



免疫

IMMUNOASSAYS

分析工具



多個測定平台

- Cytometric Bead Array (CBA)
- ELISA
- ELISPOT



騰達行



0800-211-819



BD 免疫分析工具

多個測定平台

- Cytometric Bead Array (CBA) ————— P.03
- ELISA ————— P.15
- ELISPOT ————— P.18

Tool/Technology	BD Cytometric Beads Array (CBA)	ELISA	ELISPOT
樣品偵測型式	分泌型	分泌型	分泌型 (In situ)
多參數同時偵測	可	不行	不行
提供單一細胞訊息	不行	不行	可
抗原專一性	有	有	有
蛋白濃度定量	可	可	相對定量
使用機型	流式細胞儀	分光光度計	ELISPOT reader

BD Cytometric Bead Array (CBA)

Multiplexed Bead-Based Immunoassays

利用流式細胞儀同時定性定量多種蛋白

BD™ Cytometric Bead Array (CBA) 技術能在單一份血清，血漿，組織培養上清液或細胞裂解樣品中定量檢測多種分析物。

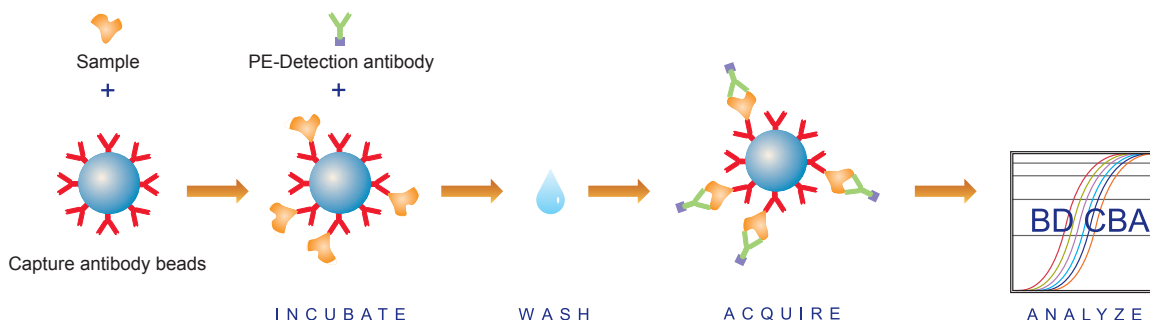
產品特色

- CP 值超高** 一份檢體即可同時定性定量多種蛋白質，最多可達 **30 種**
- 樣本超省** 一個 **test** 的檢體體積至多只需要 **20-50 µl**
- 步驟簡單** 步驟簡單人人可上手，最後僅需清洗一次即可上機讀取 **data**
- 時間超省** 花費時間僅需要 **3-4 小時**，與他牌或傳統的 **ELISA** 相比相當省時
- 分析快速** 搭配 **FCAP array** 軟體，**5 分鐘**完成數據分析

原理

CBA 定量是利用大小相同、螢光強度不同且帶有專一性辨識特定蛋白的抗體 (capture antibody) 的微球，來辨認樣本裡的特定蛋白，再與 PE 螢光抗體 (Detection antibody) 作用後，便可藉由流式細胞儀來偵測各種微珠所表現的 PE 平均螢光強度 (MFI)，然後利用標準品所得到的 PE 平均螢光強數值便可回推樣本裡的蛋白質濃度。

流程



產品型式

CBA Kit	CBA Flex Set
將熱賣品項合併成套組，套組最多可測 7 種 cytokine，套組不需再搭配其他試劑。流式細胞儀僅需要 1-laser 機型 (488 nm laser)	提供開放式的彈性實驗設計，可自由搭配想要偵測的項目，最多可搭配到 30 種 beads，flex set 須搭配專屬 buffer。流式細胞儀需要 2-laser 機型 (488 nm + 640/633 nm laser)

ELISA vs BD CBA 比較

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：個樣本最多可 30 種	1 個樣本最多可 7 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	
100µl 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 Reader	測定儀器	2-Laser Flow Cytometry	1-Laser Flow Cytometry

Step-by-Step 建立適合您實驗的 CBA 平台！

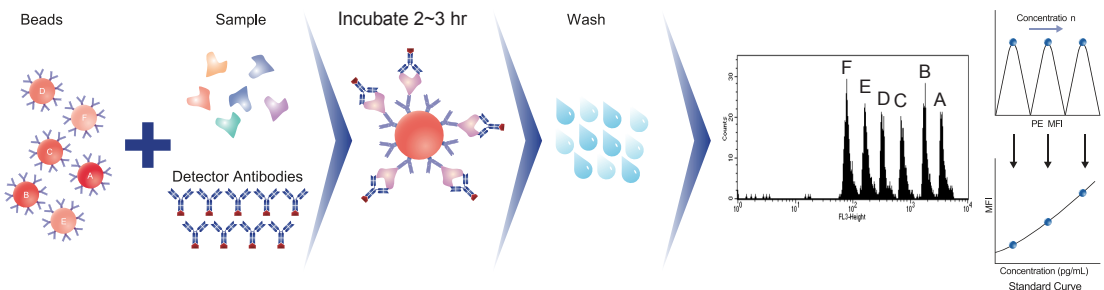
STEP 1	根據欲偵測的蛋白，選擇適合的 CBA 產品線，如：欲測 IL-2, IL-5, IL-13 可以選擇 Soluble Protein CBA flex set，若欲測項目有套組，則可選擇 CBA Kit。再根據樣本來源，選擇適合的物種品項及貨號。
STEP 2	確認選擇產品線是否需要搭配專屬的 Master buffer Kit，可根據樣本數選擇 100 或 500 Tests。如：在 step 1 選擇 Human Soluble Protein CBA flex set，則搭配 Human CBA Flex Set Master Buffer Kit，若選擇 CBA kit，就不需另外搭配。
STEP 3	確認 BD 流式細胞儀是否合用，如：CBA flex set 要有兩根以上雷射的機型
STEP 4	以上都確認完成即可以依據操作手冊進行實驗，並於流式細胞儀獲得實驗數據
STEP 5	利用分析軟體 FCAP array 來分析數據

START

BD CBA Kit

只需 488 nm laser 的流式細胞儀機型即可使用，Kit 是將熱賣品項合併成套組，內含 capture beads, detection antibody, standard, buffer，all-in-one 不需再搭配其他試劑。上機時，利用近紅外光 (NIR/FL-3) 或是紅光 (Red-A/FL-4) 做 beads 分群，再利用 PE/FL-2 來偵測平均螢光強度。

