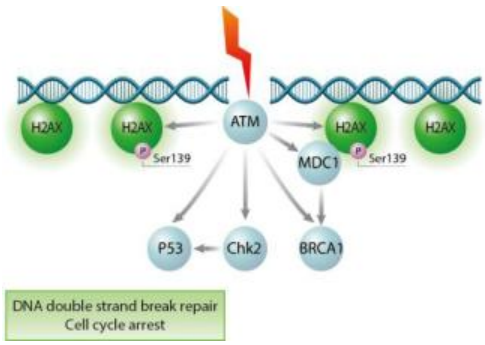
DNA damage causes caspase independent Cell Cycle arrest



H2AX(Ser139) is a key to detect DNA double-stranded breaks (DSBs)

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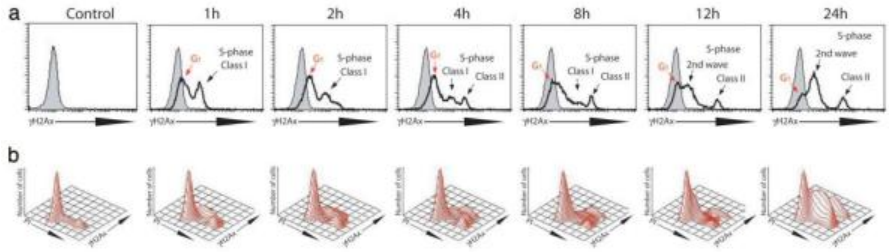
Time course of γH2AX fluorescence after UV irradiation



PNAS, June 2006, Vol 103, 9891-9896

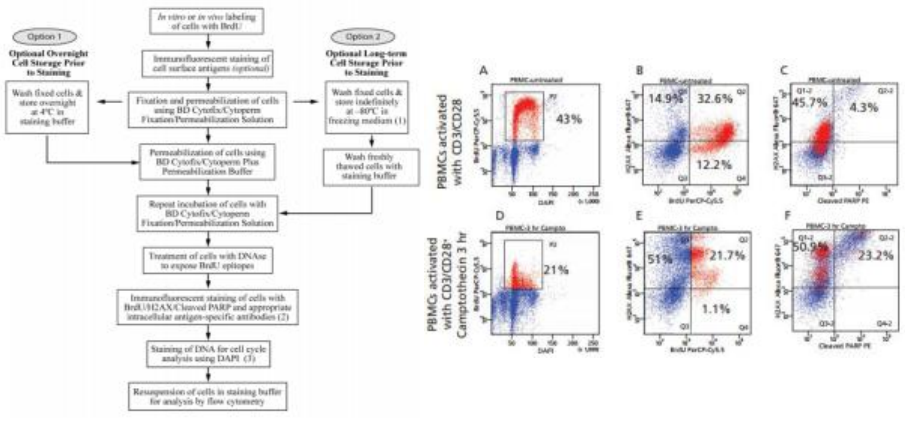
γH2AX products

Cat.No	Description
564720	BD Horizon™ BV421 Mouse Anti-H2AX (pS139)
562377	BD Pharmingen™ PE Mouse Anti-H2AX (pS139)
560447	BD Pharmingen™ Alexa Fluor® 647 Mouse anti-H2AX (pS139)
560445	BD Pharmingen™ Alexa Fluor® 488 Mouse anti-H2AX (pS139)
562253	**BD Pharmingen™ Apoptosis, DNA Damage and Cell Proliferation Kit**

