

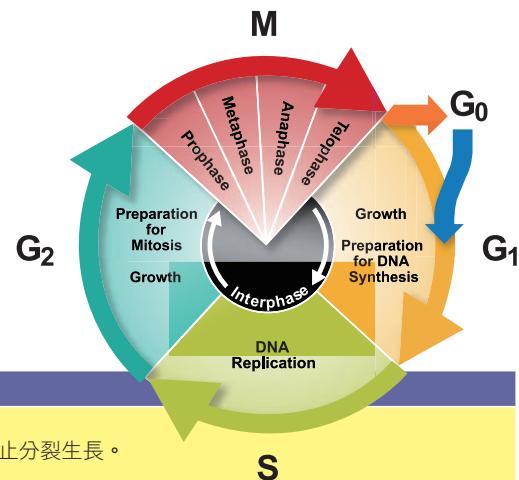
Tools for Assessing Cell Events



細胞週期 Cell cycle

真核細胞，cell cycle 主要分成兩個階段

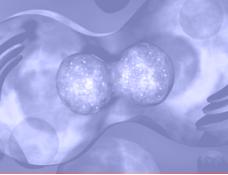
1. 間期 (Interphase)：在此時期，細胞生長、累積進行有絲分裂所需要的養分及複製 DNA。
2. 分裂期 (Mitosis Phase)：細胞在這個時候，分裂成兩個子細胞。藉由 cell cycle，生物體可以由單一受精卵變成一個成熟的個體。並且藉此，生物體可以在生長發育時，更新毛髮、皮膚、血球及一些器官和組織。



狀態	期間	簡稱	說明
靜止 / 老化 (quiescent/senescent)	Gap 0	G0	休止期；細胞離開週期階段，停止分裂生長。
間期 (Interphase)	Gap 1	G1	細胞變大；合成 RNA 及蛋白質，預備進行 DNA 合成。
	Synthesis	S	此時 DNA 進行複製。
	Gap 2	G2	在 DNA 合成和有絲分裂之間，細胞繼續成長。準備進入分裂期進行分裂。
細胞分裂 (Mitosis phase)	Mitosis and cytokinesis	M	這個時期，細胞停止生長。細胞集中進行有絲分裂，由一個母細胞變成兩個子細胞。

偵測 cell cycle 的工具和方式

Measures	Reagents	Mechanism	Technology	Sample Types
DNA	Propidium Iodide (PI)	Interaction into DNA double strands	Flow cytometry	Fixed, permeabilized, and for live/dead discrimination in intact cells
Newly Synthesized DNA	BrdU and antibodies to BrdU	Bromodeoxyuridine replaces thymidine (T) in dividing DNA. It is then detected by antibodies to BrdU.	Flow cytometry, cell imaging, immunohistochemistry	Fixed and permeabilized cells, treated tissues (cell imaging, immunohistochemistry only)
Protein Level	Antibodies to cyclins, retinoblastoma (Rb), other cell cycle markers	Levels go up and down at different stages of the cell cycle.	Flow cytometry, bioimaging, immunohistochemistry, Western blot	Fixed cells, tissues, and extracts
Protein Modification	Antibodies to phosphorylated histone H3, cyclin dependent kinases (cdk)	Proteins become phosphorylated as a result of proliferation or changes to the cell cycle.	Flow cytometry, bioimaging, immunohistochemistry, Western blot	



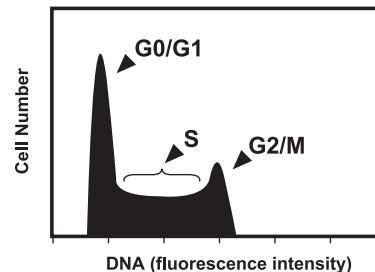
細胞週期 Cell cycle

Flow cytometry 偵測

細胞週期指標：DNA content 的測定

PI是最常用來偵測DNA含量的螢光染劑。其作用原理是細胞固定打洞後，PI會染上核酸(DNA & RNA)，並搭配RNase處理後，再進行染色後，以流式細胞儀測定DNA含量。

- PI可用488 nm雷射激發，散射波長約617 nm。
- Hoechst 33342需用UV雷射激發，散射波長約461 nm。



BD 核酸染劑產品

Cat. No.	Description	Flow Cytometry		Excitation Laser	Fluorescence Channel	Ex (nm)	Em (nm)				
		DNA Content (cell cycle)									
		Live	Fixed								
561908	BD Pharmingen Hoechst 33342 Solution	√	√	UV/Violet	DAPI(UV)/BV421(V)	350	461				
564902 564903	BD Pharmingen DRAQ5™	√	√	Red/YG	Alexa Fluor® 700/ APC/PE-Cy7	600/646	697				
564907	BD Pharmingen DAPI Solution		√	UV/Violet	DAPI(UV)/BV421(V)	358	461				
565799 565802	BD Via-Probe Green Nucleic Acid Stain		√	Blue	FITC	503	526				
550825	BD Pharmingen PI /RNase Staining Buffer		√	YG/Blue	PE	535	617				
559925 555815 555806	BD Pharmingen 7-AAD		√	YG/Blue	PerCP-Cy™ 5.5	546	647				
565803 565804	BD Via-Probe Red Nucleic Acid Stain		√	Red	APC	642	660				
564904	BD Pharmingen DRAQ7™		√	Red/YG	Alexa Fluor® 700/ APC/PE-Cy7	599/644	694				

DRAQ5 and DRAQ7 are also suboptimally excited by the blue laser.

Flow cytometry 偵測

MERCK Cell Cycle Analysis Kit

利用細胞核染劑來偵測DNA含量的螢光染劑。其作用原理是細胞固定打洞，再進行染色後，以流式細胞儀(excitation filter: 488 nm)測定DNA含量。

- 操作簡單：僅需室溫反應30分鐘。
- 安全：非放射性材料，安全無虞。

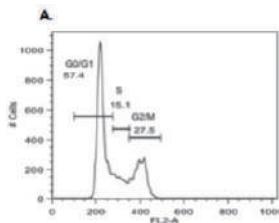


Figure 1A.
Cell cycle analysis of 3T3 cells in 10% FBS culture medium.

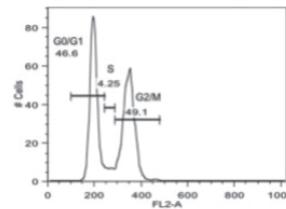
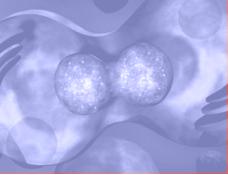


Figure 1B.
Cell cycle analysis of 3T3 cells in 10% FBS culture medium containing 20 M of SKPin C1.

Cat. No.	Description	Content	Size	Detection
MAK344	Cell Cycle Analysis Kit	10x Cell Cycle Assay Buffer 50 mL Enzyme A Solution Nuclear Dye	100 Assays	Fluorometric



細胞週期 Cell cycle



Immunofluorescence 偵測

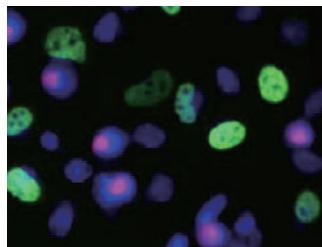
Cell cycle kit

(Cat. No. 558662) – 偵測標的 : BrdU & Histone H3 (pS28)

Histone 會和 DNA 複合形成染色質 (Chromatin)，在哺乳類細胞進行有絲分裂或減數分裂時候，Histone H3 會在 serine 28 (S28)、S10 和 threonine 11 等位置會磷酸化。S10 會在 G2 phase 晚期到 anaphase 期間發生磷酸化；S28 會在 prophase 到 anaphase 期間發生磷酸化。

BD Cell cycle kit 結合了 Alexa Fluor® 488 Mouse anti-BrdU 與 Alexa Fluor® 647 Rat anti-Histone H3 (pS28)，利用免疫螢光染色方式偵測實驗細胞其細胞週期的變化。

- **BrdU**：偵測 **S phase - Bromodeoxyuridine (BrdU)** 是核苷酸胸線嘧啶 (**Thymidine**) 的類似物，當細胞要合成新的 DNA 時 (**S phase**)，BrdU 可以被細胞攝入並取代 Tymidine 合成 DNA，利用 **anti-BrdU** 融光抗體以辨識 BrdU，以鑑定細胞之 **S phase**。
- **Histone H3 (pS28)**：偵測 **M phase - 細胞在有絲分裂期發生染色體濃縮 (chromosome condensation)** 時，其 **Histone H3 serine 28** 位置發生磷酸化現象，故利用 **Phospho-Histone H3** 抗體鑑定細胞之 **Mphase**。



Confocal image, using the BD Pathway™ 435 Bioimaging system and a 20x (0.75 NA) objective, of HeLa cells that were stained with the three kit components, Alexa Fluor® 488 Mouse anti-BrdU (pseudo-colored green), Alexa Fluor® 647 Rat anti-Histone H3 (pS28) (pseudo-colored red) and Hoechst 33342 (pseudo-colored blue). Costaining of Hoechst 33342 and Histone H3 (pS28) appears pink.

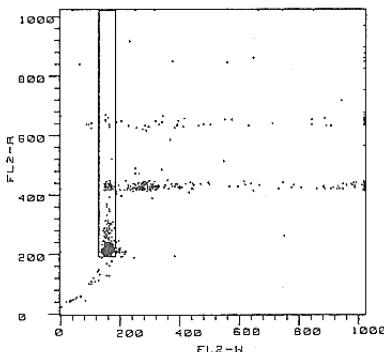
Cat. No.	name	Content	Size
558662	Cell Cycle Kit	Alexa Fluor® 488 Mouse anti-BrdU Alexa Fluor® 647 Rat anti-Histone H3 (pS28) 5x Fixation Buffer Perm Buffer II Stain Buffer (FBS) PBS (10X) Concentrate Hoechst 33342 Solution BrdU DNase	100 Tests

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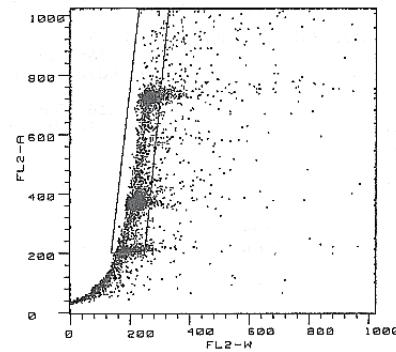
BD Cyclatest™ Plus DNA Reagent Kit

適用於新鮮或冷凍固態組織亦或是懸浮細胞，來進行細胞核染色。利用流式細胞儀可以分析正常細胞與腫瘤細胞染色上的差異。藉由此技術來鑑定異常 DNA stemline 的 DNA 指數 (DNA index, DI) 和細胞週期相分佈。搭配 BD DNA QC particles kit，可進行儀器電壓設定調整，確認儀器線性度及靈敏度。

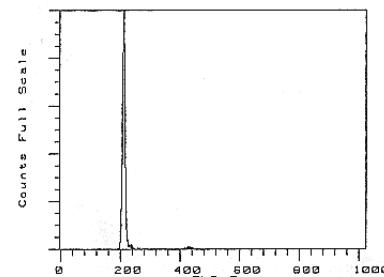
- ① FL2-W vs FL2-A dot plot of PBMCs showing a singlet gate, which excludes aggregates.



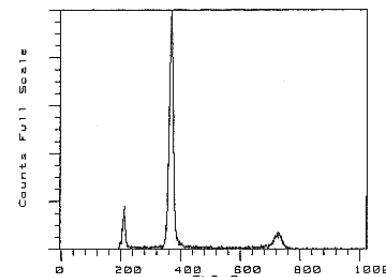
- ③ FL2-W vs FL2-A dot plot of breast tumor tissue showing a singlet gate, which excludes aggregates.



- ② FL2-A DNA histogram of PBMCs, which has been gated to exclude aggregates.

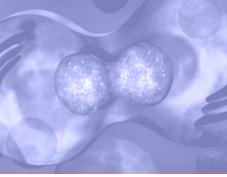


- ④ FL2-A DNA histogram of breast tumor tissue, which has been gated to exclude aggregates.