實驗流程



Human

Cat. No.	Description	contents	Size
561418	Human Anaphylatoxin Kit	C4a, C3a, C5a	80 Tests
552990	Human Chemokine Kit	IL-8, RANTES, MIG, MCP-1, IP-10	80 Tests
551811	Human Inflammatory Cytokine Kit	IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70	80 Tests
550749	Human Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IL-10, TNF, IFN- γ	80 Tests
551809	Human Th1/Th2 Cytokine Kit II	IL-2, IL-4, IL-6, IL-10, TNF, IFN- γ	80 Tests
560484	Human Th1/Th2/Th17 Kit	IL-2, IL-4, IL-6, IL-10, TNF, IFN- γ , IL-17A	80 Tests

Mouse

Cat. No.	Description	contents	Size
551287	Mouse Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IFN- γ , TNF	80 Tests
560485	Mouse Th1/Th2/Th17 Kit	IL-2, IL-4, IL-6, IFN- γ , TNF, IL-17A, IL-10	80 Tests
552364	Mouse Inflammation Kit	IL-6, IL-10, MCP-1, IFN- γ , TNF, IL-12p70	80 Tests
550026	Mouse Immunoglobulin Isotyping Kit	Heavy and light chain isotypes of IgG1, IgG2, IgG2b, IgG3, IgA, IgM, IgE	100 Tests

*Mouse Immunoglobulin Isotyping Kit 為定性，無法定量

Non-Human Primate

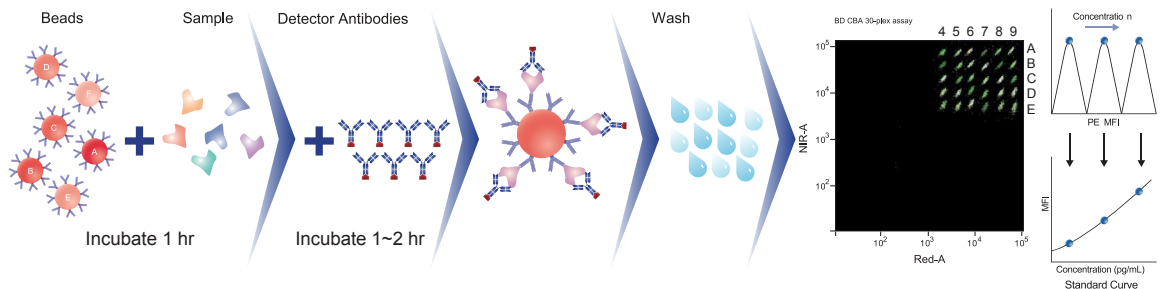
Cat. No.	Description	contents	Size
557800	Non-human Primate Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IL-6, TNF, IFN- γ	80 Tests

* Non-human Primate : Baboon, Cynomolgus, Rhesus

CBA flex set Soluble Proteins assay

Flex set 需要 488 及 640/633 nm 兩根 laser 的流式細胞儀機型才可以使用，至多可以選擇 30 種品項同時做實驗。由於不同品項的 capture beads 即為不同螢光強度的 beads，上機時，利用近紅外光 (NIR/FL-3/APC-Cy7) 及紅光 (Red-A/FL-4/APC) 做 beads 分群，再用 PE/FL-2 來偵測平均螢光強度。產品內容物包含 capture beads, detection antibody, standard，Flex set 不含 buffer，需依照不同物種及產品線搭配專用的 " master buffer kit "。

實驗流程



Human

Human Soluble Proteins

須搭配 " Human soluble protein master buffer kit " 使用 (cat. no.558264 or 558265)

Cat. No.	Description	Bead location	Size
558328	Angiogenin	C4	100 Tests
558327	Basic FGF	C5	100 Tests
560269	CD54 (ICAM-1)	A4	100 Tests
560419	CD62E (E-Selectin)	D9	100 Tests
560420	CD62L (L-Selectin)	D8	100 Tests
560426	CD62P (P-Selectin)	D7	100 Tests
560281	CD121b (IL-1 RII)	B7	100 Tests
558330	CD178 (Fas Ligand)	C6	100 Tests
558329	Eotaxin	C7	100 Tests
560265	Fractalkine	C6	100 Tests
558326	G-CSF	C8	100 Tests
558335	GM-CSF	C9	100 Tests
560304	Granzyme B	D7	100 Tests
560379	IFN- α	B8	100 Tests
560111	IFN- γ	B8	100 Tests
558269	IFN- γ	E7	100 Tests
558682	IgE	E9	100 Tests
560153	IL-1 α	D6	100 Tests
558279	IL-1 β	B4	100 Tests
558270	IL-2	A4	100 Tests
558355	IL-3	D5	100 Tests
558272	IL-4	A5	100 Tests
558278	IL-5	A6	100 Tests
558276	IL-6	A7	100 Tests
558334	IL-7	A8	100 Tests
558277	IL-8	A9	100 Tests
558333	IL-9	B6	100 Tests
558274	IL-10	B7	100 Tests

Cat. No.	Description	Bead location	Size
560228	IL-11	D5	100 Tests
560154	IL-12/IL-23p40	E5	100 Tests
558283	IL-12p70	E5	100 Tests
558450	IL-13	E6	100 Tests
560383	IL-17A	B5	100 Tests
562151	IL-17F	C6	100 Tests
560358	IL-21	B8	100 Tests
558280	IP-10	B5	100 Tests
560083	LT- α	D5	100 Tests
560364	I-TAC	A7	100 Tests
558287	MCP-1	D8	100 Tests
558286	MIG	E8	100 Tests
558325	MIP-1 α	B9	100 Tests
558288	MIP-1 β	E4	100 Tests
558324	RANTES	D4	100 Tests
560305	sCD154 (sCD40 Ligand)	C7	100 Tests
560276	Soluble CD121a (IL-1 RI)	B6	100 Tests
560418	Soluble CD14	D7	100 Tests
560156	Soluble TNFRI	C4	100 Tests
560155	Soluble TNFRII	C5	100 Tests
560112	TNF	C4	100 Tests
558273	TNF	D9	100 Tests
558336	VEGF	B8	100 Tests
560427	CD106(VCAM-1)	D6	100 Tests
560299	Granzyme A	D9	100 Tests