Apoptosis, DNA Damage and Cell Proliferation Kit (Cat.No 562253) Components

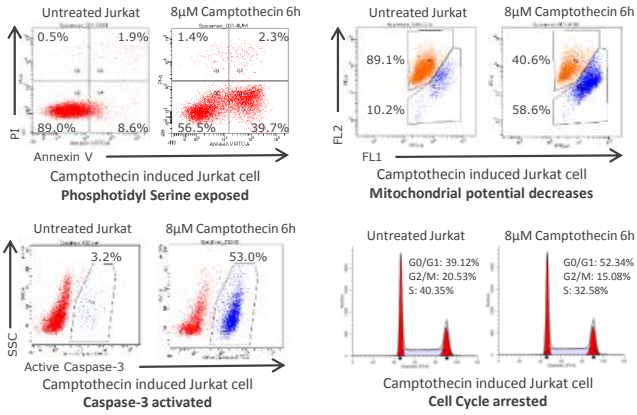
Part	Components	Size
Part A	PerCP-Cy™5.5 Mouse Anti-BrdU	50T
	Alexa Fluor® 647 Mouse Anti-H2AX (pS139)	50T
	PE Mouse Anti-Cleaved PARP (Asp214) Antibody	50T
	BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution	25ml
	BD Perm/Wash™ Buffer (10X)	25ml
	BD Cytofix/Cytoperm™ Plus Permeabilization Buffer	10ml
	DAPI	100ul
Part B	BudU(10mg/ml)	5mg
	DNase	300ul

Apoptosis, DNA Damage and Cell Proliferation Kit (Cat.No 562253) protocols and data



Cell function assay example

Camptothecin induced Jurkat cell apoptosis



1X10^6 Jurkat cells were treated with 8 µM capmtothecin for 6 hours, harvest cells and wash twice with cold PBS, then perform assays according protocols provided by Cat.No. 556547, Cat.No. 551302, Cat.No. 550914 and Cat.No. 340242 (BD Biosciences). Data from BDB SH COE, acquired on BD FACSCanto II, analyzed by BD FACSDiva software and ModFit software.

Example:
CPTH6 induced apoptosis in U937 cell on Accuri C6

Cancer Therapy: Preclinical

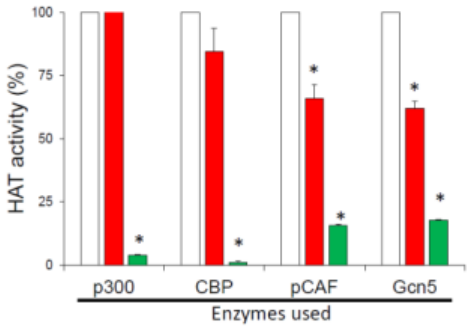
Clinical Cancer Research

CPTH6, a Thiazole Derivative, Induces Histone Hypoacetylation and Apoptosis in Human Leukemia Cells

Daniela Trisciuoglio[1], Ylenia Ragazzoni[1], Andrea Pelosi[2], Marianna Desideri[1], Simone Carradori[3], Chiara Gabellini[1,3], Giovanna Maresca[2], Riccardo Nescatelli[3], Daniela Secci[3], Adriana Bolasco[3], Bruna Bizzarri[4], Chiara Cavallere[5], Igea D'Agnano[2], Patrizia Filetici[6], Lucia Ricci-Vitiani[6], Maria Giulia Rizzo[2], and Donatella Del Bufalo[1]

Clin Cancer Res. 2012 Jan 15;18(2):475-86

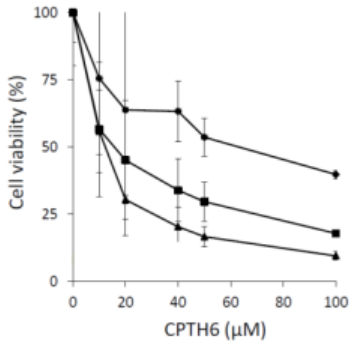
CPTH6 is a specific Gcn5/pCAF inhibitor



HAT activity assay: In vitro assay using purified recombinant enzymes with H3 histone and [^{3}H]acetyl-CoA

- Vehicle
- CPTH6
- AA (Anacardic acid - panHAT inhibitor)

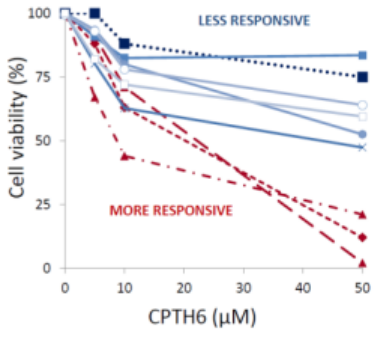
CPTH6 reduces in vitro cell viability of U937 cells



MTT Cell viability assay:
Colorimetric assay based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium.