Cat. No.	Name	Content	Size
340242	BD Cyclatest™ Plus DNA Reagent Kit	Trypsin Solution, Trypsin Inhibitor/Ribonuclease A Solution, Propidium Iodide Solution, Citrate Buffer.	40 Tests RUO (GMP)
349523	BD™ DNA QC Particles	Vial A: 1-mL suspension of CEN in buffer and ethanol	25 Tests RUO (GMP)
		Vial B: 1-mL suspension of CTN in buffer with formaldehyde and 0.01% thimerosal	
		Vial C: 1-mL suspension of 2-μm fluorescent beads in buffer with gelatin and 0.1% sodium azide	
		Vial D: 50 mL of 50-μg/mL solution of PI in buffer (25 tests using CEN and CTN)	

Chicken erythrocyte nuclei (CEN)| calf thymocyte nuclei (CTN)| instrument QC beads| propidium iodide solution.
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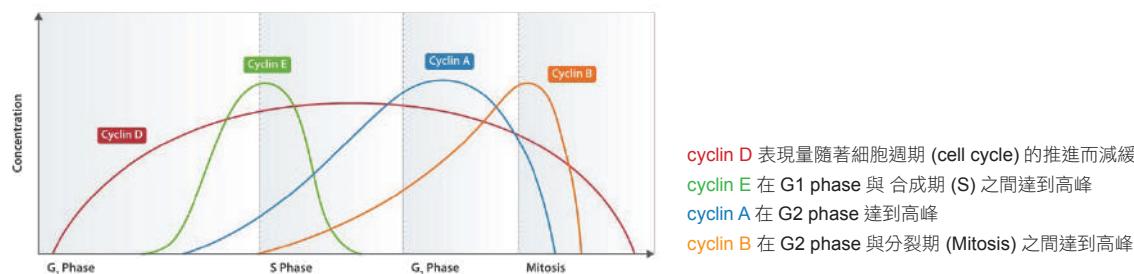


細胞週期 Cell cycle

MERCK

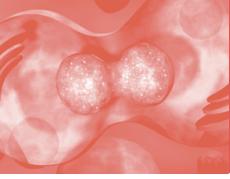
Cell cycle 調控蛋白

- 主要由 CDK、Cyclins 與 CKI 三種蛋白組成。
- CDK(Cyclin-dependent Protein Kinase)**：細胞週期調控中主要執行磷酸化的激酶，受到 CKI 與 Cyclins 調節。
- CKI (Inhibitor of CDK)**：負責與 CDK 結合，以抑制 CDK 的活性。
- Cyclins**：細胞週期蛋白，用來活化 CDK 的活性。



Cell Cycle 相關抗體

Cat. No.	Description	Species Reactivity	Key Applications	Host	Format	Antibody Type
05-507	Anti-Cdc25C Antibody, clone TC-15	H, M	IP, WB, ICC	M	Purified	Monoclonal Antibody
MAB8878	Anti-Cdk1 Antibody, clone A17.1.1	Ch, H, M, R, Xn	IF, IP, Enzyme Assays, WB, IH(P)	M	Purified	Monoclonal Antibody
06-923	Anti-Cdk1/Cdc2 (PSTAIR) Antibody	Am, Ch, H, M, R, Sh, Dr, Mk, Ca	ICC, IP, WB	Rb	Purified	Polyclonal Antibody
06-923	Anti-Cdk1/Cdc2 (PSTAIR) Antibody	Am, Ch, H, M, R, Sh, Dr, Mk, Ca	ICC, IP, WB	Rb	Purified	Polyclonal Antibody
06-966	Anti-CDK1/CDC2 Antibody (C-Term)	M	IP, WB	Rb	Serum	Polyclonal Antibody
07-631	Anti-cdk2 Antibody	H	IP, WB	Rb	Purified	Polyclonal Antibody
05-596	Anti-Cdk2 Antibody, clone AN4.3	H, M, Xn	IP, WB	M	Purified	Monoclonal Antibody
MAB8879	Anti-Cdk4 Antibody, clone DCS-35	H, M, Po, R	IF, IP, Enzyme Assays, WB, IHC	M	Purified	Monoclonal Antibody
05-364	Anti-Cdk5 Antibody, clone DC17	Dr, H, M, R	IP, WB, ICC	M	Purified	Monoclonal Antibody
06-1398	Anti-CDK5RAP2 Antibody	H	WB, ICC	Rb	Affinity Purified	Polyclonal Antibody
06-138	Anti-Cyclin A Antibody	H, M, R, Sh	IP, WB, ICC	Rb	Purified	Polyclonal Antibody
MAB3680	Anti-Cyclin A Antibody, clone E23.1	B, H, M	WB, ICC	M	Ascites	Monoclonal Antibody
MAB3682	Anti-Cyclin A Antibody, clone E67.1	B, H, M, Mi	IP, WB	M	Ascites	Monoclonal Antibody
05-373	Anti-Cyclin B1 Antibody, clone GNS3 (8A5D12)	H, M	IP, WB	M	Purified	Monoclonal Antibody
MAB3684	Anti-Cyclin B1 Antibody, clone V152	H, M	WB, IH(P)	M	Ascites	Monoclonal Antibody
06-137	Anti-Cyclin D Antibody	H, M, R	ICC, IP, WB	Rb	Purified	Polyclonal Antibody
ABE52	Anti-Cyclin D Antibody	H, M, Pm	WB, ICC, IP	Rb	Affinity Purified	Polyclonal Antibody
CC12-100UGCN	Anti-Cyclin D1 (Ab-3) Mouse mAb (DCS-6)	H, M, R	WB, IHC, IH(P), FC, IF, IP, Neut	M	Purified	Monoclonal Antibody
05-362	Anti-Cyclin D1/2 Antibody, clone 5D4	H, Ht, M, R	ICC, IP, WB	M	Purified	Monoclonal Antibody
05-363	Anti-Cyclin E Antibody, clone HE12	H	IP, WB, ICC	M	Purified	Monoclonal Antibody
06-1289	Anti-Cyclin T1 Antibody	H, M	WB	Sh	Serum	Polyclonal Antibody
MABE229	Anti-phospho-Cdk1 (Thr14, Tyr15) Antibody, clone CP3.2	H	WB, ICC	M	Purified	Monoclonal Antibody



細胞增生 Proliferation

Cell Proliferation

是指細胞生長、分裂導致細胞數目變多，可藉由細胞週期 (cell cycle) 來調控。

在原核細胞，cell cycle 主要藉由分裂生殖 (binary fission) 來完成；

在真核細胞，cell cycle 主要可分成兩個階段：

1. 間期 (Interphase)：在此時期，細胞生長、累積進行有絲分裂所需要的養分及複製 DNA。

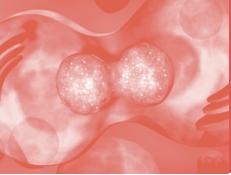
2. 分裂期 (Mitosis Phase)：細胞在這個時候，分裂成兩個子細胞。藉由 cell cycle，生物體可以由單一受精卵變成一個成熟的個體。並且藉此，生物體可以在生長發育時，更新毛髮、皮膚、血球及一些器官和組織。

Cell proliferation rate 細胞增生速率對於細胞研究相當重要，

包括細胞生長、細胞分化、細胞毒殺試驗等等，是探討細胞健康的重要指標之一。

偵測 Cell Proliferation 的工具和方式

試劑	樣品類型	機轉	偵測方式
BD Horizon™ cell proliferation dyes CFSE & Violet Proliferation Dye 450 (VPD 450)	Live proliferating cells	Diffuses into live cells and is hydrolyzed by intracellular non-specific esterases to become fluorescent products.	Flow cytometry Cell imaging
BrdU/EdU incorporation	DNA Synthesis	Bromodeoxyuridine replaces thymidine (T) in dividing DNA and is then detected by antibodies to BrdU	Flow cytometry Cell imaging Immunohistochemistry
Antibodies to Ki-67, PCNA	Protein level	Levels increase as a result of proliferation	Flow cytometry Cell imaging Immunohistochemistry Western blot



細胞增生 Proliferation

BD
MERCK

Flow cytometry 偵測

BD Horizon™ Cell Proliferation Dyes

CFSE & Violet Proliferation Dye 450 (VPD 450)

原理

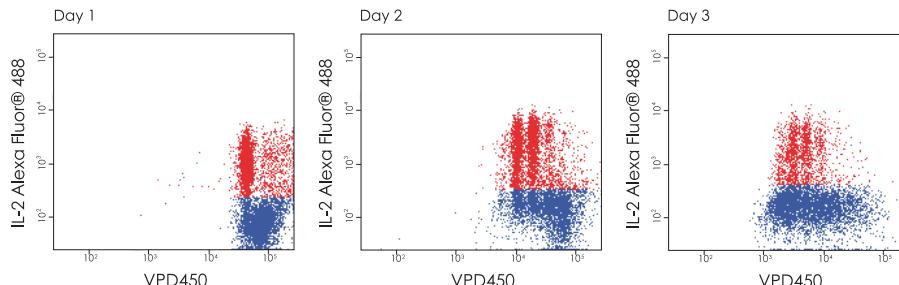
染劑本身是非螢光型態的物質，經由擴散作用到細胞內，其 ester group 會被非專一性的酯酶 (esterase) 水解 (hydrolysis) 成螢光型態，並以 succinimidyl ester group 共價結合細胞內蛋白質的氨基 (amino group)。死細胞就不會發螢光。

- 活細胞分裂時，每個子細胞所含螢光量約為母細胞的一半，可藉由螢光量的減少偵測細胞增生。
- 能耐受細胞固定液與打洞液，能搭配 multi-color stain 增加實驗設計彈性。

實驗數據

Staining of intracellular phenotypes on activated mouse splenocytes

BD #562158



CD4+ mouse splenocytes 加入 1 μ M VPD450。10 分鐘後，細胞用 anti-CD3/CD28 刺激。在收集細胞前的 4-6 小時，細胞加入 PMA 和 ionomycin、BD Golgi-Stop™ (protein transport inhibitor)。細胞在固定、打洞後，進行 IL-2 染色。可見隨著時間增加，細胞增殖，VPD450 的螢光強度也漸漸變弱。

Cat. No.	Description	Laser	Equivalent Fluorochromes*	Size
562158	BD Horizon Violet Proliferation Dye 450	Violet	V450, Pacific Blue™, BV421	1 mg
565082	BD Horizon CFSE	Blue	FITC, Alexa Fluor® 488	1 mg

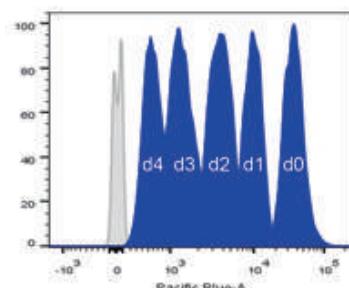
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Flow cytometry/Microscope 偵測

MERCK 活細胞染劑

BioTracker 405 Blue SE Cell Proliferation Kit

- 可用於長期細胞標記。
- 偵測體內或體外細胞分裂。
- 利用螢光顯微鏡觀察被標記的細胞質。
- 利用 ELISA reader 定量細胞數。



Cat. No.	Description	應用	螢光
SCT110	BioTracker 488 Green CFSE Cell Proliferation Kit	偵測 carboxyfluorescein succinimidyl ester (CFSE) used for fluorescent cell labeling in applications.	Green
SCT111	BioTracker 405 Blue SE Cell Proliferation Kit	Live cell imaging cell proliferation kit used for fluorescent cell labeling in flow cytometry applications.	Blue

Flow cytometry 偵測

偵測新合成 DNA : BrdU

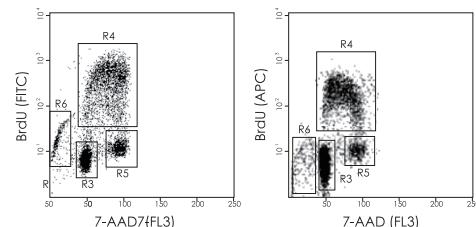
直接測定 DNA 合成是細胞增殖檢測的最準確方法之一，是測定物質毒性、評估藥物安全評價、細胞健康的基本方法，目前常用的方式就是在實驗中加入 thymidine 的類似物，Bromodeoxyuridine (BrdU)。在 S 期的時候，BrdU 可以代替 thymidine，崁入新合成的 DNA 內。再用 BrdU 的抗體與其結合，以流式細胞儀 (Flow Cytometry)、免疫化學染色 (Immunohistochemistry) 及酵素免疫分析 (ELISA) 等常用的細胞分析方式來系列量化 BrdU-positive cell，進而得知細胞週期 S 期的表現。

可適用於體外偵測

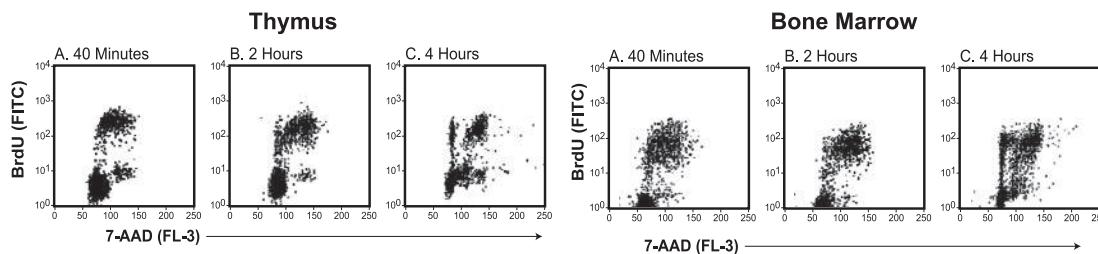
Cell cycle analysis of a population stained for incorporated BrdU and total DNA levels (7-AAD).