Human TGF- β 1 Single Plex Flex Set

需先將檢體做酸化中和處理，所以要單獨偵測無法和其他品項一起偵測，附有完整步驟 (含酸化處理) 。須搭配 " Human soluble protein master buffer kit " 使用 (cat. no. 558264 or 558265)

Cat. No.	Description	Size
560429	TGF- β 1 Single Plex Flex Set	100 Tests

Human Immunoglobulins Flex set

內容物包含 capture beads, detection antibody, standard 。可定量，但無法和其他產品線同時使用。須搭配 " Human Immunoglobulins Master Buffer Kit "(cat. no. 558683) 使用

Cat. No.	Description	bead location	Size
558681	IgA	C9	100 Tests
558676	IgG2	C5	100 Tests
558677	IgG3	C6	100 Tests
558678	IgG4	C7	100 Tests
558680	IgM	C8	100 Tests
558679	Total IgG	C6	100 Tests

Human flex set 搭配 buffer

Cat. No.	Description	contents	Size
558264	Human Soluble Protein Master Buffer Kit	Assay diluent, capture bead diluent, capture bead diluent for serum/plasma samples, detection reagent diluent, wash buffer, instrument setup reagents	100 Tests
558265			500 Tests
558683	Human Immunoglobulin Master Buffer Kit	Assay diluent, capture bead diluent, detection reagent diluent, wash buffer, instrument setup reagents	100 Tests

Mouse / Rat

Mouse Soluble Proteins

內容物包含 capture beads, detection antibody, standard 。

須搭配 " Mouse/Rat soluble protein master buffer kit " 使用 (cat. no. 558266 or 558267)

Cat. No.	Description	bead location	Size
560384	CD62E (E-Selectin)	E6	100 Tests
560385	CD62L (L-Selectin)	E7	100 Tests
560152	G-CSF	D4	100 Tests
558347	GM-CSF	B9	100 Tests
558296	IFN- γ	A4	100 Tests
560157	IL-1 α	E4	100 Tests
560232	IL-1 β	E5	100 Tests
558297	IL-2	A5	100 Tests
558346	IL-3	A8	100 Tests
558298	IL-4	A7	100 Tests
558302	IL-5	A6	100 Tests
558301	IL-6	B4	100 Tests
558348	IL-9	B5	100 Tests
558300	IL-10	C4	100 Tests

Cat. No.	Description	bead location	Size
560151	IL-12/IL-23p40	D7	100 Tests
558303	IL-12p70	D7	100 Tests
558349	IL-13	B8	100 Tests
560283	IL-17A	C5	100 Tests
562174	IL-17F	D6	100 Tests
560160	IL-21	B6	100 Tests
562575	IL-23 p19/p40	D7	100 Tests
558340	KC	A9	100 Tests
558342	MCP-1	B7	100 Tests
558341	MIG	D9	100 Tests
558449	MIP-1 α	C7	100 Tests
558343	MIP-1 β	C9	100 Tests
558345	RANTES	D8	100 Tests
558299	TNF	C8	100 Tests

Rat Soluble Proteins

內容物包含 capture beads, detection antibody, standard ◦

須搭配 " Mouse/Rat soluble protein master buffer kit " 使用 (cat. no. 558266 or 558267)

Cat. No.	Description	bead location	Size
558305	Rat IFN- γ	A6	100 Tests
560159	Rat IL-1 α	A4	100 Tests
561420	Rat IL-2	E5	100 Tests
558307	Rat IL-4	B9	100 Tests
558306	Rat IL-10	A8	100 Tests
558309	Rat TNF	C8	100 Tests

Mouse/Rat Soluble flex set 搭配 buffer

Cat. No.	Description	contents	Size
558266	Mouse/Rat Soluble Protein Master Buffer Kit	Assay diluent, capture bead diluent, detection reagent diluent, wash buffer, instrument setup reagents	100 Tests
558267			500 Tests

CBA Flex Set 搭配建議

免疫疾病	參考指標	參考文獻
multiple sclerosis lesions	IL-1, IL-2, IL-4, IL-10, TNF- α , TGF- β , and IFN- γ	The adhesion molecule and cytokine profile of multiple sclerosis lesions B Cannella, CS Raine - Annals of neurology, 1995 - Wiley Online Library
Rheumatoid Arthritis	IL-2, IL-4, IL-5, IL-10, IFN, and TNF	Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNF α therapy MR Ehrenstein, JG Evans, A Singh... - The Journal of ..., 2004 - jem.rupress.org
Langerhans cell histiocytosis	IL-1 β , IL-2, IL-4, IL-5, IFN- γ , GM-CSF, IL-10, IL-3, and IL-7	Differential in situ cytokine profiles of Langerhans-like cells and T cells in Langerhans cell histiocytosis: abundant expression of cytokines relevant to disease RM Egeler, BE Favara, M van Meurs, JD Laman... - Blood, 1999 - Am Soc Hematology
allergic asthma	IL-2, IL-4, IL-5 or IFN-gamma	T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. Am J Respir Cell Mol Biol. 1996 Apr;14(4):319-26.
diabetic	IP-10, MCP-1, MIP-1 α , MIP-1 β , RANTES, VEGF	Multiplex bead analysis of vitreous and serum concentrations of inflammatory and proangiogenic factors in diabetic patients F Leonard, EM Collnot, CM Lehr - Molecular pharmaceutics, 2010 - ACS Publications

細胞治療	參考指標	參考文獻
CD19-Targeted T Cells with Chemotherapy-Refractory Acute Lymphoblastic Leukemia	IL-2, IFN-r, IP-10, IL-6	CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia RJ Brentjens, ML Davila, I Riviere... - Science translational ..., 2013 - stm.sciencemag.org
human regulatory T cell	IL-1R2, IL-1 β , IL-6, and IL-8	Expression and function of TNF and IL-1 receptors on human regulatory T cells F Mercer, L Kozhaya, D Unutmaz - PloS one, 2010 - journals.plos.org