◆ 24h
■ 48h
▲ 72h

CPTH6 decrease cell viability of NSCLC cells in vitro



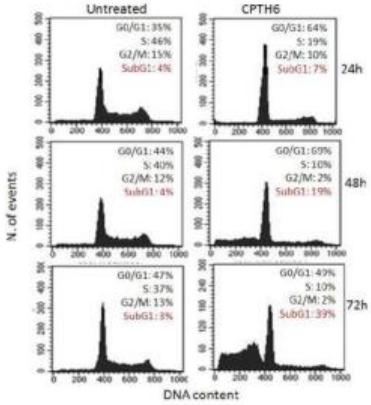
Established commercial cell lines,
Colorimetric assay based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium.

H1299
H460
Calu-1
Calu-3
A549

Patient-derived stem-like cells,
Luminescent Cell Viability Assay based on quantitation of the ATP present.

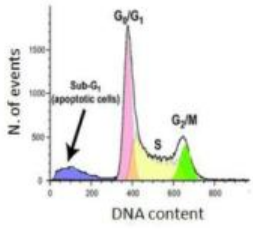
LCSC 136
LCSC 36
LCSC 34
LCSC 143

CPTH6 induces cell-cycle perturbation in U937 cells

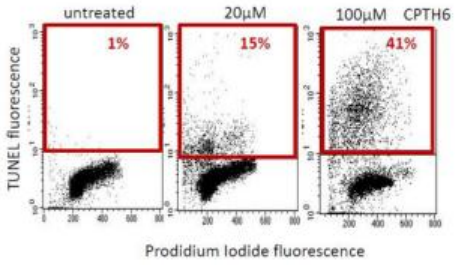


Flow cytometric analysis of DNA content:
Determination of fluorescent intensity of propidium iodide intercalated in DNA.

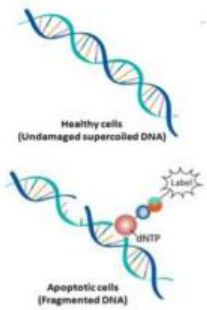
Example of profile:



CPTH6 induces apoptosis in U937 cells

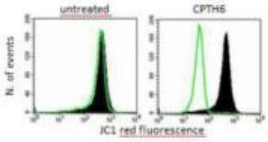


Flow cytometric TUNEL assay:
Apoptosis detection by a two-color assay for labeling DNA breaks and total cellular DNA content.

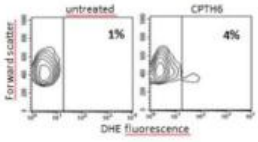


Determination of apoptotic mechanisms induced by CPTH6 treatment in U937 cells

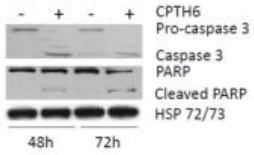
Mitochondrial membrane potential flow cytometric assay (JC1 probe)



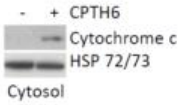
Flow cytometric assay detecting Reactive Oxygen Species production (DHE)



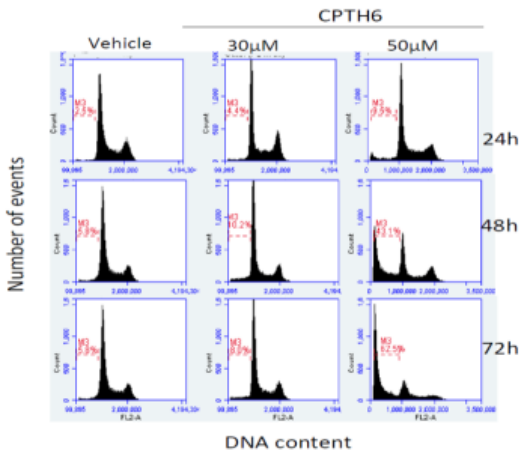
Western Blot analysis:
Detection of apoptotic markers



