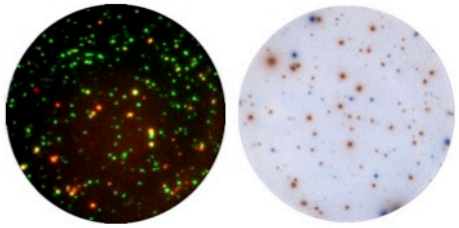
The Most Sensitive Cytokine Detection

C.T.L. ImmunoSpot System - ELISpot & FluoroSpot

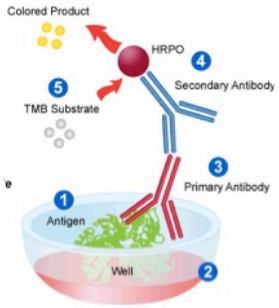


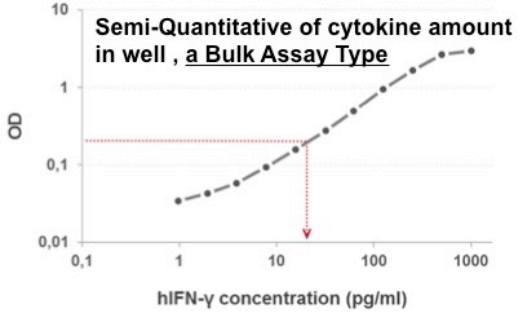
尚博生物科技有限公司
Cell-Bio Biotechnology Co., Ltd.

Glenn Yang

Product Manager
glenn.cellbio@gmail.com

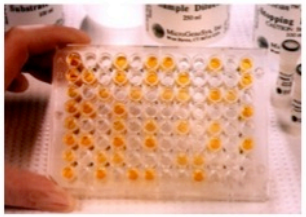
ELISA – Enzyme-Linked ImmunoSorbent Assay





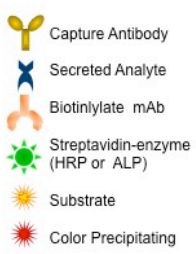
ELISA is a method for quantification of cytokines and other analytes in solution. It was first developed in 1971 and has since become one of the most widely used techniques in clinical and research laboratories. The ELISA can be used in a variety of formats from testing only a few individual samples to fully automated high-throughput screening.

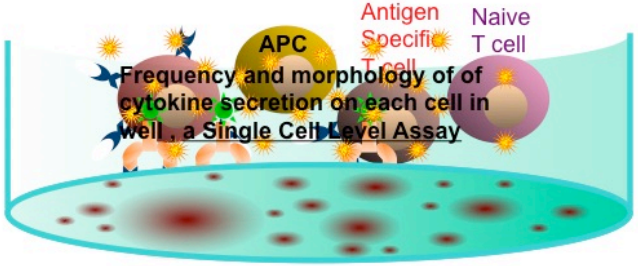
The sensitivity of an ELISA mainly depends on the affinity of the antibodies. The accuracy of the cytokine ELISA is commonly established by calibration with an external reference standard.



Principle of Enzyme-Linked ImmunoSpot = ELISPOT

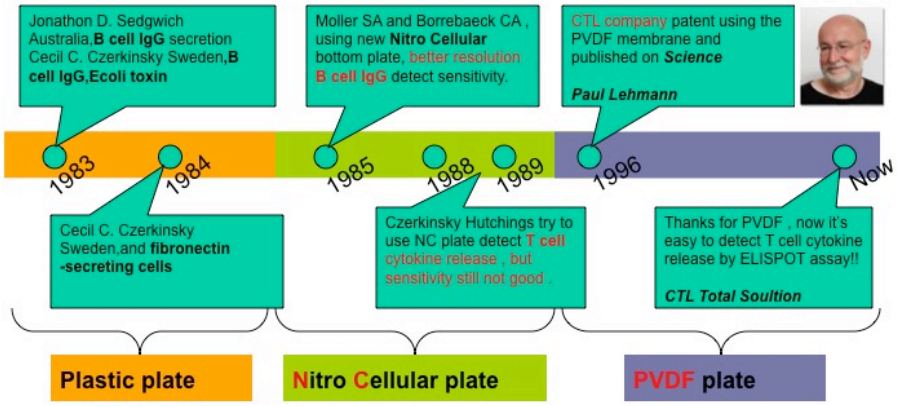
<Day1>
Capture Antibody : Coat micro well with antibody
<Day2>
(a)Blocking : Block unoccupied well sites with protein
(b)Add Cells : Incubate cells in well with Ag stimulus or APC.
<Day3>
(a)Wash : Cells are washed off
(b)Detection antibody : Add biotinylated anti-cytokine detection antibody
(c)Enzyme-Avidin : Add streptavidin-enzyme
(d)Develop With Substrate : Add substrate and monitor colored spots formation





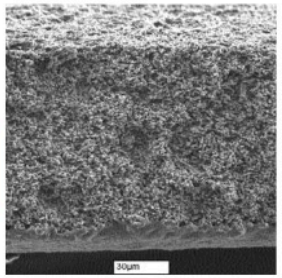
History of ELISpot

Enzyme-Linked ImmunoSpot = ELISPOT, an old technique base on sandwich ELISA, was used to detect antibody release frequency of B cell.