Human PBMC 用 anti-CD3 和 anti-CD28 刺激 48 小時後，再用 PMA、Ionomycin 處理 4 小時。
在最後一個小時加入 BrdU。收集細胞後進行 BrdU 染色步驟。

Region	Cell Cycle Phase
R3	G0/G1
R4	S
R5	G2+M
R6	Sub-G0/G1



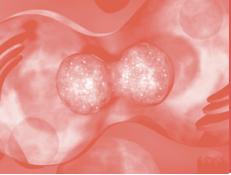
可適用於體內偵測



C57BL/6 在不同的時間點，IP 注射 1mg BrdU。小鼠分別在注射後 40 分鐘、2 小時、4 小時犧牲，取胸腺 (Thymus) 及骨髓 (Bone Marrow) 細胞懸浮液進行 anti-BrdU FITC 和 7-AAD 染色。結果如左，40 分鐘細胞呈現馬蹄型。此種型態為短時間標定 BrdU 的經典型態。隨著時間變常，原本在 S 期的細胞會回到 G0/G1，在 4 小時可以觀察到明顯 BrdU+ G0/G1 週期的細胞。

Cat. No.	Description	Content	Size
559619	FITC BrdU Flow Kit	FITC-conjugated Anti-BrdU Antibody, BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution, BD Perm/Wash™ Buffer (10×), BD Cytoperm™ Plus Permeabilization Buffer, 7-AAD, BrdU, Dnase	50 Tests
557891	FITC BrdU Flow Kit		200 Tests
552598	APC BrdU Flow Kit	APC-conjugated Anti-BrdU Antibody, BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution, BD Perm/Wash™ Buffer (10×), BD Cytoperm™ Plus Permeabilization Buffer, 7-AAD, BrdU, Dnase	50 Tests
557892	APC BrdU Flow Kit		200 Tests

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細胞增生 Proliferation



Immunohistochemistry 偵測

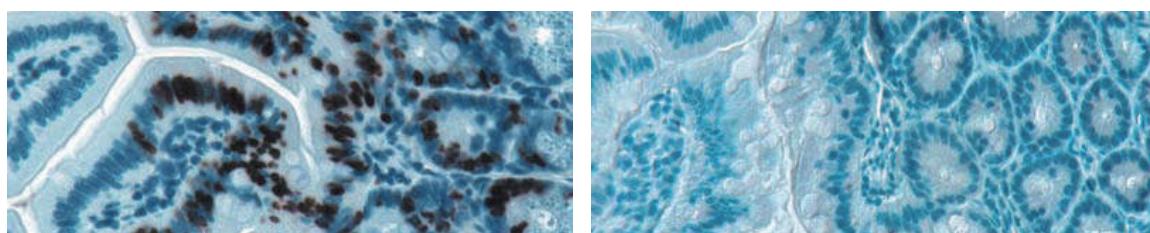
BrdU In-Situ Detection Kit

- Kit 內附 anti-BrdU 螢光抗體辨識 BrdU，鑑定細胞之 S phase。
- 適用樣本：**frozen sections, formalin-fixed paraffin-embedded sections, cultured isolated cells on slides**。
- 適用樣本物種：**human、mouse、rat**。
- Kit 內附 BD™ Retrievagen A，可幫助有效揭露抗原位置，保留組織型態，以便成功地在 DNA stands 進行 BrdU 染色。
- 實驗彈性，視實驗設計可再加入其他抗體或是偵測表面抗原之抗體。
- 提供 Control slides 以確定實驗的準確性。

原理

偵測 S phase - Bromodeoxyuridine (BrdU) 是核苷酸胸線嘧啶 (Thymidine) 的類似物，當細胞要合成新的 DNA 時 (S phase)，BrdU 可以被細胞攝入並取代 Tymidine 合成 DNA，利用 anti-BrdU 融光抗體以辨識 BrdU，以鑑定細胞之 S phase。

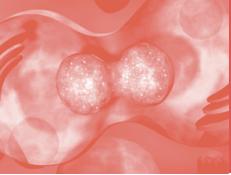
實驗數據



Immunohistochemical staining of BrdU in paraffin sections. BALB/c mice were injected with 1 mg of BrdU via the intra-peritoneal route. After 24 hrs the spleen, thymus, and gastrointestinal tract were harvested and processed for paraffin sections. Mice injected with PBS served as the negative control. Immunohistochemical staining of BrdU was performed using the BrdU In-Situ Detection Kit on paraffin sections of the mouse gastro-intestinal tract. Proliferating cells in the crypts that incorporated BrdU can be identified by the dark brown color in their cell nuclei (A) in contrast to the control (B). Magnification 400X.

Cat. No.	Name	Contents	Size
550803	BrdU In-Situ Detection Kit	Biotin anti-BrdU antibody, BD™ Retrievagen A antigen retrieval solution, streptavidin-horseradish peroxidase (SAv-HRP) solution, DAB buffer, DAB chromogen, fixation buffer, diluent buffer, and control slides	50 Tests

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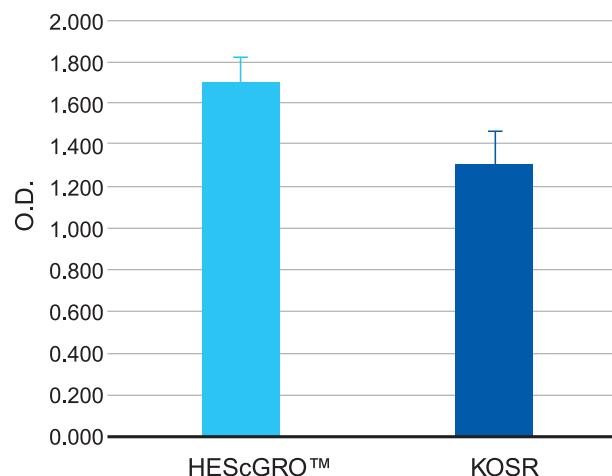
細胞增生 Proliferation

MERCK

ELISA 偵測

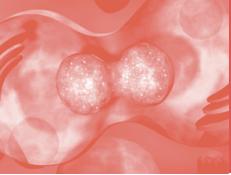
BrdU Cell Proliferation Kit

- 適用於酵素免疫分析試驗 (ELISA)。
- 安全：無須使用同位素即可進行體外定量。
- 應用性廣：適用物種為 **Mouse、Human、Rat、Pig、Horse、Rabbit、Guinea Pig、Hamster、Non-human Primate、Canine**。
- 適用樣本：貼附型 (Adherent) 與懸浮型 (Suspension) 細胞。
- 方便：**Kit** 內附 **BrdU、Fixing Solution、Stop Solution** 以及實驗所需之抗體與試劑。
- 偵測波長：**dual wavelength of 450/550 nm**
(替代波長 **450/540 nm、450/595 nm** 或 **450 nm single**)。



The BrdU cell proliferation kit (Cat. No. 2750) was used to measure proliferation of H9 human embryonic stem cells in HEScGRO™ and KOSR media, after cells were enzymatically expanded for 12 passages. Increased BrdU incorporation indicated faster cell proliferation in HEScGRO™ medium.

Cat. No.	Description	Content	Size	Detection
2750	BrdU Cell Proliferation Kit	BrdU Reagent (500X) 、Fixing Solution 、Prediluted BrdU Detection Antibody 、Stop Solution 、Goat anti-Mouse IgG, Peroxidase labeled (2000X) 、Conjugate Diluent 、Substrate 、Plate Wash Concentrate (50X)	200 Assays	Colorimetric
2752			1000 Assays	



細胞增生 Proliferation



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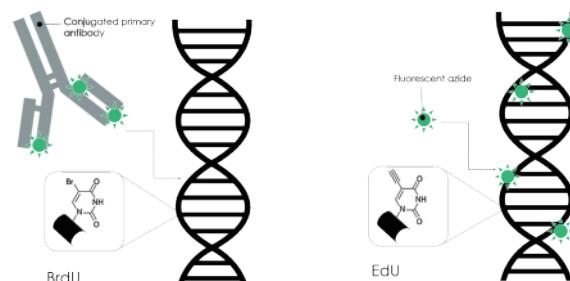
偵測新合成 DNA : EdU

EdU (5-ethynyl-2'-deoxyuridine) 也是一種 thymidine 類似物，在細胞增殖時能夠插入正在複製的 DNA 分子中，基於 EdU 與染料的共軛反應可以有效地檢測處於 S 期的細胞。EdU 染料分子比 BrdU 抗體小，在細胞內更容易擴散，不需要嚴格的樣品變性（酸解、熱解、酶解）處理，有效地避免了樣品損傷，有助於在組織、器官的整體水準上觀測細胞增殖的真實情況，具有更高的靈敏度和更快的檢測速度。

BrdU 與 EdU 比較表

	EdU	BrdU
檢測分子	很小	大
檢測方式	化學反應	免疫反應
DNA 變性	不需	需要
靈敏度	靈敏	一般

BrdU 與 EdU 檢測原理示意圖



Cat. No.	Description	Content	Size	Detection	Equivalent Fluorochromes
565456	BD Pharmingen™ 647 EdU Click Proliferation Kit	EdU (5-ethynyl-2'-deoxyuridine)、Eterneon™ Red 645 azide (10 mM)、Buffer Additive (10×)、Saponin-based Permeabilization and Wash Reagent (10×)、Fixative Solution (4% paraformaldehyde-based)、Catalyst Solution、DMSO	50 Tests	Flow cytometry Bioimaging	APC
565455	BD Pharmingen™ 488 EdU Click Proliferation Kit	EdU (5-ethynyl-2'-deoxyuridine)、6-FAM Azide (10 mM)、Buffer Additive (10×)、Saponin-based Permeabilization and Wash Reagent (10×)、Fixative Solution (4% paraformaldehyde-based)、Catalyst Solution、DMSO	50 Tests	Flow cytometry Bioimaging	FITC

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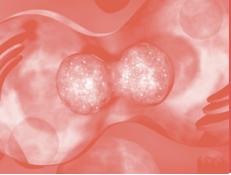
EdU in vivo Kit

- 可直接注射進生物體內，目前文獻成功使用在以下物種：

小鼠 Mouse / 大鼠 Rat / 線蟲 Nematode (C. elegans) / 昆蟲 Cricket (Gryllus bimaculatus) / 雞 Chicken (Gallus domesticus) / 斑馬魚 Zebrafish larva (Danio rerio)

- 可與常用細胞週期染劑兼容，也可同時加入辨識細胞內與細胞表面 marker 的抗體一起使用。
- 應用性廣：針對後續檢體不同分析方式，設計實驗專屬試劑。

Cat. No.	Detection			
Imaging (IM)	Flow Cytometry (FC)	Dye	Excitation/Emission (nm)	Filter
BCK488-IV-IM-S / M / L	BCK488-IV-FC-S / M / L	6-FAM-Azide	abs=496/lem=516	Green
BCK555-IV-IM-S / M / L	BCK555-IV-FC-S / M / L	5-TAMRA-PEG3-Azide	abs=546/lem=579	Violet
BCK594-IV-IM-S / M / L	BCK594-IV-FC-S / M / L	5/6-Sulforhodamine 101-PEG3-Azide	abs=584/lem=603	Orange
BCK647-IV-IM-S / M / L	BCK647-IV-FC-S / M / L	Eterneon Red 645 Azide	abs=643/lem=662	Red



細胞增生 Proliferation

Takara
Clontech Takara cellortis

MERCK

MTT

MTT 是一種黃色、接受氫離子的化合物。與活細胞粒線體中的琥珀酸脫氫酶氧化還原反應產生 Formazan 藍紫色結晶，利用 Isopropanol (含 0.04 N HCl) 將紫色結晶溶解後，藉由測定吸光值評估細胞活性及增殖。

- 價格親民。
- 偵測方法：比色法直接測量 **570nm** 波長。

Cat. No.	Description	Size	Detection
CT01	MTT Cell Growth Assay Kit	5000 Assays	colorimetric
CT02		1000 Assays	

EZMTTT

EZMTTT™ 使用改良過的 monosulfonated tetrazolium salt 的化合物。與活細胞粒線體中的琥珀酸脫氫酶作用，經氧化還原反應產生可溶性的 formazan，可直接測定吸光值 (450 nm) 評估粒線體的活性，即活細胞數目，故可用於作為細胞存活率的指標。

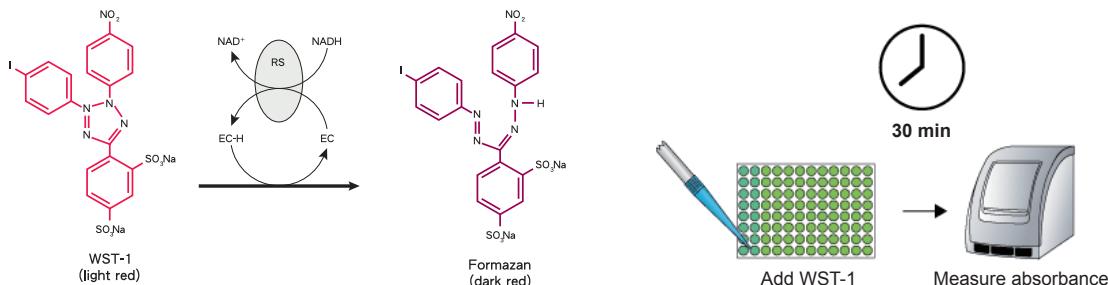
- 靈敏度較傳統 MTT 高。
- 操作簡便：無須進行 DMSO 溶解步驟。
- 偵測方法：比色法直接測量 **450nm** 波長。

Cat. No.	Description	Size	Detection
CBA410	EZMTTTM Cell Proliferation Assay, MTT based, 200X	1000 Assays	colorimetric

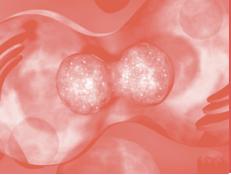
Premixed WST-1 Cell Proliferation Reagent

粒腺體呼吸鏈上的酵素活性往往成為判定細胞是否存活的依據；當細胞死亡時，酵素也跟著失去活性，只有活細胞的酵素才有活性。再加入呈色受質就可以定量存活的細胞量。

- 精確：細胞酵素代謝活性與細胞數量息息相關。
- 方便：馬上可用。
- 簡單：只要一個步驟，不用 **wash**，不需其他試劑。
- 安全：沒有放射性物質，沒有有機溶劑。
- 完全水溶：不用再用 **DMSO** 回溶結晶。



Cat. No.	Description	Size	Detection
MK400	Premix WST-1 Cell Proliferation Assay System	2500 Tests	Colorimetric
QIA127-1KIT	Rapid Cell Proliferation Kit (WST-1)	500 Tests	Colorimetric



細胞增生 Proliferation

Sigma-Aldrich®

CCK-8 (Cell Counting Kit-8)

WST-8 被活細胞內的脫氫酶還原成水溶性的橘黃色產物 WST-8 formazan，產物與活細胞數量成正比，因此可利用此特性直接進行細胞生長和毒性分析。

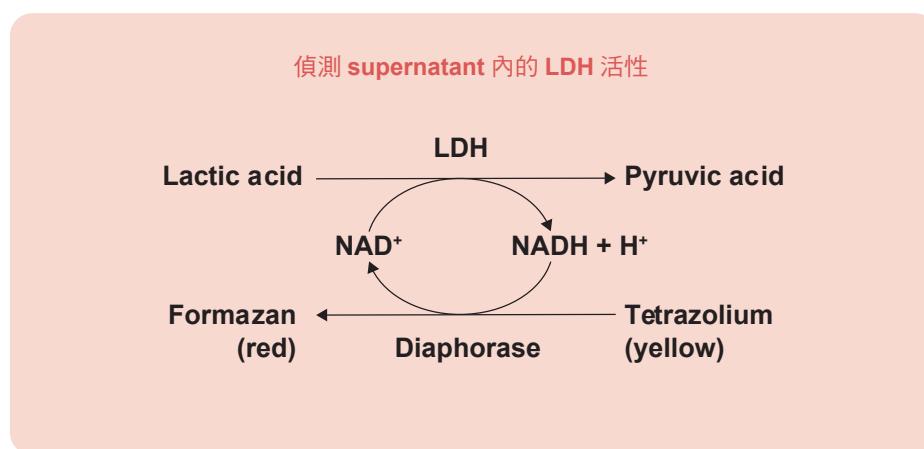
- 靈敏度佳。
- 即用型：直接加入培養液中。
- 操作簡單：不須去除上清液以及使用有機溶劑。
- 低毒性：與細胞反應 **24 - 48 小時** 也 ok。
- 省時：反應時間 **1 - 4 小時**。
- 偵測方法：比色法直接測量 **450nm** 波長。