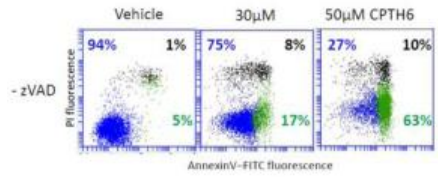
Extraction and Western Blot analysis:
Detection of an apoptotic marker in cytosol



CPTH6 induces apoptosis in patient-derived stem-like LCSC136 cells

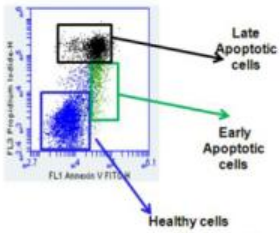


CPTH6 induces specific alterations to the plasma membrane, hallmark of apoptotic cells

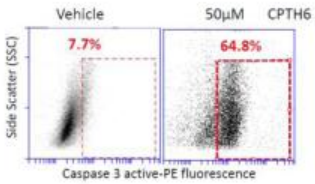


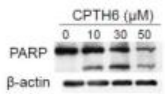
Flow cytometric AnnexinV binding assay:
Fluorescent Annexin V binding to altered plasma membrane of apoptotic cells.

Example of profile:



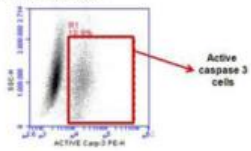
CPTH6 induces Caspase-3 activation and cleavage of PARP in LCSC136





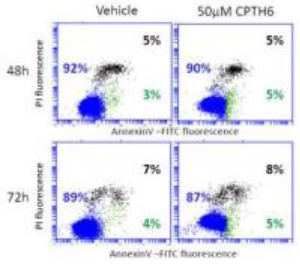
Flow cytometric active caspase 3 detection assay
Detection of the caspase 3 active form using an anti-active caspase-3 antibody.

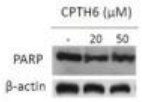
Example of profile



Western Blot analysis:
Detection of cleaved PARP protein, an apoptotic marker

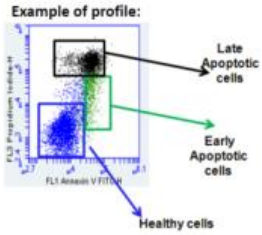
CPTH6 does NOT induce apoptosis in established human H1299 NSCLC cells





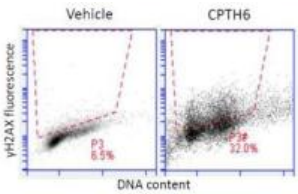
Flow cytometric AnnexinV binding assay:
Fluorescent Annexin V binding to altered plasma membrane of apoptotic cells.

Example of profile:

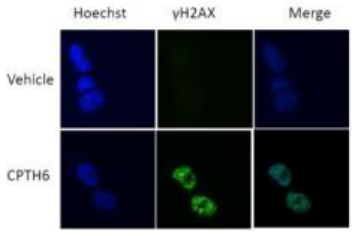


Western Blot analysis:
Detection of cleaved PARP, an apoptotic market

CPTH6 induces DNA damage in established human H1299 NSCLC cells



Flow cytometric γH2AX assay
Detection of H2AX phosporylation, a marker for DNA damage, using a H2AX antibody.



Immunofluorescence analysis of H2AX phosporylation

Summary

- Keep cells at good condition
- It is critical to determine IC50 value
- Since cell lines and drugs vary, concentration of drugs and time of treatment should be titrated
- Use multiple approaches to detect apoptosis

CBA
Cytometric Bead Array

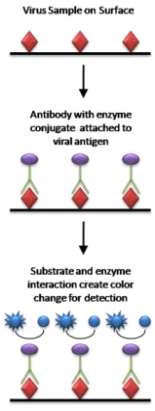
Tools and Techniques for Cytokine Quantitation