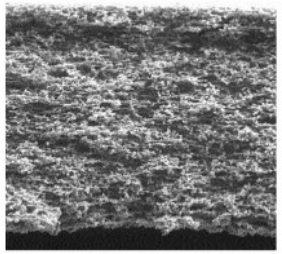


Membrane Attribute	NC (used in ELISPOT) [nominal or average values]	PVDF (used in ELISPOT) [nominal or average values]
Pore Size[a]	0.45 microns (µm)	0.45 microns (µm)
Porosity[b]	70 – 75%	65 – 70%
Thickness	150µm	135µm
B.E.T. Surface Area[c]	6.5m²/gram	6m²/gram
Surface Area Ratio[d]	250	350
Saturation Binding Capacity (IgG)	250µg/cm²	350µg/cm²
(IgG) Binding Capacity of Top 1µm	2µg	3µg
Wettability	Wettable due to the addition of surfactants or detergents to the membrane during membrane manufacture	Not directly wettable in water. Must be pre-wet with alcohol and then exchanged with water
Additives	Glycerin	None
Solvent Compatibility	Not compatible with methanol or ethanol	Broadly compatible with a wide range of aqueous and organic solvents. Avoid prolonged exposure to strong alkali (e.g., pH >12)
Mechanism of Binding	Electrostatic	Hydrophobic
Things which will interfere with or destabilize binding of anti-cytokine antibodies	Chaotropes (e.g., Tween-20, Triton-X 100, etc.). Water (if never dried), Proteins , especially larger molecular weight proteins	Detergents (e.g., SDS), low polarity solvents (e.g., dimethyl formamide, etc.)
Compatibility with different detection modes	✓ Colorimetric × Fluorescence ✓ Chemiluminescence	✓ Colorimetric ✓ Fluorescence (marginal) ✓ Chemiluminescence

PVDF membrane



NC membrane



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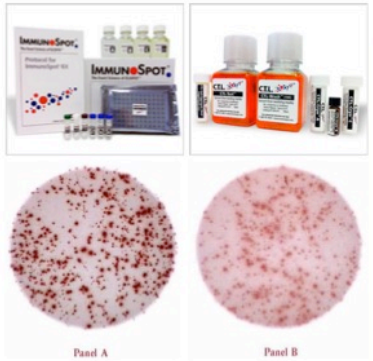
Rapidly growing ImmunoSpot



ELISPOT assay now is a powerful tool for detecting and enumerating individual cells that secrete a particular protein !

High sensitivity, specifity and affinity !

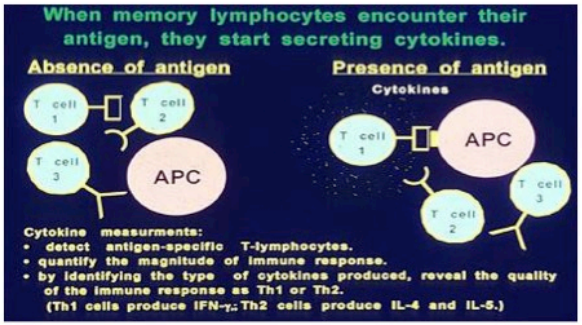
Figure 1. Frequency of publications citing ELISpot
Data were collected from a PubMed search for the keyword 'ELISpot'. Publication dates were sorted by year and a histogram showing frequency of publications per year was generated using the XML code for date of publication. The figure was last updated in early December 2010.

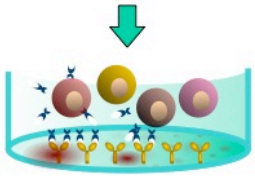
C.T.L. IMMUNOSPOT

WHY ELISpot ??

The best solution of Ex-vivo T Cell Diagnostic

ELISPOT Assay is using fresh (or Cryopreserveed) PBMC as samples , which include whole T Cell . (45mL whole blood can do over 600 antigen and peptide testing)





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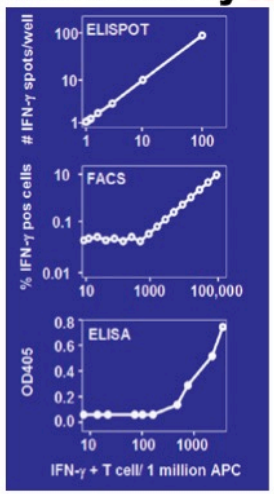
WHY ELISpot ??