Cat. No.	Description	Size	Detection
96992-100TESTS-F	Cell Counting Kit 8 (WST-8 / CCK8)	100 Tests	colorimetric
96992-500TESTS-F		500 Tests	
96992-3000TESTS-F		3000 Tests	

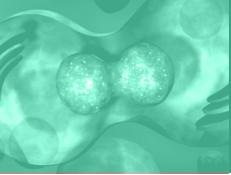
LDH Cytotoxicity Detection Kit

當細胞膜受損或破裂時，位於細胞質的 LDH (Lactate Dehydrase) 便會釋放到細胞外，藉由加入呈色受質就可以定量被毒害的細胞。

- 操作簡單：不用 wash，不用 prelabeling。
- 應用性廣：可使用組織、細胞、血清、紅血球等檢體。
- 安全：非放射性材料，安全無虞。



Cat. No.	Description	Size	Detection
MAK066	Lactate Dehydrogenase Activity Assay Kit	500 Tests	colorimetric



細胞活性 Cell viability



細胞活性 (cell viability) 偵測是分析細胞生長狀況和細胞健康重要的指標之一。

死細胞對於抗體有較強的非特異性結合，當進行流式細胞儀實驗的分析時，利用排除死細胞的 **cell viability dye**，圈出並去除死細胞，能讓分析結果的統計更加精確。

BD™ Cell Viability Kit

BD™ Cell Viability Kit with BD Liquid Counting Beads

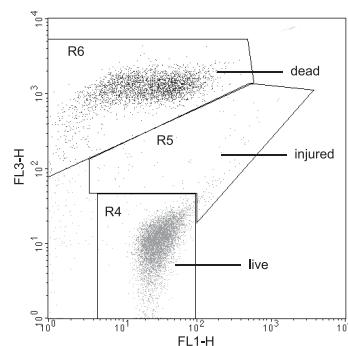
Thiazole Orange (TO) - 可染色所有細胞

Propidium Iodide (PI) - 排除死細胞。

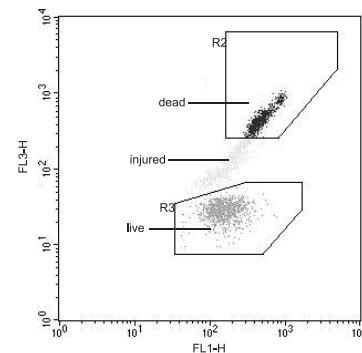
BD Liquid Counting Beads - 將計數微珠加入到樣本中以計算絕對計數。

活細胞具有完整的膜，並且對 PI 的染料是不可滲透的。TO 是一種滲透性染料，會依照細胞存活或死亡而滲透的程度不同。這兩種染料的組合提供了快速可靠區分活的和死的真核和原核細胞的方法，包括外周血單核細胞 (PBMC)，哺乳動物細胞，細菌和酵母。

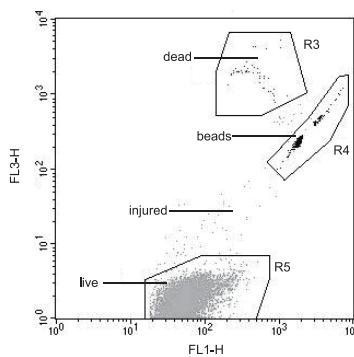
E. coli



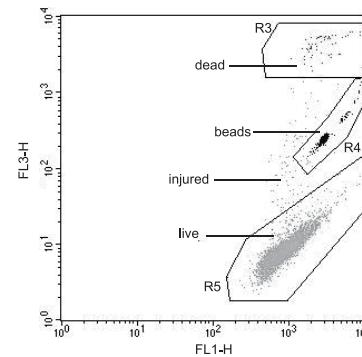
Raji cells



PBMCs



Thawed bull semen



Cat. No.	Name	Content	Size
349483	BD™ Cell Viability Kit	1 vial of 500 µL 42 µmol/L TO in dimethyl sulfoxide (DMSO) 1 vial of 500 µL 4.3 mmol/L PI in water	100 Tests
349480	BD™ Cell Viability Kit with BD Liquid Counting Beads	1 vial of 500 µL 42 µmol/L TO in dimethyl sulfoxide (DMSO) 1 vial of 500 µL 4.3 mmol/L PI in water BD Liquid Counting Beads : 1 vial of 10 mL of fluorescent microspheres in buffer with 0.1% sodium azide	100 Tests

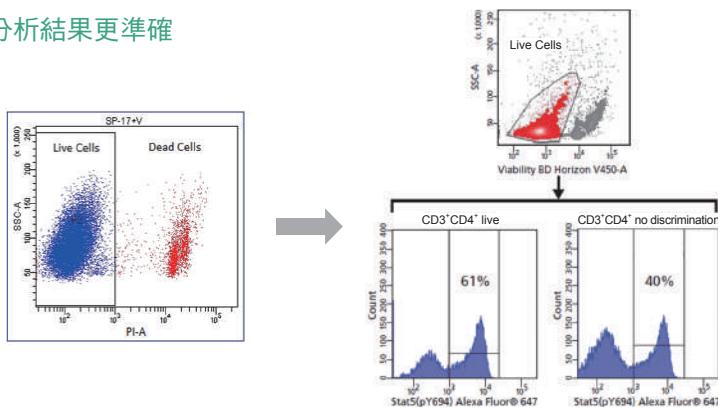
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Flow cytometry 偵測

Cell viability dye

1. PI , 7-AAD : 常用的 cell viability dye , 偵測 DNA 含量 , 但對於固定液和打洞液是無法耐受的。
2. BD Horizon™ Fixable Viability Stain (FVS) Reagents : FVS reagents 會與細胞表面或細胞內的 Amine 共價結合 , 活細胞的細胞膜沒有通透性 , FVS 僅會存於細胞膜表面 ; 但死細胞的細胞膜變得具有通透性 , 細胞表面及細胞內皆可偵測到 FVS 融光 , 比活細胞的 FVS 融光強度強 10-20 倍以上 , 因此便可以簡單辨別出細胞的死活 。最大的優點是對固定液和打洞液具耐受性 。

圈出並排除死細胞讓分析結果更準確



實驗範例

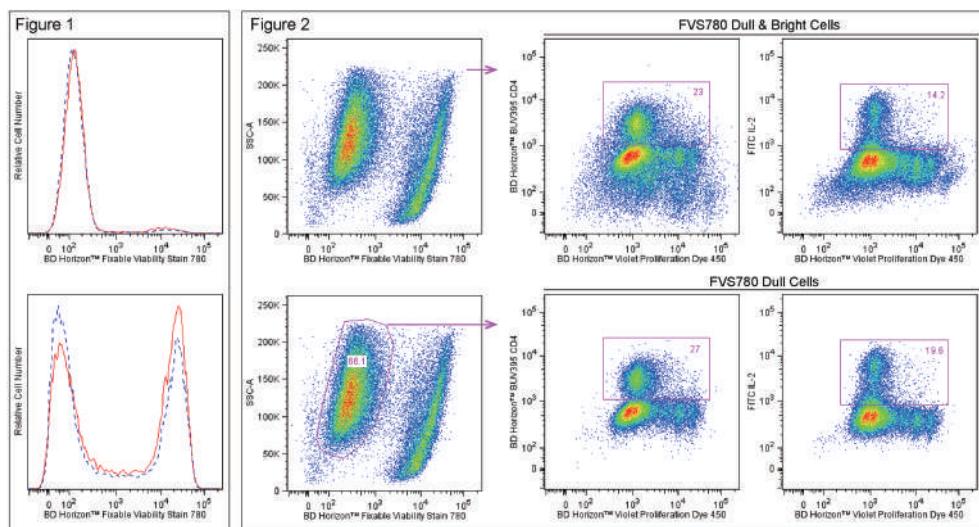
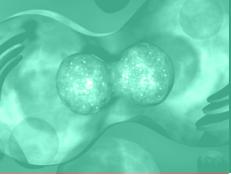


Figure 1: FVS reagent 不受固定液影響

Human Jurkat cell 以 0.025% DMSO (上圖) 或 5 μ M camptothecin (下圖) 處理 16 小時 , 並以 FVS780 染色 , 藍色虛線和紅線分別是有無用 BD Cytofix™ Fixation Buffer 做固定和有無用 BD Perm/Wash Buffer I 做打洞 。

Figure 2 : 分析小鼠脾臟細胞增生和 CD4 、 IL-2 的表現

小鼠脾臟細胞先以 BD Violet Proliferation Dye 450 (Cat. No. 562158) 做染色 , 之後以 BD anti-CD3e 和 CD28 抗體刺激細胞 (3 days) 。再用 PMA, Ionomycin, 和 BD GolgiStop™ Protein Transport Inhibitor 處理細胞 4 小時 。最後 , 將細胞染 FVS780 並用 BD Cytofix/Cytoperm™ Kit 細胞固定打洞後 , 染 BUV395 CD4 和 FITC IL-2 。



細胞活性 Cell viability



BD viability dye 產品

Cat. No.	Description	size	Laser	Equivalent Fluorochromes
對細胞固定與打洞液具耐受性				
566332	BD Horizon™ Fixable Viability Stain 440UV	200 µg	UV	BUV395 or DAPI, Hoechst dyes
562247	BD Horizon™ Fixable Viability Stain 450	0.1 mg	Violet	V450, Pacific Blue™, BV421
564406	BD Horizon™ Fixable Viability Stain 510	0.1 mg	Violet	V500, BV510
565694	BD Horizon™ Fixable Viability Stain 575V	200 µg	Violet	Brilliant Violet 605, Pacific Orange
564407	BD Horizon™ Fixable Viability Stain 520	150 µg	Blue	FITC, Alexa Fluor® 488
564995	BD Horizon™ Fixable Viability Stain 570	150 µg	YG/Blue	PE
564996	BD Horizon™ Fixable Viability Stain 620	100 µg	Yellow-Green/Blue	PE-CF594, PerCP-Cy™ 5.5
564405	BD Horizon™ Fixable Viability Stain 660	100 µg	Red	APC, Alexa Fluor® 647
564997	BD Horizon™ Fixable Viability Stain 700	100 µg	Red	APC-R700, Alexa Fluor® 700
565388	BD Horizon™ Fixable Viability Stain 780	200 µg	Red	APC-Cy7

適用於只做細胞表面染色不需內染的實驗				
565803	BD Via-Probe™ Red Nucleic Acid Stain	0.1 mL	Red	APC
565804	BD Via-Probe™ Red Nucleic Acid Stain	0.5 mL	Red	APC
565802	BD Via-Probe™ Green Nucleic Acid Stain	0.5 mL	Blue	FITC
565799	BD Via-Probe™ Green Nucleic Acid Stain	0.1 mL	Blue	FITC
564907	BD Pharmingen™ DAPI Solution	1 mg	UV/Violet	DAPI (UV)/BV421 (V)
556463	BD Pharmingen™ Propidium Iodide Staining Solution	2 mL	YG/Blue	PE
559925	BD Pharmingen™ 7-AAD	2 mL	YG/Blue	PerCP-Cy™ 5.5
555815	BD Via-Probe™ Cell Viability Solution	500 Tests	YG/Blue	PerCP-Cy™ 5.5
555816	BD Via-Probe™ Cell Viability Solution	100 Tests	YG/Blue	PerCP-Cy™ 5.5

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Flow cytometry、Microscope、螢光盤式微量分析儀 (microplate readers) 偵測

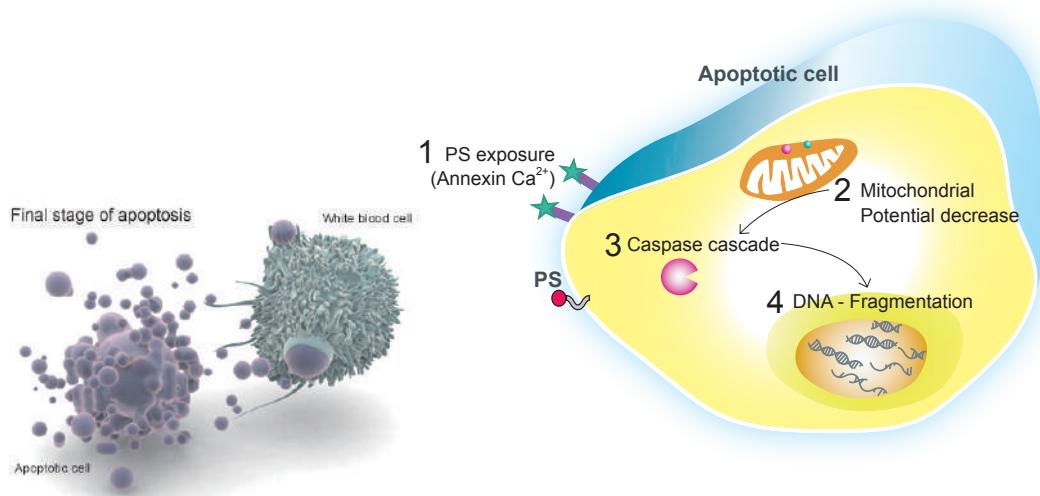
Live Dead Cell Viability Assay Kit for 3D and 2D cell culture