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

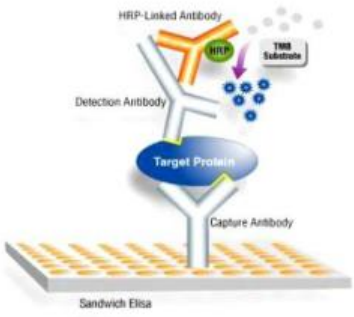
BD

ELISA

Direct

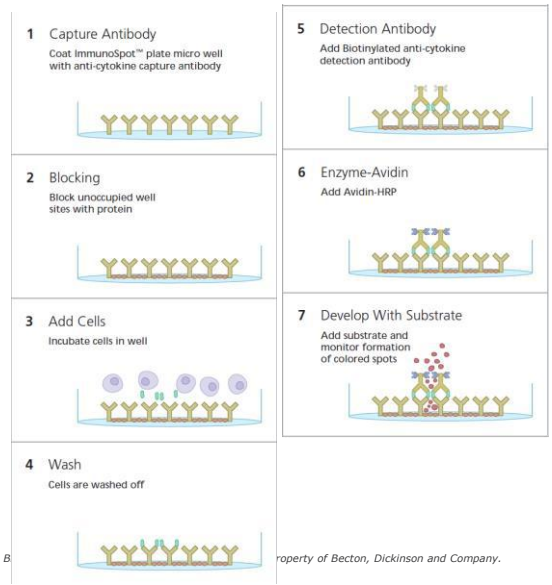


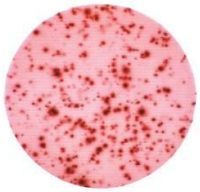
Sandwich

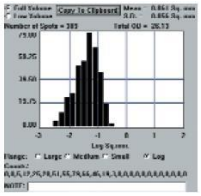


BD

ELISPOT







ELISA v.s. CBA

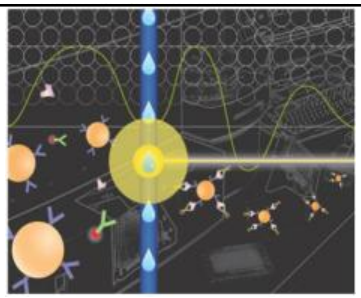
Comparison

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

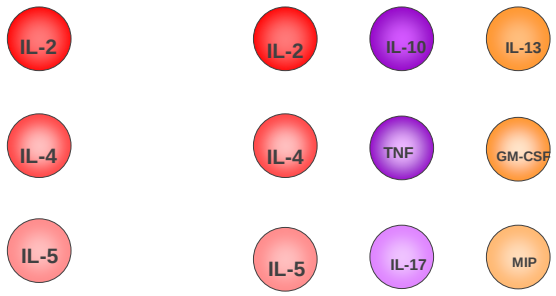


BD CBA : designed for easy and efficient multiplexing

- 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

Beads Provide a Flexible Platform

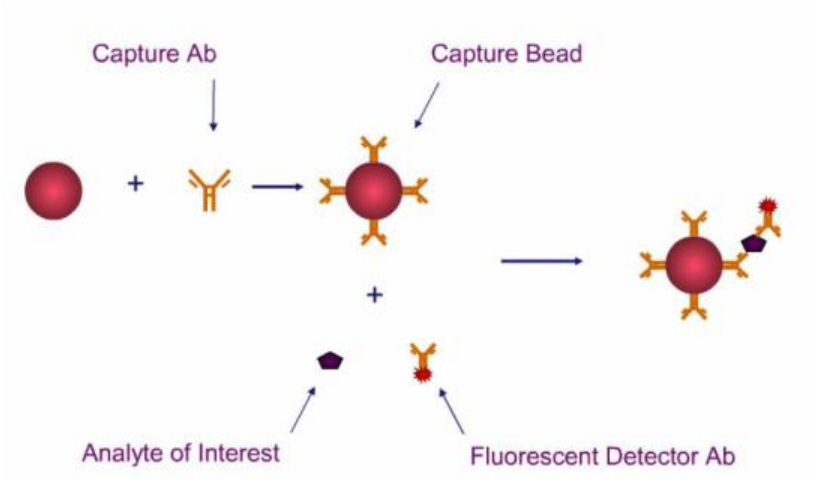


Different fluorescence intensities

Different colors with different intensities

BD

How A Bead-based Immunoassay Works



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 12:Unit12.1.