感染性疾病	參考指標	參考文獻
chronic hepatitis C virus :effects of interferon-alpha (IFN- α) treatment	IL-4, IL-6, IL-10, TNF- α and TGF- β	mRNA cytokine profile in peripheral blood cells from chronic hepatitis C virus (HCV)-infected patients: effects of interferon-alpha (IFN- α) treatment S Shapiro, V Gershtein, N Elias... - Clinical and ..., 1998 - Wiley Online Library
dengue fever and parvovirus	IL-1 RI, and IL-13,G-CSF, GM-CSF, IL-8, IP-10 and IL-7, IL-12p40 and MCP-1 TNF- α , IFN- γ , IL-6, IL-18 and MIF, IL-16, CCL2/ MCP-1, CCL5/RANTES	Profile of circulating levels of IL-1Ra, CXCL10/IP-10, CCL4/MIP-1 β and CCL2/MCP-1 in dengue fever and parvovirus Mem. Inst. Oswaldo Cruz vol.107 no.1 Rio de Janeiro Feb. 2012
pulmonary tuberculosis	MCP-1, MIP-1a,MIP-1b, RANTES, IP-10 and MIG	Chemokines induced by infection of mononuclear phagocytes with mycobacteria and present in lung alveoli during active pulmonary tuberculosis MI Sadek, E Sada, Z Toossi... - American journal of ..., 1998 - Am Thoracic Soc
SARS-CoV-2	IL-6, IL-2, GM-CSF, IL-12 p70, IL-5,IL-8, IL-1 β , IFN- α , IL-10, IP-10, MCP-1, MIP-1 α , TNF and IL-7	Shi Y, Wang Y, Shao C, et al. COVID-19 infection: the perspectives on immune responses. Cell Death Differ. 2020;27(5):1451-1454.
Human immunodeficiency virus	IL-1, IL-6, IL-2, IL-7, IL-15, and TNF	Kedzierska K, Crowe SM. Cytokines and HIV-1: interactions and clinical implications. Antivir Chem Chemother. 2001 May
癌症免疫	參考指標	參考文獻
tumor-infiltrating lymphocytes	IL-2 , IL-4 , TNF- α , TGF- β 1 , IL-10 , IFN- γ , and GM-CSF.	In situ cytokine production by breast cancer tumor-infiltrating lymphocytes Camp, B.J., Dyhrman, S.T., Memoli, V.A. et al. Annals of Surgical Oncology (1996) 3: 176. doi:10.1007/BF02305798
hormone-refractory metastatic breast cancer	vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6)	Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients British Journal of Cancer (2003) 88, 1721–1726. doi:10.1038/sj.bjc.6600956 www.bjcancer.com Published online 27 May 2003
tumour-derived factors	VEGF, TGF- β , IL-10	Kiertscher SM, Luo J, Dubinett SM, Roth MD (2000) Tumors promote altered maturation and early apoptosis of monocyte-derived dendritic cells. J Immunol 164: 1269–1276
human non-small cell lung cancer (NSCLC)-derived cytokines	IL-5 and IL-10 ,IL-4 and IL-13	Human non-small cell lung cancer cells express a type 2 cytokine pattern M Huang, J Wang, P Lee, S Sharma, JT Mao... - Cancer Research, 1995 - AACR
Serum Levels of non-small cell lung cancer	TNF- α , IL-6 and IL-8, sTNF RI, sTNF RII , IL-1RI, IL-10, VEGF	Pretreatment Serum Levels of Cytokines and Cytokine Receptors in Patients with Non-Small Cell Lung Cancer, and Correlations with Clinicopathological Features and Prognosis Oncology. 2006;70(2):115-25. Epub 2006 Apr 26.

CBA flex set Enhanced sensitivity protein assay

此系列為高靈敏度的 CBA 產品線，大部分的微球偵測系統的靈敏度約 5-10 pg/mL，而此產品線最低可測到 0.274 pg/mL (standard curve: 0.274-200 pg/mL)，其靈敏度比一般微球系統多 18 倍。可選擇同產品線的產品一起使用，須搭配 Enhanced Sensitivity Master Buffer Kit。

Human

Cat. No.	Description	bead location	Size
561515	IFN- γ	B8	100 Tests
561509	IL-1 β	B4	100 Tests
561517	IL-2	A4	100 Tests
561510	IL-4	A5	100 Tests
561511	IL-5	A6	100 Tests
561512	IL-6	A7	100 Tests
561513	IL-8	A9	100 Tests
561514	IL-10	B7	100 Tests
561518	IL-12 p70	C6	100 Tests
562143	IL-17A	B5	100 Tests
561516	TNF	C4	100 Tests

Mouse

Cat. No.	Description	bead location	Size
562233	IFN- γ	A4	100 Tests
562278	IL-1 β	B9	100 Tests
562262	IL-2	A5	100 Tests
562272	IL-4	A7	100 Tests
562234	IL-5	A6	100 Tests
562236	IL-6	B4	100 Tests
562263	IL-10	C4	100 Tests
562264	IL-12 p70	D7	100 Tests
562273	IL-13	B8	100 Tests
562261	IL-17A	C5	100 Tests
562336	TNF	C8	100 Tests

Enhanced sensitivity flex set 搭配 buffer

Cat. No.	Description	contents	Size
561521	Human Enhanced Sensitivity Master Buffer Kit	Assay diluent, capture bead diluent, detection reagent diluent, wash buffer, enhanced sensitivity detection reagent, instrument setup reagents	100 Tests
561523			500 Tests
562246	Mouse Enhanced Sensitivity Master Buffer Kit	Assay diluent, capture bead diluent, detection reagent diluents, wash buffer, enhanced sensitivity detection reagent, instrument setup reagents	100 Tests
562248			500 Tests

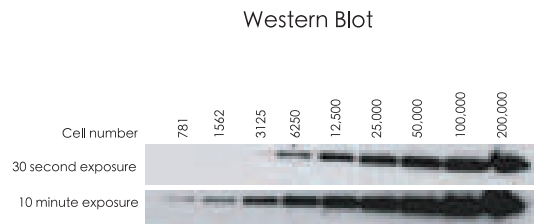
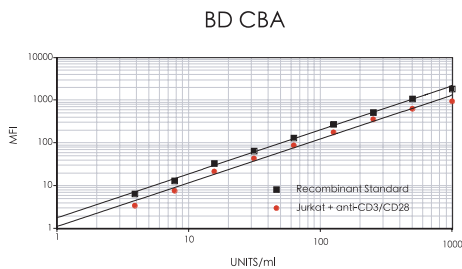
CBA flex set Cell signaling assays

此產品線是可以利用 CBA 同時定性定量偵測樣本內的磷酸化蛋白，相對於 western 及 IP 的方法，可節省大量的時間。可選擇同產品線的產品一起使用，一份檢體即可進行多種磷酸化蛋白的定量，須搭配 Cell Signaling Master Buffer Kit。

- * Cell Signaling Master Buffer Kit 內含 denaturation buffer，可方便製備 Cell lysate
- * 附完整操作手冊

產品特色

- 定性及定量：可同時偵測 Cell lysate 中多種磷酸化蛋白
- 單一樣本可進行多種分析：只要 25~50 μ L，就可以進行多種磷酸化蛋白的分析。
- 簡便省時
- 彈性的實驗設計：有 Internal control



Cell Signaling Flex Set CBA 與 Western Blot 的結果一致

Jurkat cells were activated by adding anti-CD3 and anti-CD28 which were 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.