- 高度彈性：適用於一般 3D、2D 細胞培養。
- 應用性廣：可用於流式細胞儀、螢光顯微鏡與螢光微量盤式分析儀 (microplate readers)。
- 操作方便：內含 3 種不同染劑，Calcein AM、Propidium Iodide、Hoechst 33342，可同時染上活細胞、死細胞與所有細胞。

Cat. No.	Description	size	偵測方式
CBA415	Live Dead Cell Viability Assay Kit for 3D and 2D cell culture	5 x 24 - well plates / 12 x 96 - well plates	Fluorometric

Apoptosis (細胞凋亡) 的概念在 1972 年正式由 Kerr JF, Wyllie AH; Currie AR 提出，到了 80 年代後期，隨著分子生物學技術的進步，使細胞凋亡的研究迅速發展。細胞凋亡的起因有可能是因為外界環境、生長發育等等，而細胞為維持生物體內環境的穩定，而進行有程序性的死亡現象 (Programmed cell death, PCD)。

細胞凋亡發生時，細胞會先變圓，隨後細胞皺縮、細胞質密度增加，細胞膜內的 PS (phosphatidylserine) 外翻、粒線體膜電位喪失 (depolarization)、DNA 斷裂，最後細胞會裂解成多個凋亡小體 (apoptotic body) 進而被巨噬細胞清除。



BD Apoptosis Study Tools

Mesures	Hot Sale Items		key Features
	Cat. No.	Name	
Phosphatidylserine Exposure	556547	Annexin V : FITC Apoptosis Detection Kit I	<ul style="list-style-type: none"> • Detects early Apoptosis markers • Quick and easy • Flow cytometry application
	559763	Annexin V : PE Apoptosis Detection Kit I	
Mitochondrial Changes	551302	BD™ MitoScreen (JC-1) Kit	Fast, easy, single cell resolution by flow cytometry or fluorescent microscopy
	564696	MitoStatus TMRE	
	564697	MitoStatus Red	
Caspase Activation	550480	Caspase-3, Active Form, Apoptosis Kit : FITC	<ul style="list-style-type: none"> • Quick and easy • Flow cytometry application
	550914	Caspase-3, Active Form, Apoptosis Kit : PE	
DNA Fragmentation	556381	Apoptosis Detection Kit (APO-Direct)	Works adherent cells, single cell resolution in conjunction with cell cycle analysis by flow cytometry
	556405	Apoptosis Detection Kit (APO-BRDU)	

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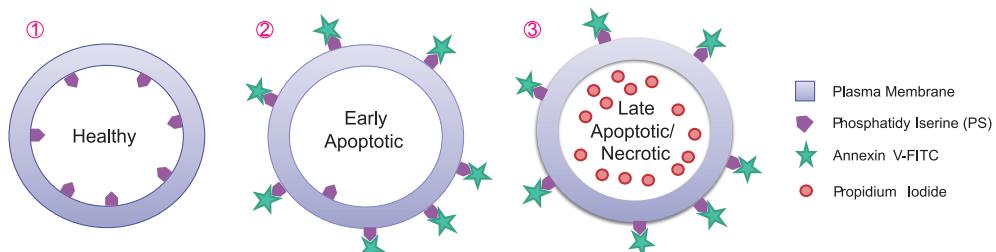
Flow cytometry 偵測

Phosphatidylserine Exposure Annexin V Binding Assay

- 細胞凋亡最早期的特徵。
- 染色操作僅需 15 分鐘。

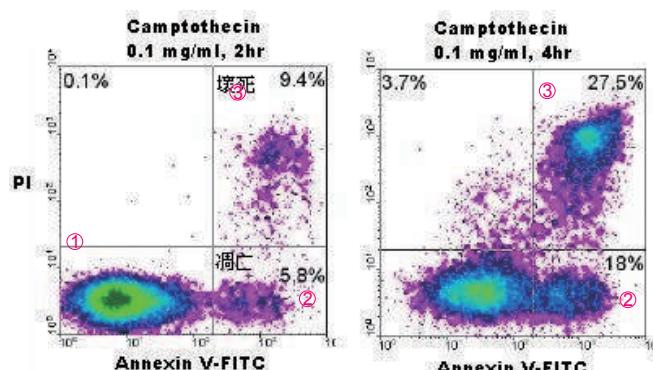
細胞膜變化是在活細胞中檢測到細胞凋亡的第一個特徵。驗證細胞凋亡可以通過檢測磷脂醯絲氨酸（PS）的存在，其通常位於細胞膜的細胞質表面（如 ①）。細胞凋亡過程中，PS 會轉移到質膜的外層（如 ②③），與螢光標記的 Annexin V (Ca²⁺ dependent) 具有高度親和而結合，之後即可用流式細胞儀和細胞成像儀檢測。

原理示意圖



①代表健康的細胞 ②代表細胞凋亡早期的細胞 ③代表細胞凋亡晚期 / 壞死的細胞

實驗範例
BD #556547



熱銷推薦

BD

Cat. No.	Name	Contents	Apps	Size
556547	Annexin V : FITC Apoptosis Detection Kit I	Annexin V-FITC, Propidium Iodide Staining Solution, Annexin V Binding Buffer	FCM	100 Tests
559763	Annexin V : PE Apoptosis Detection Kit I	Annexin V-PE, 7-AAD, and Annexin V Binding Buffer	FCM	100 Tests

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MERCK

Cat. No.	Description	Content	Size	Detection	Flow cytometry	Fluorescence microscopy
CBA059-1KITCN	Annexin V-FITC Apoptosis Detection Kit II	Annexin V-FITC, Binding Buffer, Propidium Iodide	20 Tests	Fluorescence	✓	
APT750	ApopNexin Annexin V FITC Apoptosis Kit	ApopNexin™ FITC, Binding Buffer, 4X ,Propidium Iodide	100 Assays	Fluorescence	✓	✓

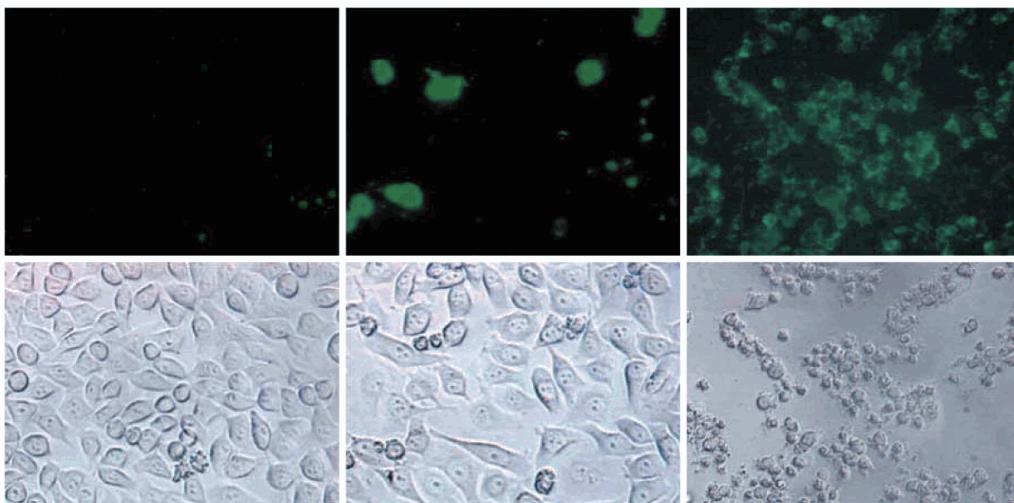
Immunofluorescence 及 Immunohistochemistry 偵測

Phosphatidylserine Exposure Annexin V Binding Assay

- 細胞凋亡最早期的特徵。
- 染色操作僅需 15 分鐘。

實驗範例

#BD 550911



Fluorescence Microscopy Analysis of Annexin V-FITC Staining. HeLa cells were left untreated [top left panel] or induced to undergo apoptosis with camptothecin ($8 \mu M$ camptothecin, 24 h) [top middle panel]; or staurosporine ($1 \mu M$, 4 h) [top right panel] and stained with Annexin V-FITC according to the protocol provided. bottom left panel, bottom middle panel, and bottom right panel represent phase contrast correlates of top left panel, top middle panel, and top bottom panel, respectively. The data shows that untreated cells were negative for Annexin V-FITC staining, whereas positive cells were seen following either camptothecin or staurosporine treatment. The data also shows that more positive cells were seen in the staurosporine- than the camptothecin-treated populations. The staurosporine treatment was also associated with dramatic morphological changes. The amount of Annexin V-FITC positive cells and the cell morphology can vary according to the cell type, model system, treatment type, or time after apoptosis induction.



Cat. No.	name	contents	Apps	sizes
550911	Annexin V-FITC Fluorescence Microscopy Kit	Annexin V-FITC, 10X Annexin V Binding Buffer, 10X PBS Buffer	IF	25 slides

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直接偵測 Phosphatidylserine Exposure

Cat. No.	name	Apps	sizes
05-719	Anti-Phosphatidylserine Antibody, clone 1H6	FC, IHC	200 µg
16-256	Anti-Phosphatidylserine Antibody, clone 1H6, Alexa Fluor® 488	FC	100 µg

Flow cytometry 偵測

Mitochondrial Changes



BD MitoScreen (JC-1) Kit

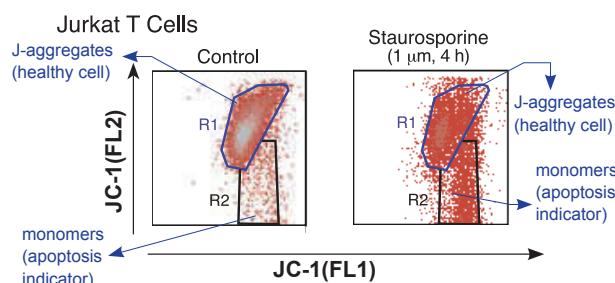
- 可偵測粒線體膜電位變化。
- 適用於 Human 和 Mouse 檢體。
- 染色 15 分鐘，輕鬆操作。

JC-1 (5,5' ,6,6' - tetrachloro-1,1' ,3,3' - tetraethylbenzimidazolcarbocyanine iodide) 的兩種形式：

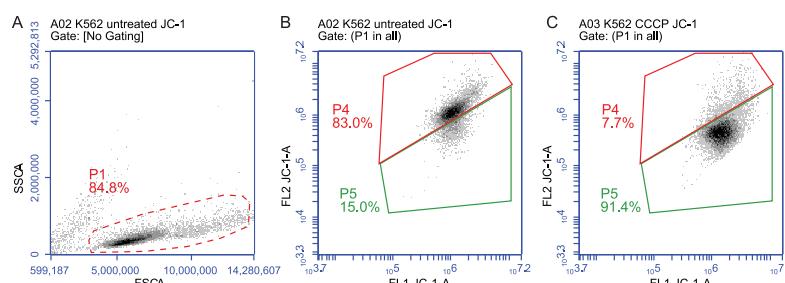
- JC-1 單體 (Monomer)：呈現綠色螢光，在大多數的流式細胞儀，可用 FL-1 偵測器偵測。
- JC-1 多聚體 (Aggregates)：呈現黃色螢光，在大多數的流式細胞儀，可用 FL-2 偵測器偵測。

正常生理狀態下，細胞粒線體負電性高，細胞質內的 JC-1 單體 (monomer) 進入粒線體以多聚體 (Aggregates) 存在，此時會偵測到同時有綠色螢光跟黃色螢光的健康細胞較多，只呈現綠色螢光的細胞較少。當細胞走向凋亡時，粒線體去極化產生，負電性降低，JC-1 從粒線體釋放到細胞質，呈現單體形式，因此會偵測到呈現綠色螢光的細胞增加，同時具有綠色螢光跟黃色螢光的細胞變少。

實驗範例 #551302



JC-1 Staining in Control and Apoptotic Cells.
Cells (1×10^6 cells/ml) were untreated or treated with staurosporine ($1 \mu\text{m}$, 4 h to induce apoptosis. Cells were stained with JC-1 according to the protocol and analyzed on a BD FACSCalibur™.



BD MitoScreen (JC-1) Kit analysis on the BD Accuri C6. K562 cells were treated with $100 \mu\text{M}$ of CCCP (in DMSO) for 5 minutes at 37°C to induce mitochondrial membranes to decouple. Results: Compared to untreated controls (B), CCCP treatment (C) resulted in a shift in mitochondrial membrane potential.