Ultra-High Sensitivity for Detecting Cytokine Production by Rare Antigen Specific T Cell



- **ELISpot permits accurate frequency measurements down to the 1/10⁶ cell range**
- **ICS and ELISA (also CBA) reach detection limit at 1/1000**

- **Why detection limit is critical ?**
  - In 80s people believe we lose the memory cell.
  - Antigen Specific T cell
  - Minor population but important job

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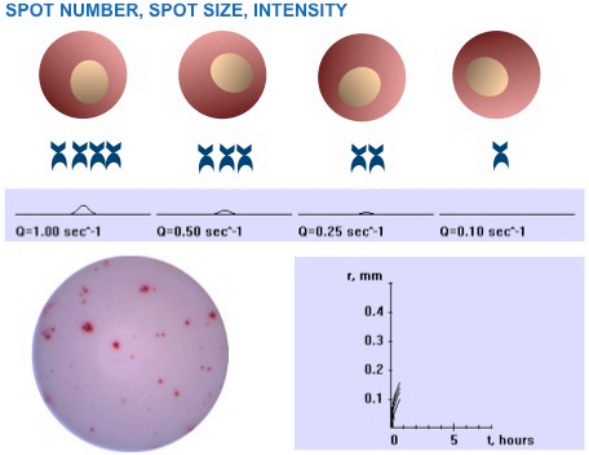
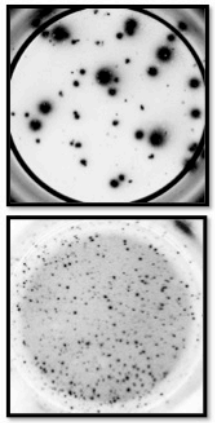
WHY ELISpot ??

Spot morphology reflect Single Cell productive FREQUENCY

SPOT NUMBER, SPOT SIZE, INTENSITY





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Competing Techniques vs. ELISPOT

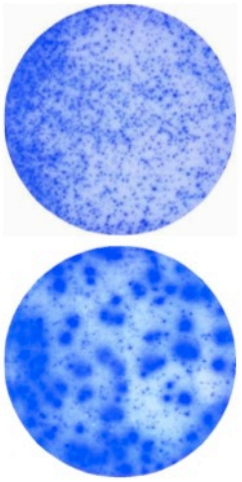
ELISA

Enzyme-linked Immunosorbent Assays

- **Much less sensitive** which makes it frequently fail for ex-vivo T cell measurements
- **No frequency information**
- **End point assay**
- **Low signal to noise resolution**: does not permit to distinguish between many cells making little cytokine (frequently "background" spots in medium control) vs. few cells making lots of cytokine (the relevant T cells in the antigen-induced "foreground").

Strength: low cost, ease of use

NK cells - IFNγ



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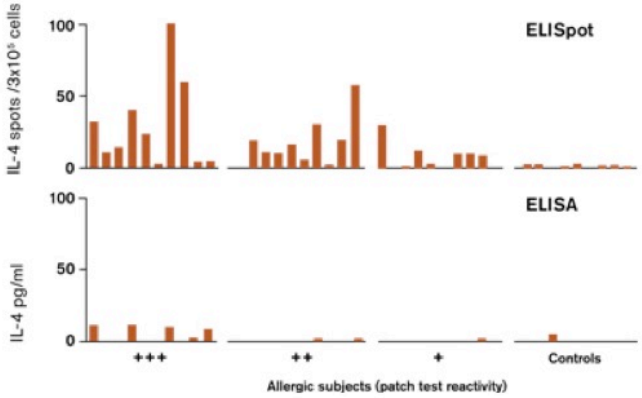
Competing Techniques vs. ELISPOT

ELISA

Enzyme-linked Immunosorbent Assays



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Competing Techniques vs. ELISPOT

ICS (FlowCytometry)

Intracytoplasmic Staining

- **Does not account for post-transcriptional regulation: synthesis of does not mean biologically relevant secretion, e.g., IL-2.**
- **Does not permit to distinguish between storage and release of protein e.g., Granzyme B and Perforin.**
- **Does not permit endpoint measurement: e.g., actual killing (unlike Lysispot)**
- **Pharmacological treatment: altered cell functions vs. the untreated cells in ELISPOT. Cells do not survive analysis: can not be rescued and recultured like in ELISPOT.**
- **Very long analysis time when it comes to low frequency cells.**
- **Expensive analysis instrumentation, high maintenance fee.**

Strength: permits simultaneous determination of CD4/CD8 lineage (other cell surface markers are uninformative if these are activation dependent since all cytokine expressing cells have been recently activated).