Cat. No.	Description	bead location	Reactivity	Size
560012	Phospho ERK1/2 (T202/Y204)	C4	Hu, Ms, Rat	100 Tests
560013	Phospho JNK1/2 (T183/Y185)	B5	Hu, Ms, Rat	100 Tests
560010	Phospho p38 (T180/Y182)	B6	Hu, Ms, Rat	100 Tests
560093	Phospho Stat3 (Y705)	C8	Hu, Ms, Rat	100 Tests
560145	Total p38- α	B6	Hu	100 Tests
560017	Total STAT1 (Bead)	C5	Hu, Ms, Rat	100 Tests

* 此產品線的 QC testing 皆為 "Hu" (Human)，Ms (Mouse) 及 Rat 為 "Tested in Development"

Cell signaling flex set 搭配 buffer

Cat. No.	Name	Contents	Size
560005	Cell Signaling Master Buffer Kit	Assay diluent, capture bead diluent, detection reagent diluent, denaturation buffer, wash buffer, instrument setup reagents	100 Tests
560006			500 Tests

Q BD CBA capture beads 的大小？材質？

A BD CBA capture beads 為單一大小的 7.5 μm 、PS 材質的 beads，他牌的 beads size 分大小，可能會因為 beads aggregation 而使小 size beads 變成大 size beads，在分析數據時可能會有誤差。

Q 哪一種 BD 流式細胞儀的機型適用 BD CBA 呢？

Product Type	Platform
BD CBA kits	BD FACScan™, BD FACSCalibur™, BD FACSArray™, BD FACSCanto™, BD FACSCanto™ II, BD™ LSR II, BD LSRFortessa™, BD FACSria™, BD FACSria™ II, BD FACSria™ III
Flex Sets	BD dual-laser FACSCalibur, BD FACSArray, BD FACSCanto, BD FACSCanto II, BD LSR II, BD LSRFortessa, BD FACSria™, BD FACSria™ II, BD FACSria™ III

Q 如果用 CBA flex set-soluble protein assay 產品線偵測不到欲測蛋白濃度，是否有其他方法可以測到嗎？

A 可以改用高靈敏度的 CBA flex set-Enhanced sensitivity protein assay，此產品線最低可測到 0.274 pg/mL (standard curve: 0.274-200 pg/mL)。

Q 什麼樣的檢體可以適用於 CBA 呢？

A BD 原廠在 CBA 的 QC test 是以血清、血漿及細胞培養液做為檢體，其他檢體如 cell/tissue lysates、小鼠肺泡沖洗液等已有許多使用 CBA 當作偵測平台的參考文獻。cell/tissue lysates 當然適用於 Cell signaling CBA flex set。

參考文獻如下：

Milano F, Jorritsma T, Rygiel AM, et al. Expression pattern of immune suppressive cytokines and growth factors in oesophageal adenocarcinoma reveal a tumor immune escape-promoting microenvironment. *Scand J Immunol.* 2008;68:616-623.

Bhowmick S, Duseja R, Das S, Appaiahgiri MB, Vrati S, Basu A. Induction of IP-10 (CXCL10) in astrocytes following Japanese encephalitis. *Neurosci Lett.* 2007;414:45-50.

Ghoshal A, Das S, Ghosh S, et al. Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. *Glia.* 2007;55:483-496.

Q 溶血 (hemolysis) 的檢體是否會影響 CBA 的偵測結果？

A 因為 CBA 是以偵測 PE 螢光來換算欲測蛋白濃度，不是 TMB/HRP 呈色，因此不會受溶血影響，而出現偽陽性。些微的溶血並不會影響結果。

Q human CBA Kits 組裡面的 serum enhancement buffer 用途為何？

A 用來阻斷 Human serum or plasma 中的內源性抗體與試劑中的 capture or detector antibodies 反應而導致偽陽性。

Q 回溶後的標準品要如何保存？

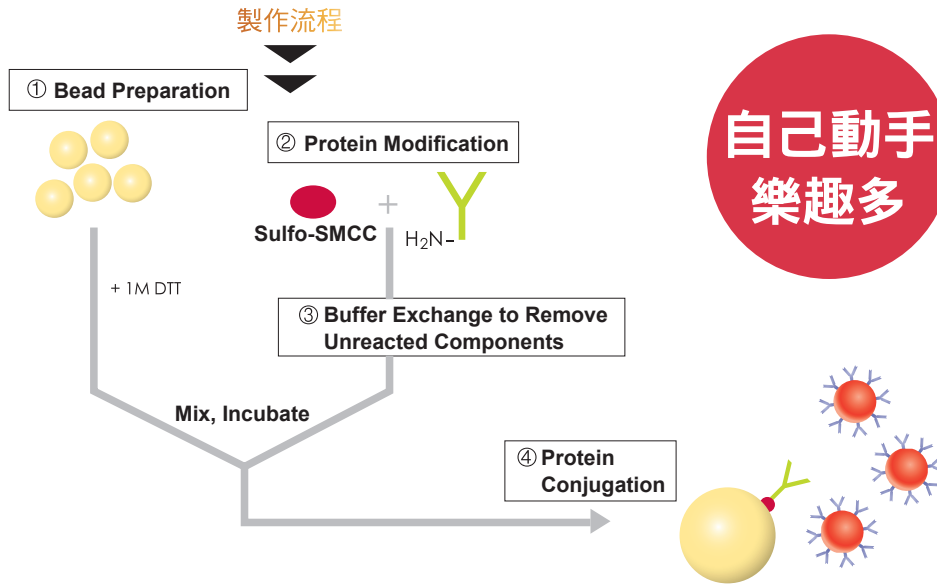
A 建議在回溶後十二小時內進行實驗，不建議反覆冷凍與解凍標準品。細胞訊息傳遞的標準品回溶後可在 4°C 保存三個月。