熱銷推薦

Cat. No.	Name	Contents	Apps	Size
551302	BD™ MitoScreen (JC-1)	JC-1 dye and assay buffer	FCM	100 Tests

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MitoLight® Mitochondrial Apoptosis Detection Kit

使用專利 MitoLight™ 粒線體活性染劑，原理與 JC-1 相同，可偵測粒線體膜電位變化。

- 適用於 Mammals 檢體。
- 應用 : Flow cytometry、fluorescence microscopy。
- FITC channel 觀察 green monomers (Ex/Em = 488/530)
- PI channel 觀察 red aggregates (Ex/Em = 488/585)

Cat. No.	Description	Content	Size	Flow cytometry
APT142	MitoLight® Mitochondrial Apoptosis Detection Kit	MitoLight dye, 10X Incubation Buffer	25 Assays	✓
APT242			100 Assays	✓

Flow cytometry 和 Immunofluorescence 偵測

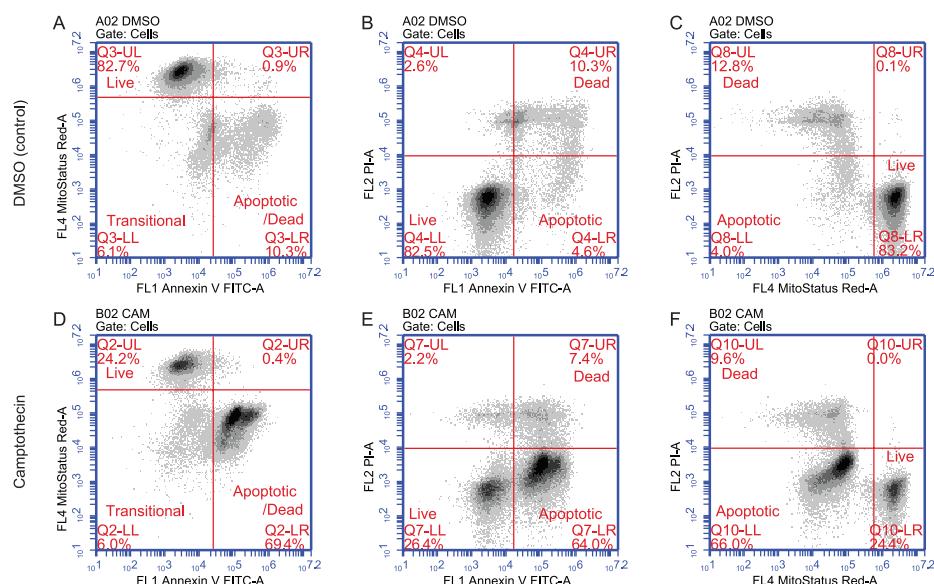
Mitochondrial Changes

BD Pharmingen™ MitoStatus Reagents

BD Pharmingen™ MitoStatus reagents 是一種陽離子、脂溶性的螢光染劑，會在健康細胞的粒線體 (mitochondria) 內累積；但當細胞處於細胞凋亡的狀態或是接觸到 mitochondrial uncoupler 而使粒線體內膜電位 (inner mitochondrial membrane potential, $\Delta \psi m$) 去極化的時候，MitoStatus reagents 就不會累積。在流式細胞儀分析時，健康細胞的螢光強度會比失去粒線體內膜電位不健康細胞的螢光強約 10 倍。利用此一特性，MitoStatus reagents 可以偵測粒線體去極化的情形，用來研究細胞凋亡 (Apoptosis)，自體吞噬 (autophagy)，衰老 (senescence) 等現象。

實驗範例

Detecting apoptosis with MitoStatus Red and Annexin V



可節省細胞樣本及時間

*MitoStatus Red 建議可與 Annexin V FITC Apoptosis Detection Kit I (Cat. No. 556547) 同時偵測；
MitoStatus TMRE 建議可與 Annexin V APC (Cat. No. 550475) 及 FVS 520 (Cat. No. 564407) 同時偵測。

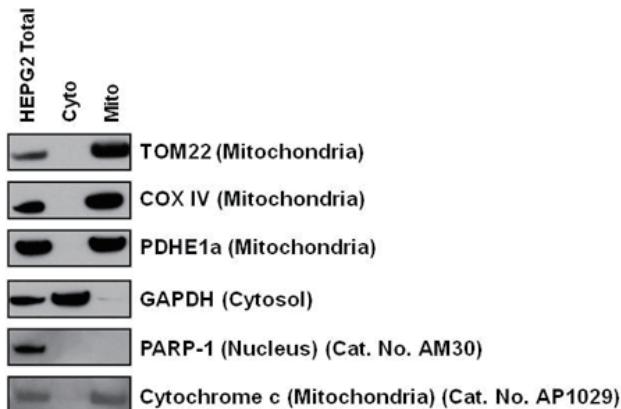
Cat. No.	MitoStatus TMRE	MitoStatus Red
	564696	564697
Characteristic		
Excitation peak	549 nm	622 nm
Emission peak	574 nm	648 nm
Laser	488 nm (blue)	640 nm (red)
Detector	FL2	FL4
Equivalent fluorochromes*	PE	APC Alexa Fluor® 647

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粒線體純化

ProteoExtract® Cytosol/Mitochondria Fractionation Kit

- 節省：一組 Kit 同時可得兩個不同位置的蛋白質。Cytosol 及 Mitochondria fraction。
- 可得 Native 的蛋白：適用於 WB, ELISA, Protein translocation 研究。
- 過程簡單：不需經過超高速離心。



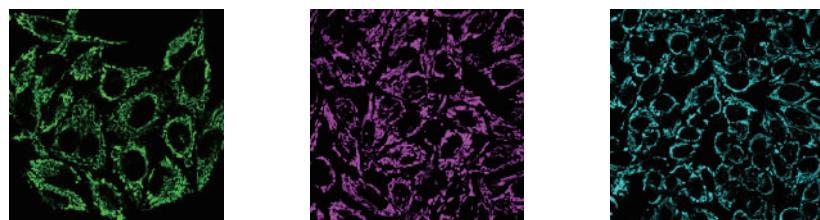
Cat. No.	Description	Size
QIA88-1KITCN	ProteoExtract® Cytosol/Mitochondria Fractionation Kit	100 Extractions

Microscope 偵測

粒線體活細胞染色

活細胞影像觀察相較於固定樣本觀察，更能實現動態變化觀察，3D 結構觀察，避免固定細胞步驟對樣品的影響。

- 粒線體膜活細胞染劑，無須固定即可直接檢測。
- 應用：測細胞存活率，代謝活性和觀測整體細胞健康。



Cat. No.	Description	螢光	Channel
SCT136	BioTracker 488 Green Mitochondria Dye	Green	FITC/GFP
SCT137	BioTracker 633 Red Mitochondria Dye	Red	Cy5
SCT135	BioTracker 405 Blue Mitochondria Dye	Blue	DAPI

細胞凋亡 Apoptosis

Flow cytometry 偵測

Caspase Activation

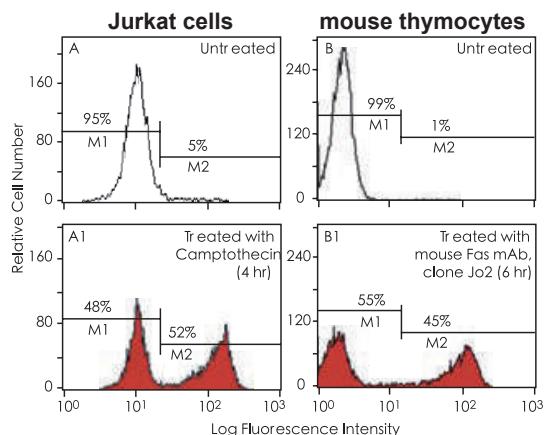
Caspase 家族的活化

caspase 的活化在細胞凋亡反應扮演著重要的角色，活化的 caspase 會進行一連串的蛋白質酶作用 (proteolysis cascade)，將死亡訊號傳遞至下游，最後引發細胞凋亡。Caspase 以未活化態存在 (procaspase)，當細胞凋亡的訊號啟動，procaspase 會自行裂解或被其他的蛋白酶切割而變成活化態的 caspase，進而進行一連串的下游反應。其中 caspase 8, 9, 3 為此過程的關鍵角色，上游的 caspase 8 和 caspase 9 是細胞凋亡起始訊號的 caspase，caspase 8 透過不同路徑來活化 caspase 3，而活化 caspase 3 會將訊息放大傳遞至下游，最終執行細胞凋亡。

Caspase-3 Assay

Caspase 3：細胞凋亡的關鍵人物

Caspase 3 在細胞凋亡早期是主要的蛋白酶，未活化的 caspase 3 會自行裂解或因其他蛋白酶 (如：caspase 9) 切割而成兩個次單位 (17-22kDa 和 10-12 kDa) 的二聚體，此活化態的 caspase 3 會進一步活化下游的目標蛋白，例如胞漿內的 Bcl-2 和 D4-GDI 及核內的 PARP。



Flow cytometric analysis of apoptotic and non-apoptotic populations using anti-active caspase-3 antibodies.

Jurkat T cells (A, A1) or mouse thymocytes (B, B1) were left untreated (A, B) or treated for 4 h with camptothecin (A1) or a mouse Fas monoclonal antibody, clone Jo2 (Cat. No. 554254) to induce apoptosis (B1). Cells were permeabilized and then stained with PE-conjugated active caspase-3 antibodies (Cat. No. 557091). Untreated cells were primarily negative for the presence of active caspase-3, whereas about half of each population of cells induced to undergo apoptosis had detectable active caspase-3.

熱銷推薦

Cat. No.	Name	Contents	React.	Apps	Size
550480	Caspase-3, Active Form, Apoptosis Kit : FITC	FITC anti-active Caspase-3 antibody, BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution, and BD Perm/Wash™ Buffer	Hu, Ms	IC/FCM	100 Tests
550914	Caspase-3, Active Form, Apoptosis Kit : PE	Anti-Active Caspase-3 Antibody, BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution, and BD Perm/Wash™ Buffer	Hu, Ms	IC/FCM	100 Tests

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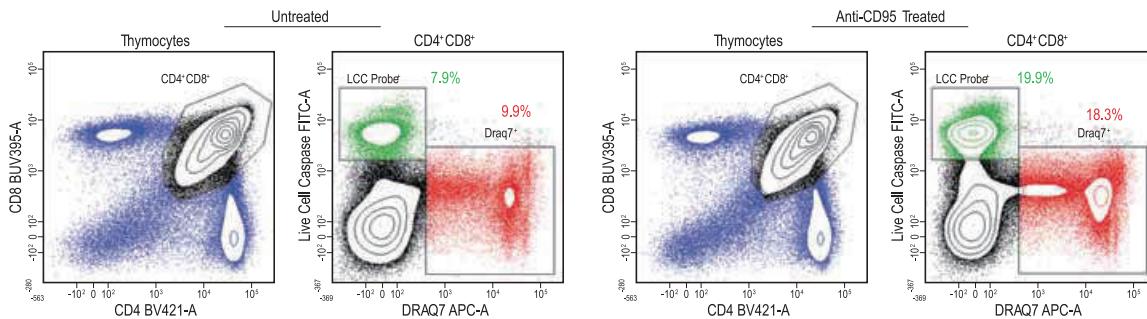
Flow cytometry 偵測

Caspase Activation

Active caspase family (caspase-1, -2, -3, -6, -8, -9, and -10)

Live Cell Caspase Probes

Live Cell Caspase Probe 可用以偵測活細胞的 active caspases，Live Cell Caspase Probe 含有螢光的 fluorochrome-labeled caspase inhibitor：包括 3-amino acid sequence (VAD) 以及 fluoromethyl ketone (FMK)；可偵測 active caspase family (caspase-1, -2, -3, -6, -8, -9, and -10) 的活性。



Multicolor Flow Cytometric Analysis of Viability in Mouse Thymocytes

Balb/c thymocytes were incubated with (right, treated) or without (left, untreated) BD Pharmingen™ Purified NA/LE Hamster Anti-Mouse CD95 (anti-FAS, Cat. No. 554254) for 6 hours in culture, followed by staining with BD Pharmingen™ Blue Live Cell Caspase Probe (Cat. No. 565519). Cells were washed and stained with BD Horizon™ BV421 Rat Anti-Mouse CD4 (Cat. No. 562891) and BD Horizon™ BUV395 Rat Anti-Mouse CD8a (Cat. No. 563786), followed by staining with 1 μ M of DRAQ7. Samples were analyzed on a BD LSRII Fortessa cell analyzer and gated based on scatter properties to exclude debris. Anti-FAS-treated cells show an increase in the amount of CD4+CD8+ apoptotic and dead cells, as assessed by staining with Blue Live Cell Caspase Probe and DRAQ7, respectively.

Cat. No.	name	Laser	Equivalent Fluorochromes	sizes
565519	Blue Live Cell Caspase Probe	Blue	FITC (eg, 530/30 nm)	50 Tests
565521	Violet Live Cell Caspase Probe	violet	BV421, V450 (eg, 450/40 nm)	50 Tests
565520	Yellow-Green Live Cell Caspase Probe	Blue or yellow-green	PE (eg, 575/26 nm) or PE-CF594 (eg, 610/20 nm)	50 Tests

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特別推薦 Caspase Inhibitor

Cat. No.	Name	Size
550377	General Caspase Inhibitor, Z-VAD-FMK	1 mg
563828	General Caspase Inhibitor, Q-VD-OPh	1 mg
550380	Caspase-8 Inhibitor, Z-IETD-FMK	1 mg
550381	Caspase-9 Inhibitor, Z-LEHD-FMK	1 mg
550378	Caspase-3 Inhibitor, Z-DEVD-FMK	1 mg
550411	Caspase Inhibitors Negative Control, Z-FA-FMK	1 mg

特別推薦 Activator

Cat. No.	Description	Size
178497-10MGCN	Apoptosis Activator VII, Apotozole - CAS 1054543-47-3- Calbiochem	10 mg
178493-10MGCN	Apoptosis Activator III, Embelin - CAS 550-24-3 - Calbiochem	10 mg
178496-5MGCN	Apoptosis Activator VI, CD437/AHPN - CAS 125316-60-1 - Calbiochem	5 mg
5.08774.0001	Apoptosis Activator VIII, TP421 - Calbiochem	10 mg
APT800	Apoptosis Inducer Set	<ul style="list-style-type: none"> · Actinomycin D (10 mM): 50 µL · Camptothecin (2 mM): 1 mL · Cycloheximide (100 mM): 1 mL · Dexamethasone (10 mM): 1 mL · Etoposide (10 mM): 100 µL

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Flow cytometry 偵測

Caspase Tag Caspase 3,7,8,9 In Situ Assay Kit, Fluorescein

- 應用 : 96-well plate-based fluorometry、fluorescence microscopy、Flow cytometry、Activity Assay。
- 適用樣本：貼附型 (Adherent) 與懸浮型 (Suspension) 細胞。
- 方便：Kit 內附 FLICA Reagent、固定液、PI 染劑、Hoechst Stain 以及 Wash buffer。

原理

Fluorochrome Inhibitors of Caspases (FLICA) 方法學，抑制劑帶有螢光標記，可通透細胞且不具有毒性。抑制劑進入細胞後會共價結合上活性的 caspase，未結合的試劑將擴散出細胞並被洗掉，螢光強度可直接代表 caspase 活性。