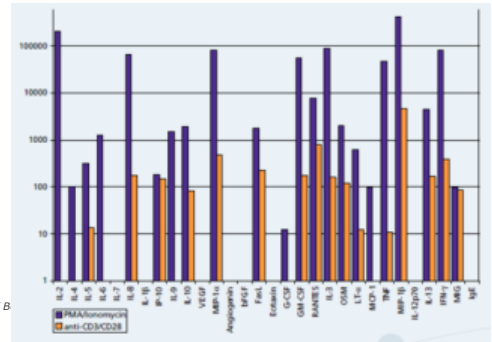
Clinical Immunology Volume 110, Issue 3, March 2004, Pages 252-266

一根釣竿就可以釣起所有想要的魚

BD

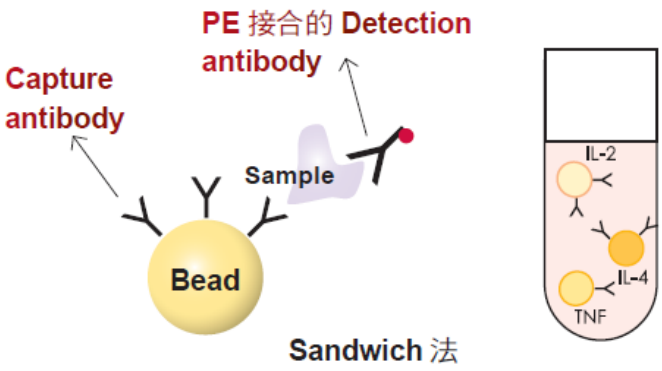
BD™ Cytometric Bead Array (CBA)

- 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.

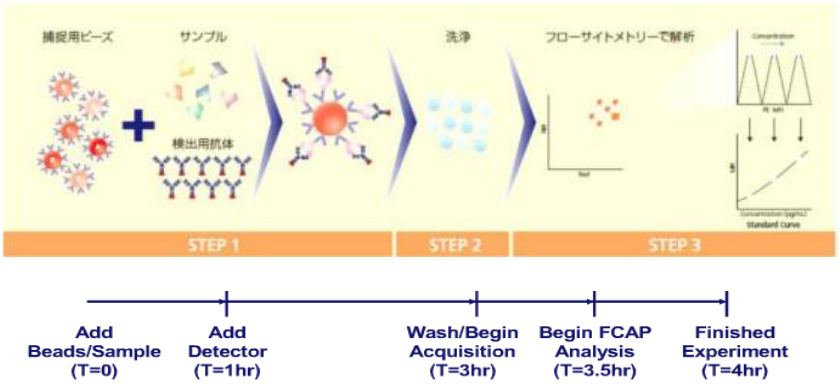


BD™ CBA Flex Set を用いたタンパクレベル解析
ヒト末梢血単核細胞（PBMC）を異なる2つの条件下で刺激し、上清を回収後 BD™ CBA Flex Set assay（ 30 plex ）で測定した。

Assay Principle



Assay Procedure



Product Format

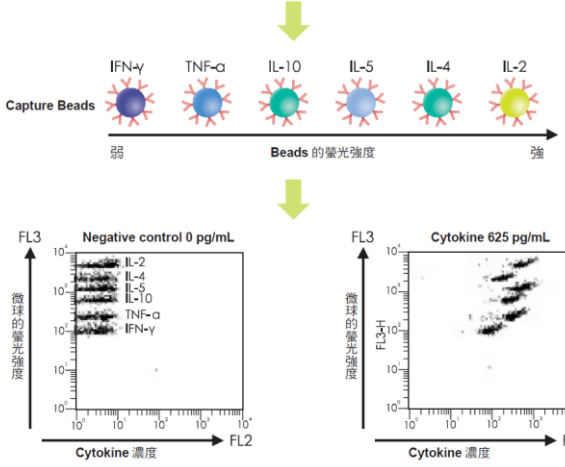
BD CBA Flex Sets	提供開放式的彈性實驗設計，可自由搭配想要偵測的試劑。
BD CBA Kits	將常會一起測試的 **CBA** 試劑合併成套裝組合，例如，**Th1/Th2 cytokine kit**、**Inflammation Kit**，可節省時間。