Q 標準品的重組蛋白來源為何？

A 重組蛋白的生產系統為 insect cells 或 *E. coli*。

CBA Functional Beads

BD Biosciences 提供沒有 coating antibody 的 beads，讓您可以將手邊現有的抗體 conjugate 到 beads 上，做成專屬您的 capture beads，建議新的實驗平台！原廠附有完整的 protocol，讓您輕鬆享受 DIY 的樂趣喔！



**製作
Functional Beads
您需要準備**



1. Beads (BD 提供不同位置的 beads)
2. Functional Bead Conjugation Buffer Set (cat. no. 558556)
3. Antibody pairs or protein
4. 抗體與 beads 接合劑：Sulfo-SMCC
5. 其他試劑耗材：1M DTT, DMSO, NEM, bio-spin columns
6. 評估接合結果的試劑 (依 capture antibody 來做選擇)

**1, 2, 6 項 BD 有產品可供選擇

Beads

Cat. No.	Description	Size
558578	Functional Bead (A4)	0.9 mL
560031	Functional Bead (A5)	0.9 mL
560032	Functional Bead (A6)	0.9 mL
560033	Functional Bead (A7)	0.9 mL
560034	Functional Bead (A8)	0.9 mL
558579	Functional Bead (A9)	0.9 mL
560035	Functional Bead (B4)	0.9 mL
558586	Functional Bead (B5)	0.9 mL
558645	Functional Bead (B6)	0.9 mL
558580	Functional Bead (B7)	0.9 mL
558581	Functional Bead (B8)	0.9 mL
558646	Functional Bead (B9)	0.9 mL
558647	Functional Bead (C4)	0.9 mL
558648	Functional Bead (C5)	0.9 mL
558582	Functional Bead (C6)	0.9 mL

Cat. No.	Description	Size
558587	Functional Bead (C7)	0.9 mL
558649	Functional Bead (C8)	0.9 mL
558650	Functional Bead (C9)	0.9 mL
560036	Functional Bead (D4)	0.9 mL
558583	Functional Bead (D5)	0.9 mL
558654	Functional Bead (D6)	0.9 mL
558655	Functional Bead (D7)	0.9 mL
558656	Functional Bead (D8)	0.9 mL
558657	Functional Bead (D9)	0.9 mL
558584	Functional Bead (E4)	0.9 mL
560037	Functional Bead (E5)	0.9 mL
560038	Functional Bead (E6)	0.9 mL
560039	Functional Bead (E7)	0.9 mL
560040	Functional Bead (E8)	0.9 mL
558585	Functional Bead (E9)	0.9 mL

接合用 buffer

Cat. No.	Description	Contents	Size
558556	Functional Bead Conjugation Buffer Set	Coupling buffer and storage buffer	15 Tests

評估接合結果的試劑

Cat. No.	Description	Reactivity	Size
558550	Mouse Ig Detector PE	Mouse	25 Tests
558553	Rabbit IgG Detector PE	Rabbit	25 Tests
558551	Rat Ig Detector PE	Rat	25 Tests
554061	Streptavidin PE		0.5 mg

Functional beads Q&A

Q 製備專屬 beads 需要花多久時間呢？

A 大約 4 小時即可完成。

Q Protein 是否也可接合到 beads 上呢？

A 任何 protein (需有 free amino group) 都可以。

Q 完成接合反應的 beads 可以保存多久呢？

A 保存在 4 度，可長達一年。

Q 接合到 beads 的抗體或蛋白質，需要多少量呢？

A 最多只需要準備 100 μ g protein (in 1 mg/mL)。

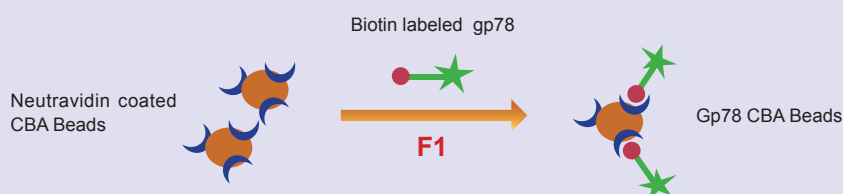
Q 每管 functional Beads(0.9 mL) 為多少 test？

A 足夠做 6000test，每次接合反應建議使用 500-1000 test，因此可做 6-12 次。

Functional beads 應用實例

有研究者利用 CBA functional beads 建立鼻咽癌病毒檢測平台，其專利 (CN101113979 B) 聲明如下：

一種檢測 EB 病毒 gp78 抗體的 CBA 試劑盒，其特徵是：包括有 CBA 偶聯微珠，該偶聯微珠是 CBA 空白微珠與 NeutrAvidin 偶聯而成，且所述 NeutrAvidin 與偶聯有生物素的 EB 病毒 gp78 抗原結合，該 EB 病毒 gp78 抗原為人工合成的由 Biotin-Ahx-Thr Ser Thr Ser His Arg Pro His Arg Arg Pro Val Ser Lys Arg Pro Thr His Lys 組成的多肽。



資料來源

BD OptEIA™ ELISA

ELISA (Enzyme-Linked ImmunoSorbent Assay) 即酵素免疫分析法，是藉由抗原抗體的之間專一性的鍵結，對檢體（例如：血清、血漿或體液）進行檢測，再利用酵素呈色的深淺進行定量，即可知道檢體裡標的物的濃度。

BD Biosciences 的 ELISA kits/sets 提供片段型專一性抗體 (specifically fragmented antibody)，能夠減少樣本中其他蛋白質的干擾，降低非專一性的結合，進而準確定量血清、血漿及細胞培養液裡的細胞激素。

ELISA 解憂錦囊

1. 在 ELISA 試劑的技術數據表 (TDS) 中，通常建議使用 2µg/mL 的 capture antibody 和 1µg/mL 的 detection antibody，為得到最佳信號與最低背景值，客戶須自行提相互滴定 (titration) capture antibody (1-4µg/mL) 和 detection antibody (0.25-2µg/mL) 以及調整細胞激素標準品的連續稀釋範圍
2. 細胞激素標準品處理：請仔細閱讀隨試劑盒所附的 dilution instructions on Instruction / Analysis Certificate，標準品濃度會隨批號而有差異
3. 為了獲得最佳的靈敏度，標準品和樣品可以反應隔夜
4. 使用過氧化物酶 (peroxidase) 作為顯色酶，在洗滌緩衝液和稀釋劑中避免疊氮化鈉 (sodium azide)，因為這是過氧化物酶活性的抑制劑