其他 Caspase assay kit

Cat. No.	Description	Content	Size	Detection
APT400	Caspase Tag Pan-Caspase In Situ Assay Kit, Fluorescein	FLICA Reagent (FAM-VAD-FMK),	100 Assays	Fluorescence
APT420		10X Wash Buffer, Fixative, Propidium Iodide, Hoechst Stain	25 Assays	
APT403	Caspase Tag Caspase 3,7 In Situ Assay Kit, Fluorescein	FLICA Reagent (FAM-DEVD-FMK),	100 Assays	Fluorescence
APT423		10X Wash Buffer, Fixative, Propidium Iodide, Hoechst Stain	25 Assays	
APT408	Caspase Tag Caspase 8 In Situ Assay Kit, Fluorescein	FLICA Reagent (FAM-LETD-FMK),	100 Assays	Fluorescence
APT428		10X Wash Buffer, Fixative, Propidium Iodide, Hoechst Stain	25 Assays	
APT409	Caspase Tag Caspase 9 In Situ Assay Kit, Fluorescein	FLICA Reagent (FAM-LEHD-FMK),	100 Assays	Fluorescence
APT429		10X Wash Buffer, Fixative, Propidium Iodide, Hoechst Stain	25 Assays	

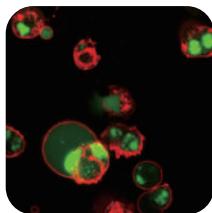
Flow cytometry/Microscope 偵測

Caspase 活細胞染劑

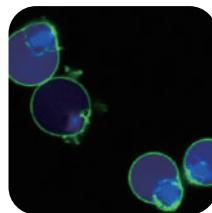
原理

當細胞凋亡啟動時，caspase-3/7 會切 caspase-3 tracker，使得螢光 DNA dye 能夠游離，並進入到細胞核內與 DNA 結合而發出螢光。Caspase-3 tracker 由螢光 DNA dye 與 caspase-3/7 DEVD 辨識序列所組成，可同時觀察到凋亡時 caspase-3/7 活性，以及細胞核的型態變化。

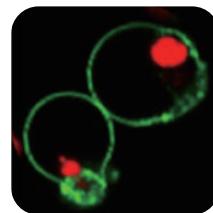
- 提供兩種版本：溶於 DMSO 或 PBS，PBS 版本較推薦給對於 DMSO 耐受性較低之細胞使用。



Apoptotic Jurkat cells stained with BioTracker™ NucView® 488 Green Caspase-3 Dye and CF™594 Annexin V (Red)



Apoptotic Jurkat cells stained with BioTracker™ NucView® 405 Blue Caspase-3 Dye and CF™488 Annexin V (Green)



Apoptotic Jurkat cells stained with BioTracker™ NucView® 530 Red Caspase-3 Dye and CF™488 Annexin V (Green)

Cat. No.	Description	Size	Detection	Detection
SCT100	BioTracker NucView® 488 Green Caspase-3 Dye (DMSO)	100 µL	Fluorescence	Ab: 500 nm Em: 530 nm
SCT101	BioTracker NucView® 488 Green Caspase-3 Dye (PBS)	100 µL	Fluorescence	Ab: 429 nm Em: 469 nm
SCT102	BioTracker NucView® 405 Blue Caspase-3 Dye (DMSO)	100 µL	Fluorescence	Ab: 528 nm Em: 563 nm
SCT104	BioTracker NucView® 405 Blue Caspase-3 Dye (PBS)	100 µL	Fluorescence	
SCT103	BioTracker NucView® 530 Red Caspase-3 Dye (DMSO)	100 µL	Fluorescence	
SCT105	BioTracker NucView® 530 Red Caspase-3 Dye (PBS)	100 µL	Fluorescence	

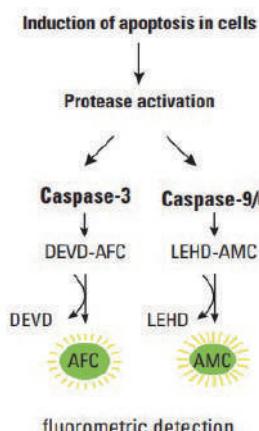
ApoAlert Caspase Assay Kits

提供簡單便利的 螢光法 or 比色法 檢測 Caspase 活性！

分析哺乳動物細胞凋亡過程中的兩大要角：Caspase-3 & Caspase-9。

- 可直接使用 **cell lysates** 操作。
- 可作用於 **tube** 或 **96-well**，輕鬆達成高通量應用。
- 只需少量樣本， 1×10^6 顆貼附或懸浮細胞。
- **Substrate** 無須額外前處理。

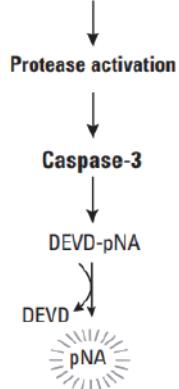
從細胞收集到螢光 or 比色結果呈現，只需要花 90 分鐘



螢光法

Caspase-3 被活化後，切割釋出的 AFC 可透過 Ex 400-nm 濾片及 Em 505-nm 濾片檢測 AFC 的螢光變化。
 Caspase-9/6 被活化後，切割釋出的 AMC 可透過 Ex 380-nm 濾片及 Em 460-nm 濾片檢測 AMC 的螢光變化。
 螢光法的靈敏度較高，可用於測定微量的 Caspase 活化態！

Induction of apoptosis in cells



比色法

Caspase-3 被活化後，切割 Peptide substrate DEVD-pNA，釋出的發色團 pNA 可透過 405 nm 吸光值進行比色監測。

PARP monoclonal antibody

於細胞凋亡前期，CPP32/Caspase-3 被活化後將切割 Poly (ADP-ribose) polymerase (PARP)，PARP 的切割即為 Apoptosis 的前期指標。PARP 單株抗體可辨認 Native PARP(116-kDa) 與切割後的 PARP(85-kDa)，適用於 Western Blot、ELISA 等抗體檢測。

Cat. No.	Event	Measure	Product	Package Size	Detection
630212	Apoptosis	Caspase proteolytic activity	ApoAlert™ Caspase-9/6 Fluorescent Assay Kit	100 Assays	Fluorometer, microplate reader
630215			ApoAlert™ Caspase-3 Fluorescent Assay Kit	100 Assays	
630216			ApoAlert™ Caspase-3 Colorimetric Assay Kit	25 Assays	Spectrophotometer, microplate reader
630217				100 Assays	
630210		Native and cleaved PARP	PARP Monoclonal Antibody (Mouse IgG1, C-2-10)	50 µL	Western Blotting, immunocytochemistry, ELISA

Flow cytometry 偵測

DNA Fragmentation

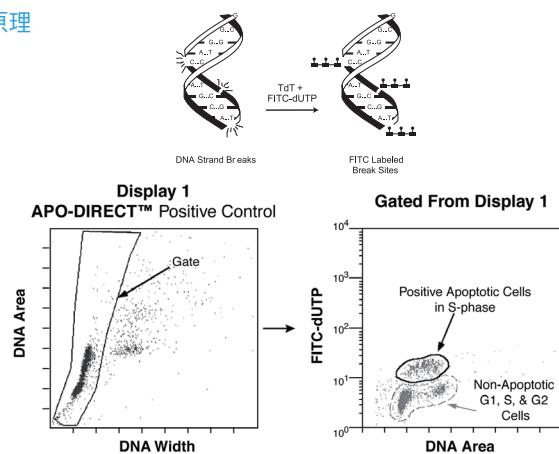
TUNEL Assay (Terminal deoxynucleotidyltransferase dUTP Nick End Labeling)

- 可同時偵測核內 DNA Fragmentation 及細胞週期 (Cell Cycle)。
- 適用於懸浮型或貼附型細胞的實驗。

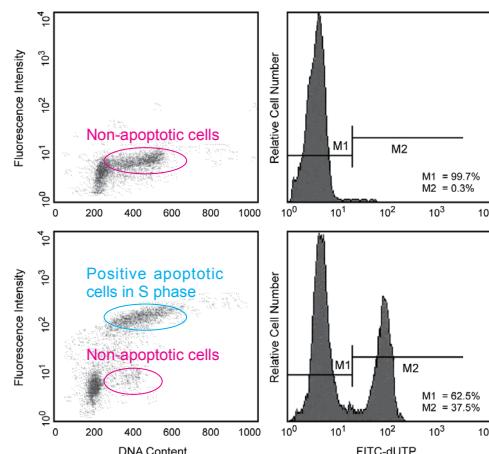
到了細胞凋亡後期，細胞核內的 DNA 會被內源性核酸內切酶降解，使 DNA 發生片段化的現象 (DNA fragmentation)。當 DNA 被內切酶作用後，會產生有 3' 末端的切口或是斷裂 DNA 片段，在 TdT (terminal transferase，末端轉移酶) 的作用下，使已標定螢光的核苷酸 (ex : FITC-dUTP) 連結到 DNA 片段的 3' 末端，螢光強度與 DNA 片段量成正比。另外，可再搭配 PI 染色來得知檢體中凋亡細胞的百分比及凋亡細胞所在的細胞週期 (G0/G1, S, G2/M phase)。

APO-DIRECT™ Kit

實驗原理



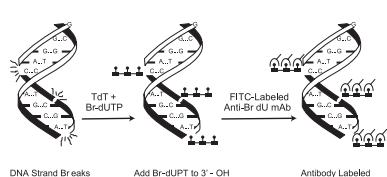
實驗數據



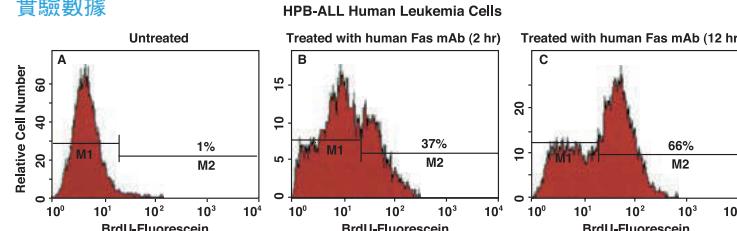
Cells are labeled with both PI (DNA) and FITC-dUTP (Apoptotic Cells). Display 1: Non-clumped cells are gated.

APO-BRDU™ Kit

實驗原理

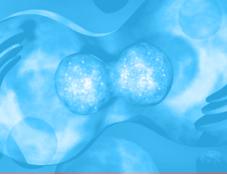


實驗數據



套組			
Cat. No.	Description	Contents	Size
556381	APO-DIRECT™	FITC dUTP, PI/RNase staining buffer, reaction buffer, rinsing buffer, wash buffer, TdT enzyme, negative control cells, positive control cells	50 Tests
556405	APO-BRDU™	FITC anti-BrdU antibody, PI/RNase staining buffer, reaction buffer, rinsing buffer, wash buffer, Br-dUTP, TdT Enzyme, negative control cells, positive control cells	60 Tests

For Research Use Only. Not for use in diagnostic procedures.