BD

CBA Kit

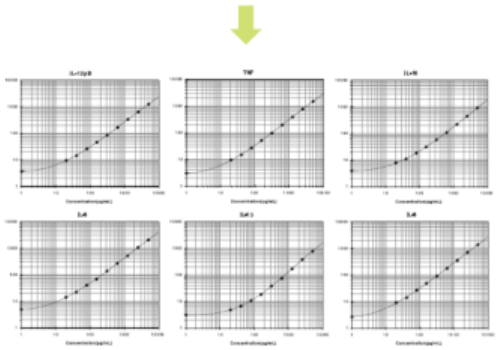
將常常同時偵測的種類，合併成套裝組合

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。

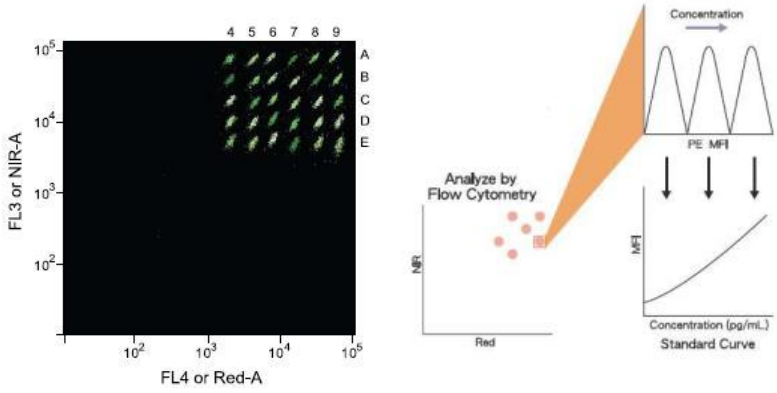
BD

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

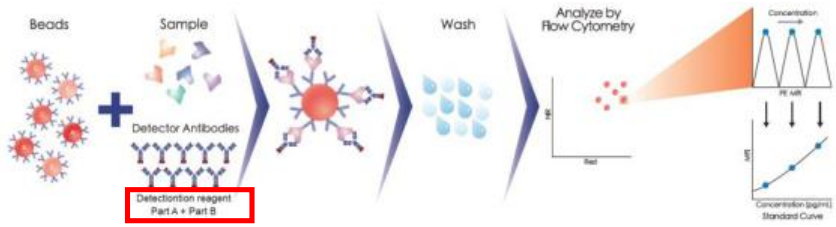
CBA FLEX SET



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**

Enhanced Sensitivity Flex Set System

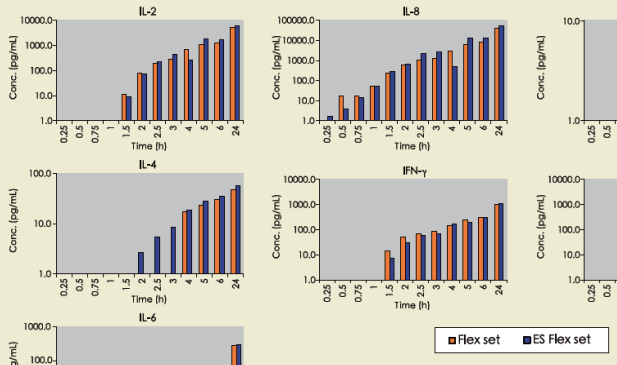


Features

- 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

Time course of human PBMCs stimulated with PMA and Calcium Ionophore

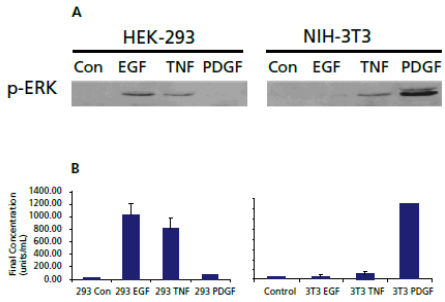


進行不同時間點（Time Course）培養實驗時，利用 BD CBA Enhanced Sensitivity Flex Set system（ES Flex Set）可以準確地在早期偵測到濃度特低的細胞激素變化。