ELISA 異常排除指南

Observation	Probable cause	Observation	Probable cause
吸光度低	不正確的抗體製備	精確度差	pipetting 不準確
	標準品準備不當		試劑混合不充分
	酶或呈色試劑異常		樣品含有沉澱物
	使用不當的緩衝液 / 稀釋劑		實驗操作過程中讓 Well 乾掉
	試劑尚未回溫到室溫就加到 Well		Well 底部太髒
	試劑反應時間不正確	標準曲線差	標準品的稀釋或保存不當
	實驗操作過程中讓 Well 乾掉		使用不正確的稀釋劑
	自動 ELISA plate 清洗機的 沖洗力道太強		Well 清洗不完全
	機器讀取波長設定錯誤		pipetting 不準確
高背景值	不正確的抗體製備	樣品或標準品 讀值差異大	樣品混合不均勻
	Well 清洗不完全		Well 間交叉汙染
	呈色試劑汙染		pipetting 不準確

BD OptEIA™ ELISA Kit

- All in one，內附完整試劑及完整操作手冊
- 附有 pre-coated plate，讓實驗結果更穩定、更一致
- 產品內容：pre-coated 96 well plates, detection antibody, standard, ELISA diluent, standard/sample diluent, Enzyme reagent (Streptavidin-HRP), one-step TMB substrate reagent, Stop Solution, Plate Sealers.

Human

Cat. No.	Description	Size	Sensitivity (pg/mL)
550499	C3a Kit	1 plate	7.3
550947	C4a Kit	1 plate	6.2
557965	C5a Kit	1 plate	0.047
550612	IFN- γ Kit	2 plates	1
557966	IL-1 β Kit	2 plates	3.9
550611	IL-2 Kit	2 plates	1
550614	IL-4 Kit	2 plates	2
550799	IL-6 Kit	2 plates	2.2
550999	IL-8 Kit	2 plates	0.8
550613	IL-10 Kit	2 plates	2
551116	IL-12 (p40) Kit	2 plates	3.9
559258	IL-12 (p70) Kit	2 plates	4
559017	MCP-1 Kit	2 plates	1
550610	TNF Kit	2 plates	2

Mouse

Cat. No.	Description	Size	Sensitivity (pg/mL)
558258	IFN- γ Kit	2 plates	0.762
550950	IL-6 Kit	2 plates	3.8
560478	TNF Kit	2 plates	1.07
550487	Immunoglobulin Isotyping Kit	80 Tests	

Rat

Cat. No.	Description	Size	Sensitivity (pg/mL)
557825	C-reactive protein (CRP) Kit	1 plate	
560479	TNF Kit	2 plates	4.59
557081	Immunoglobulin Isotyping Kit	80 Tests	

Canine

Cat. No.	Description	Size	Sensitivity (pg/mL)
557826	C-reactive protein (CRP) Kit	1 plate	

BD OptEIA™ ELISA Set

- 每組 set 包含 20 個 96 well plates 的試劑，並附完整操作手冊 (cat. no. 558315 為 5 個 plates)
- 產品內容：capture antibody, detection antibody, standard, streptavidin-HRP
- 可選購 BD ELISA reagent set 一起使用 (cat. no. 550536 (set A), cat. no. 550534 (set B))

Human

Cat. No.	Description	Assay range (pg/mL)	Reagent Set
550948	CD253 Set (TRAIL)	62.5~4000	B
555175	Eotaxin Set	6.3~400	B
555126	GM-CSF Set	4.7~300	B
555142	IFN- γ Set	4.7~300	B
557953	IL-1 β Set	3.9~250	B
555190	IL-2 Set	7.8~500	B
559104	IL-2 sR α Set	7.8~500	B
555194	IL-4 Set	7.8~500	B
555202	IL-5 Set	7.8~500	B
555220	IL-6 Set	4.7~300	B
555244	IL-8 Set	3.1~200	B
555157	IL-10 Set	7.8~500	B
555171	IL-12 (p40) Set	31.3~2000	B
555183	IL-12 (p70) Set	7.8~500	B
559268	IL-15 Set	7.8~500	B
550926	IP-10 Set	7.8~500	B
555179	MCP-1Set	7.8~500	B
559119	TGF- β 1 Set	62.5~4000	B
555212	TNF Set	7.8~500	B
558315*	C5b-9 Set		B

*Reagent size for 5 plates

Mouse

Cat. No.	Description	Assay range (pg/mL)	Reagent Set
555167	GM-CSF Set	15.6~1000	B
555138	IFN- γ Set	31.3~2000	B
551866	IFN- γ Set (AN-18)	3.1~200	B
555248	IgE Set	1.6~100	B
552576	IgG2a Set	3.1~200	B
555148	IL-2 Set	3.1~200	B
555232	IL-4 Set	7.8~500	B
555236	IL-5 Set	15.6~1000	B
555240	IL-6 Set	15.6~1000	B
555252	IL-10 Set	31.3~2000	A
555165	IL-12 (p40) Set	15.6~1000	A
555256	IL-12 (p70) Set	62.5~4000	A
555260	MCP-1 Set	15.6~1000	A
555268	TNF (Mono/Mono) Set	15.6~1000	A
558534	TNF (Mono/Mono) Set II	15.6~1000	B

*Mouse TNF mono/mono ELISA set (Cat. No 555268) 和 mouse TNF ELISA set II (cat. No.558534) 的差別是 TNF mono/mono set 是只能偵測到 soluble TNF 而 mouse TNF ELISA set II 能偵測到 soluble TNF 與結合到 soluble TNF receptor 的 TNF。

Monkey

Cat. No.	Description	Assay range (pg/mL)	Reagent Set
551492	IFN- γ Set	15.6~1000	B

Rat

Cat. No.	Description	Assay range (pg/mL)	Reagent Set	Cat. No.	Description	Assay range (pg/mL)	Reagent Set
555198	IL-4 Set	1.6~100	B	555130	MCP-1 Set	31.3~2000	B
550319	IL-6 Set	78~5000	B	558535	TNF Set	31.3~2000	B
555134	IL-10 Set	15.6~1000	A				