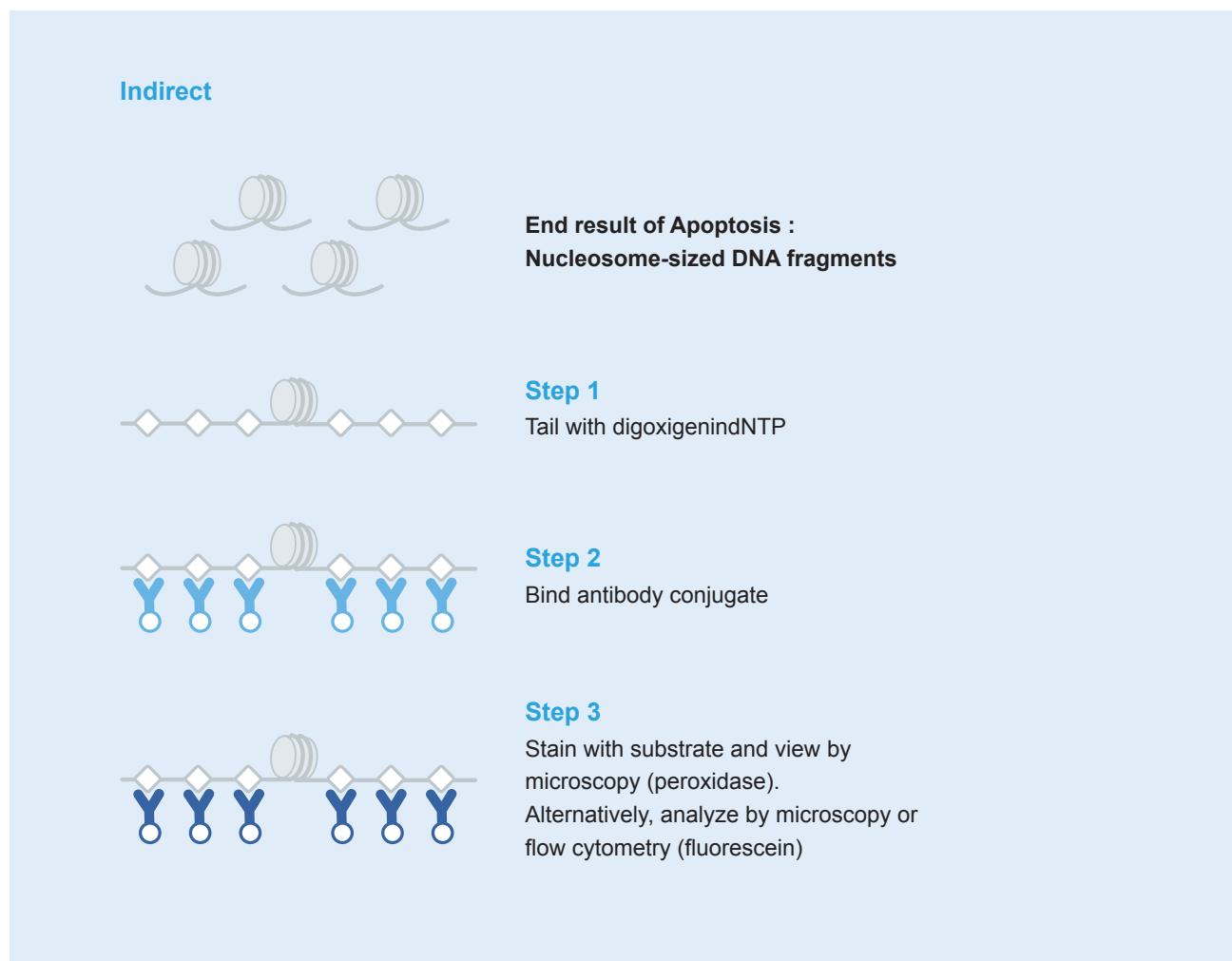


細胞凋亡 Apoptosis

ICC / IHC 偵測

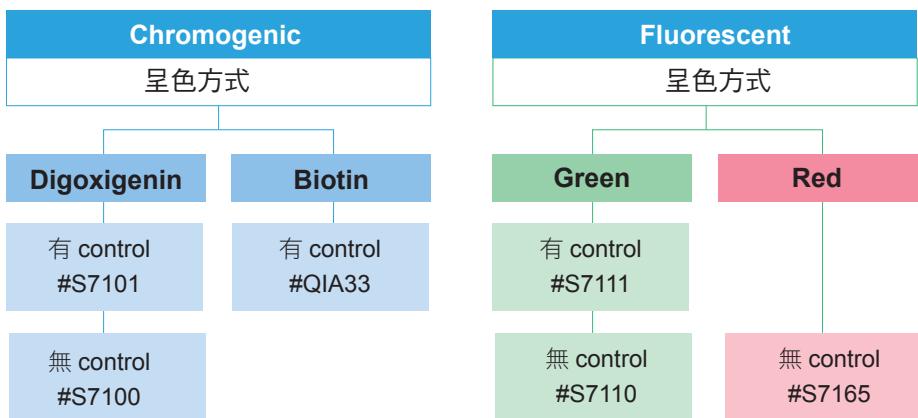
DNA Fragmentation TUNEL Assay

利用間接偵測 (Indirect) 法，將有接上 Digoxigenin (Dig) 或 Biotin 的 dNTP 連結到 DNA 片段的 3' 端，接著利用帶有 HRP 且可辨識到 Dig 或 Biotin 的抗體，加入受質進行呈色。



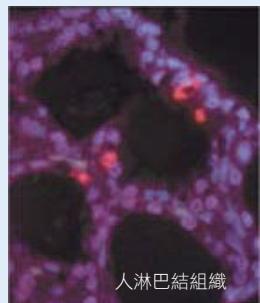
Cat. No.	S7100	S7101	QIA33
Description	ApopTag Peroxidase In Situ Apoptosis Detection Kit	ApopTag Plus Peroxidase In Situ Apoptosis Kit	FragEL DNA Fragmentation Detection Kit, Colorimetric - TdT Enzyme
Assays	40 Assays (~5 cm ² tissue specimens)	40 Assays (~5 cm ² tissue specimens)	50 Assays
Application	ICC, IHC, IH(P)	ICC, IHC, IH(P)	IHC, IH(P), ICC
Methodology	indirect	indirect	indirect (Biotin-labeled)
Detection Methods	Chromogenic (HRP)	Chromogenic (HRP)	Chromogenic (HRP)
特點	Anti-Digoxigenin 抗體，不含 control slide 與 DAB 試劑	Anti-Digoxigenin 抗體，內含 control slide 與 DAB 試劑	Anti-Biotin 抗體，內含 control slide 與 DAB 試劑
抗體	Anti-Digoxigenin-HRP	Anti-Digoxigenin-HRP	Anti-Biotin-HRP
Control slide 有無		√	√

選擇指南

**ApopTag® Peroxidase In Situ Apoptosis Detection Kit**

(Cat. No. S7100 & S7101)

ApopTag® 系列產品採用 digoxigenin/anti-digoxigenin 偵測系統，比起傳統 avidin/biotin 偵測系統更加專一，背景值更低。抗體特選 sheep pAb 並且移除 Fc portion，讓非專一性降到最低。



經國內外多重驗證，適用多種檢體

1. 人前列腺，胸腺，和大腸
2. 鼠闔割後腹側前列腺
3. 鼠胸腺的淋巴細胞 (dexamethasone treatment)
4. 14 天的小鼠胚胎肢體
5. 大鼠斷奶後乳腺

Reference
達 150 篇

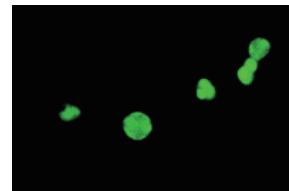
S7110	S7111	S7165
ApopTag Fluorescein In Situ Apoptosis Detection Kit	ApopTag Fluorescein In Situ Apoptosis Detection Kit	ApopTag Red In Situ Apoptosis Detection Kit
40 Assays	40 Assays	40 Assays (~5 cm ² tissue specimens)
ICC, IHC, IH(P), FC	ICC, IHC, IH(P), FC	ICC, IHC, IH(P)
indirect	indirect	indirect
Fluorescent (Fluorescein)	Fluorescent (Fluorescein)	Fluorescent (Rhodamine)
綠螢光呈色，不含 control slide	綠螢光呈色，含 control slide	紅螢光呈色，不含 control slide
Anti-Digoxigenin-Fluorescein	Anti-Digoxigenin-Fluorescein	Anti-Digoxigenin-Rhodamine
	√	

In Situ Apoptosis Detection Kit

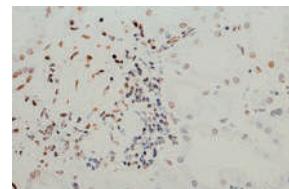
透過 TUNEL Assay (TdT-mediated dUTP Nick End Labeling)，螢光素標記的核苷酸將結合至 DNA 片段的 3' 端以檢測在細胞凋亡過程中片段化的 DNA，進而分析組織定位與單一個凋亡細胞。

組織切片 or 固定細胞 皆可用
螢光 or 光學顯微鏡 都 OK

- 快速：Ready-to-Use，TUNEL labeling 最快僅需 1 小時。
- 靈敏：可偵測單一細胞的細胞凋亡前期狀態。
- 專一：僅染色凋亡細胞，排除壞死細胞 (Necrosis)。
- 彈性：Kit 內所有組件皆可單獨購買。
- 安全：內容物不含 Cacodylic acid 等危險試劑。



細胞染色示意圖



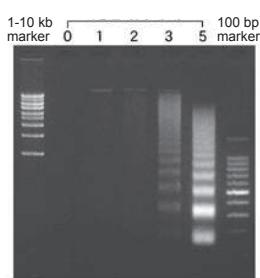
組織切片染色示意圖

ApopLadder Ex™

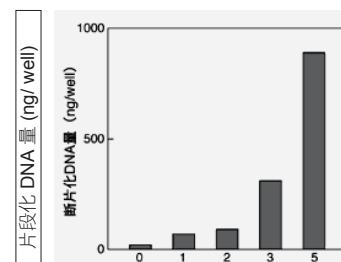
只萃取小片段 DNA 觀察 DNA 片段化現象，減少大片段染色體的干擾。

- 方便：使用 Ready-to-Use reagent，只需簡單電泳設備即可觀察。
- 快速：僅需 2.5 小時。
- 專一：選擇性萃取片段化 DNA，去除染色體 DNA 的干擾。
- 安全：不需使用 phenol-chloroform 萃取。
- 可定量：搭配 DNA 染劑 (SYBR Green I 需添購)，即可對片段化 DNA 進行定量。

Staurosporine 處理時間 (hr)



搭配 SYBR Green I 進行螢光定量



利用 ApopLadder Ex 檢測出 HL-60 細胞株處理 Staurosporine 後，產生 DNA 片段化的時間點

利用 ApopLadder Ex 萃取出的 DNA，添加 SYBR Green I 後，測定螢光強度，即可得到片段化 DNA 量。由此結果可知 Staurosporine 的確可以誘發 HL-60 細胞引起 Apoptosis。

Event	Measure	Product	Package Size	Cat. No.	Detection
Apoptosis	DNA fragmentation	In Situ Apoptosis Detection Kit	20 Assays	MK500	Fluorescence microscopy, Flow cytometry
		ApopLadder Ex™	24 Rxns	MK600	Gel electrophoresis

(Cat. No. 562253 / 50 Tests)

Apoptosis, DNA Damage and Cell Proliferation Kit

- 適用於藥物開發研究，同時偵測 3 種 marker 和細胞週期。
- 可同時偵測小分子 (small molecule)、放射物質 (Radiation) 及環境壓力 (Environmental Stressors) 等因子對於細胞週期 (Cell Cycle)、細胞增生 (Cell Proliferation)、細胞凋亡 (Apoptosis)、DNA 受損 (DNA Damage) 的影響。
- 適用於人類及小鼠檢體。

套裝組內含抗體種類

Name	Clone	Format	Laser	Purpose
Anti-BrdU	3D4	PerCP-Cy™5.5	Blue	Detection of cell proliferation
Anti-H2AX (pS139)	N1-431	Alexa Fluor® 647	Red	Detection of DNA damage
Anti-Cleaved PARP (Asp214)	F21-852	PE	Blue	Detection of apoptosis
DAPI (optional)	—	—	UV	Determination of DNA content

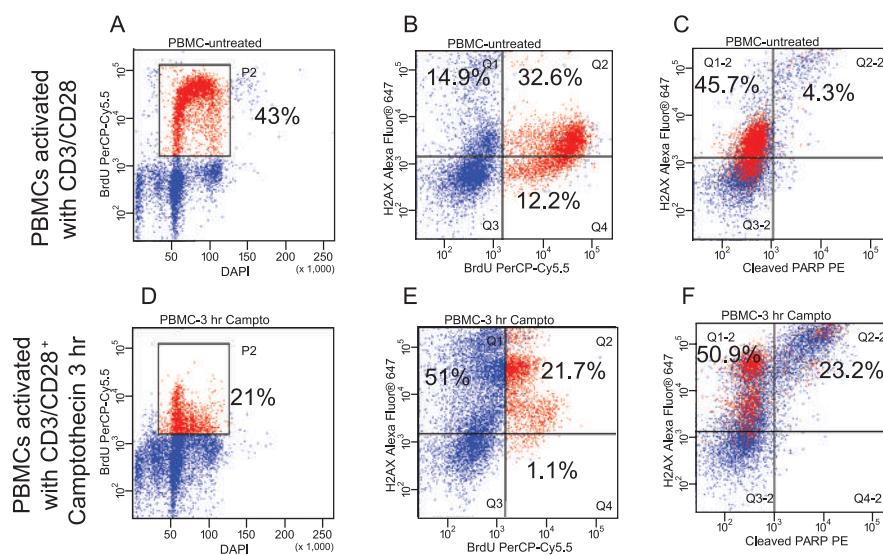


眾多文獻，請連結搜尋

This kit also contains BrdU, DNase, buffers, and a detailed protocol.

實驗範例

BD #562253



PBMCs were stimulated with Anti-CD3/CD28-coated Dynabeads for 3 days, then harvested and washed, and then replated with either 5 mM of camptothecin or left untreated as controls. The treated cell group was cultured for 3 hours with camptothecin, then washed and replated for an additional 2 hours allowing cells to recover. The untreated group was cultured for 5 hours. Both groups (control and treated) were pulsed with BrdU during the final 1 hour of culture. Cells were harvested, washed with staining buffer, then fixed and analyzed using the Apoptosis, DNA Damage, and Cell Proliferation Kit (Cat. No. 562253). Figures A–C are the untreated control group and figures D–F are the camptothecin-treated group.

ELISA 讀值好幫手



For ELISA

貨號	規格	包裝
FALCON 351172	Non-treated 96 Well Clear Flat Bottom Plate	50/ case



For ELISA

貨號	規格	包裝
EXCEL GP-PP-100	General Purpose Film	100/ box



樣品分液槽

貨號	規格	包裝
BIOTIX SR-0050-5SC	50 mL (減菌) 透明色	5/ pack , 200/ case
BIOTIX SR0055-5SNM-1	55 mL (減菌) 霧白色	5/ pack , 200/ case



1.5 mL Microcentrifuge 微量離心管



貨號	規格	包裝
NEPTUNE 4445.X	非滅菌	500/ bag
NEPTUNE 4445.S.X	滅菌	500/ bag
ANCELL 1260-00	非滅菌 (低殘留)	500/ bag



低殘留 Pipette Tips



Biotix Tip	品號	規格	包裝
Non-Filter Tip (盒裝滅菌)	M-0010-9SC	BIOTIX 10 μL, Sterile	10 trays of 96/ pack, 5 packs/ case
	M-0011-9SC	BIOTIX 10 μL XL, Sterile	10 trays of 96/ pack, 5 packs/ case
	M-0200-9SC	BIOTIX 200 μL, Sterile	10 trays of 96/ pack, 5 packs/ case
	M-0300-9SC	BIOTIX 300 μL, Sterile	10 trays of 96/ pack, 5 packs/ case
	M-1250-9SC96	BIOTIX 1250 μL, Sterile	10 trays of 96/ pack, 4 packs/ case
Non-Filter Tip (散裝)	M-0010-9BC	BIOTIX 10 μL	1000 tips/ bag, 10 bags/ case
	M-0011-9BC	BIOTIX 10 μL XL	1000 tips/ bag, 10 bags/ case
	M-0200-9BC	BIOTIX 200 μL	1000 tips/ bag, 10 bags/ case
	M-0300-9BC	BIOTIX 300 μL	1000 tips/ bag, 10 bags/ case
	M-1250-9BC	BIOTIX 1250 μL	1000 tips/ bag, 4 bags/ case



 BD

 MERCK

 Sigma-Aldrich®

 Takara
Clontech Takara cellortis

 FALCON®
A Corning Brand

 EXCEL
SCIENTIFIC

 BIOTIX
LIQUID HANDLING

 ANCCELL