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

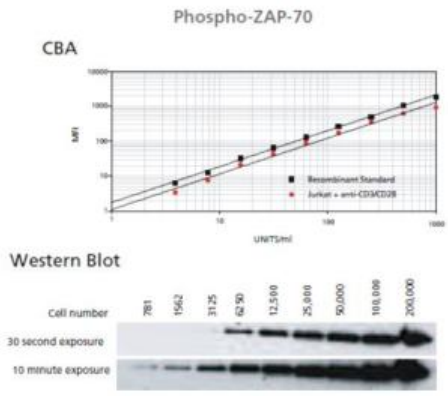


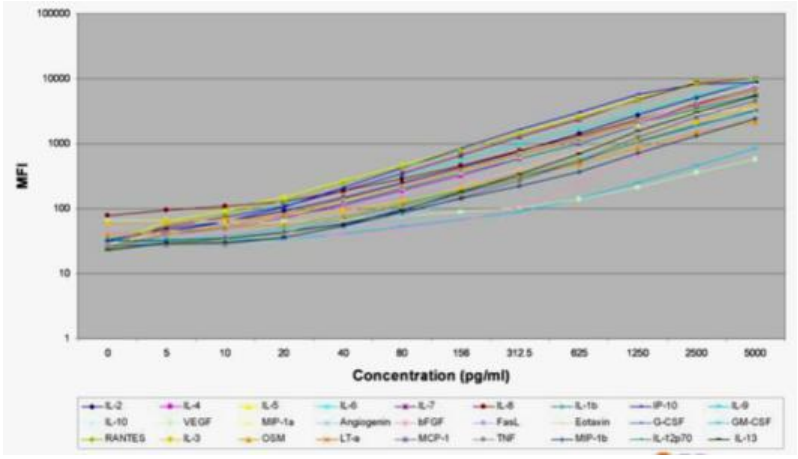
Figure 2. Jurkat cells activated with anti-CD3 and anti-CD28 and crosslinked by anti-mouse Ig for 2 minutes. The reaction was stopped by the addition of SDS (1% final) whereupon the samples were placed in a boiling water bath. A phosphorylated recombinant standard was used to generate a standard curve. 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

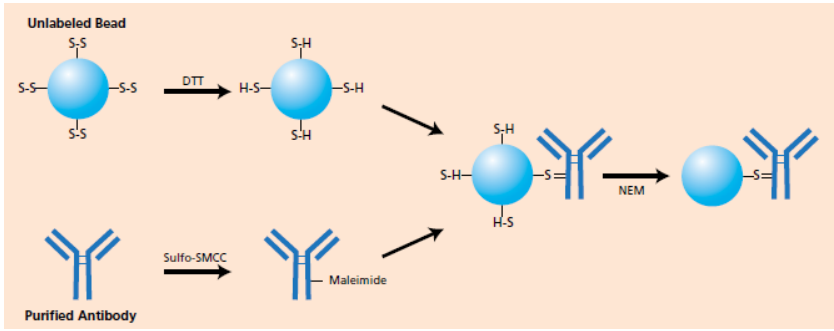
Human soluble protein 30 plex

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

Standard Curve



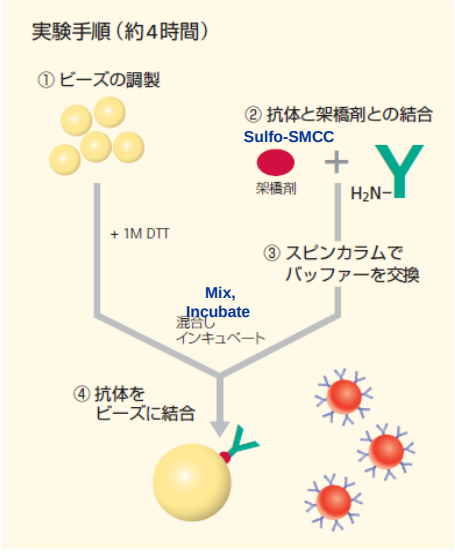
BD™ CBA Functional Bead



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

Thank you!

BD