BD OptEIA™ ELISA Set 選購試劑

Cat. No.	Description	contents	Size
550536	BD OptEIA™ Reagent Set A (pH 6.5 buffer)	Coating Buffer(pH 6.5), Assay Diluent, Wash Concentrate, Substrate, and Stop Solution	Reagents for 20 plates
550534	BD OptEIA Reagent Set B (pH 9.5 buffer)	Coating Buffer(pH 9.5), Assay Diluent, Wash Concentrate, Substrate, and Stop Solution	Reagents for 20 plates
555214	TMB Substrate Reagent Set	Two part substrate solution: Reagent A-hydrogen peroxide in buffer solution Reagent B-3,3', 5,5' tetramethylbenzidine (TMB) in an organic solvent	1 Set (300 mL each of 2 reagents)

BD ELISPOT

ELISPOT (Enzyme-Linked immunospot) 的作用原理和 sandwich ELISA 相似，可以用來偵測單一細胞所分泌的特定蛋白，例如細胞激素、趨化激素等。比起 ELISA 或是細胞內染色 (intracellular staining)，ELISPOT 有更高的靈敏度，可從 30 萬個細胞中偵測出 1 個分泌目標蛋白的細胞 (1/300,000)，是一個功能強大的偵測蛋白工具。

產品特色

高靈敏度 可偵測 30 萬分一正在分泌蛋白的細胞
嚴選抗體 capture 抗體為高品質 NA/LE 等級 (no azide /low endotoxin)，不影響細胞生長

實驗流程

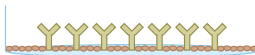
1 Capture Antibody

For Sets and Pairs : Coat microwells with anti-cytokine capture antibody.
For Kits : Go to Step 3; Steps 1 and 2 not necessary.



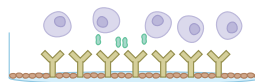
2 Blocking

Block unoccupied sites with protein



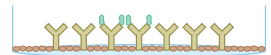
3 Add Cells

Incubate cells in well with Ag/stimulus etc.



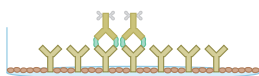
4 Wash

Cells are washed off



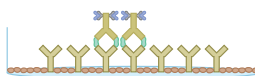
5 Detection Antibody

Add Biotinylated anti-cytokine detection antibody



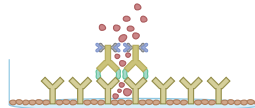
6 Enzyme-Avidin

Add Avidin-HRP



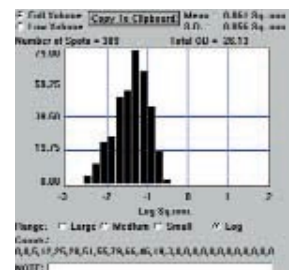
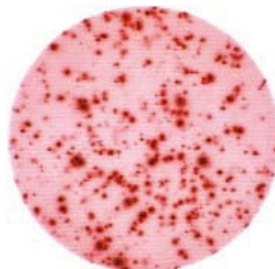
7 Develop With Substrate

Add substrate and monitor formation of colored spots



數據分析

須使用 ELISPOT reader 經過專業圖像分析軟體對斑點進行計數，便可以進行定量分析。



BD ELISPOT Kit 無須自行 coating 抗體

- 每組 kit 包含 2 個 96 well plates 的試劑。All in one，內附完整試劑及完整操作手冊
- 產品內容：Pre-coated plates, detection antibody, streptavidin-HRP, assay diluent, wash buffer, AEC substrate

Cat. No.	Description	Size
552138	Human IFN- γ Kit	2 plates
552569	Mouse IFN- γ Kit	2 plates

BD ELISPOT Set 附 96well PVDF plate，須自行 coating 抗體

- 每組 set 包含 10 個 96 well plates 的試劑，附有完整操作手冊
- 產品內容：capture antibody, detection antibody, streptavidin-HRP, ELISPOT plates.
- 可另外選購 AEC substrate (cat. no. 551951)

Human

Cat. No.	Description	Size
552572	Granzyme B Set	10 plates
551849	IFN- γ Set	10 plates
551282	IL-2 Set	10 plates
551084	IL-4 Set	10 plates
551085	IL-5 Set	10 plates
551018	IL-10 Set	10 plates
552574	IL-12p70 Set	10 plates
551446	TNF Set	10 plates

Mouse

Cat. No.	Description	Size
551083	IFN- γ Set	10 plates
551076	IL-2 Set	10 plates
551017	IL-4 Set	10 plates
551075	IL-5 Set	10 plates
552567	IL-6 Set	10 plates
558671	IL-12p70 Set	10 plates
551491	TNF Set	10 plates

BD ELISPOT Pair 須自行購買 96 well PVDF plate 進行 antibody coating

(Millipore plate, Cat. No. S2EM004M99 is recommended.)

- 每組 pair 包含 5 個 96 well plates 的試劑，附有完整操作步驟
- 產品內容：capture antibody (NA/LE 等級), detection antibody
- 可另外選購 HRP Streptavidin for ELISPOT (cat. no. 557630) 及 AEC substrate (cat. no. 551951)

Mouse

Cat. No.	Description	Size
551873	IFN- γ Pair	5 plates
551884	IL-2 Pair	5 plates
551885	IL-4 Pair	5 plates
551886	IL-5 Pair	5 plates
551883	IL-10 Pair	5 plates
551882	TNF Pair	5 plates

Cat. No.	Description	Size
551881	IFN- γ Pair	5 plates
551876	IL-2 Pair	5 plates
551878	IL-4 Pair	5 plates
551880	IL-5 Pair	5 plates
557982	IL-10 Pair	5 plates
551875	TNF Pair	5 plates

BD ELISPOT 選購試劑

Cat. No.	Description	contents	Size
551951	AEC Substrate Set	AEC Substrate and AEC Chromogen	1 set (for 10 plates)
557630	Streptavidin-HRP ELISPOT		1 mL



Accuri™ C6 Plus

Flow Cytometer



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- | 確效系統升級 | 自動化 QC、螢光補償值調整!
- | 操作軟體升級 | 功能更多，操作更方便!
- | 液流系統升級 | 上樣更穩更快!

UP!
全新升級