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Competing Techniques vs. ELISPOT

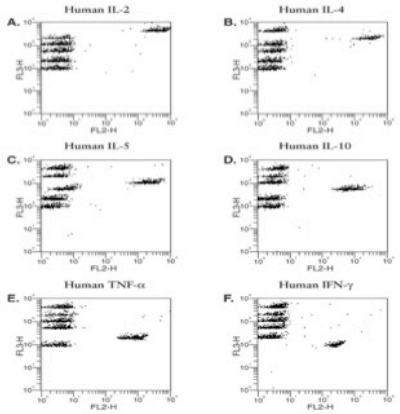
Bead Arrays

Same weakness as ELISA

- Relatively low sensitivity
- No frequency information
- Low signal to noise resolution

Plus weakness of flow cytometry

- High cost of instrumentation,
- High maintenance effort and fee



Strength: simultaneous analysis of multiple cytokines, relative fast analysis vs. other flow based measurements (but still much slower than ELISPOT analysis

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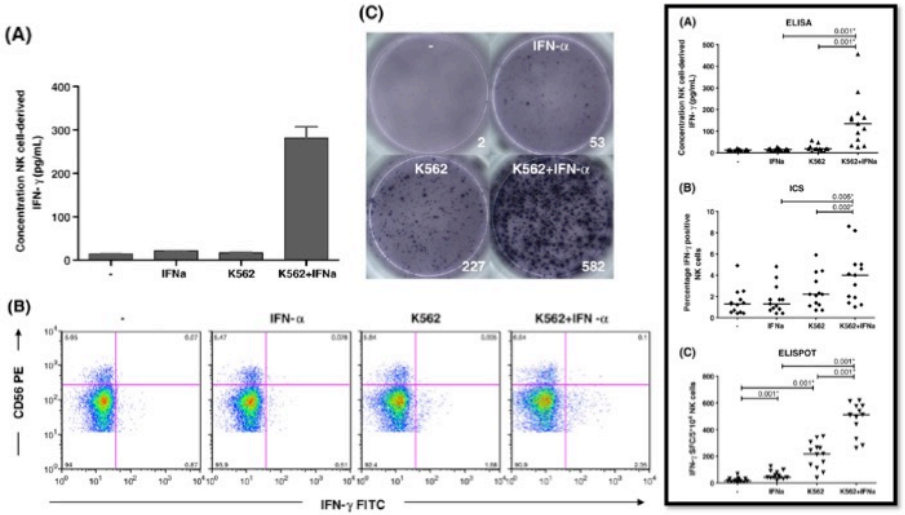
Quantification of IFN-γ produced by human purified NK cells following tumor cell stimulation: Comparison of three IFN-γ assays



E. Lion et al., *Journal of Immunological Methods* 350 (2009) 89–96

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ImmunoSpot Applications:

- **Vaccine development**
  - Vaccine efficacy assessment
  - Antigen-specific T-cell
  - Total Ab / Ag specific Ab B-cell
  - Epitope / peptide mapping
  - Vaccine-induced antibody responses
  - Antigen-specific memory B cells
- **Hypersensitive / Allergies**
  - Measuring Th0/Th1/Th2 transition
  - IL-4 induce T helper cell transition
  - IL-4, IL-5 and IL-13 secretion
  - T-cell epitopes in allergens
- **Autoimmunity / Allograft rejection**
- **Cancer**
  - Antigen-specific T cells
  - Tumor antigens
  - Cytotoxic T-cell activity (CAR-T cell)
  - Cancer vaccine efficacy
  - Granzyme B and Perforin release by T cells
  - Vaccine-induced antibody responses
- **Infectious diseases**
  - Tuberculosis
  - Malaria
  - HIV, Influenza …etc

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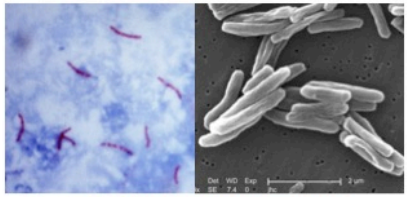
Application : Tuberculosis Diagnostics



Mycobacterium tuberculosis (MTB) 結核桿菌

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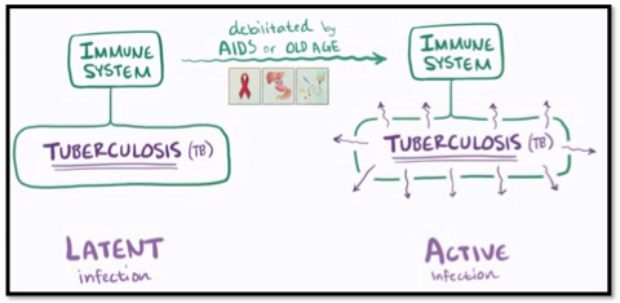
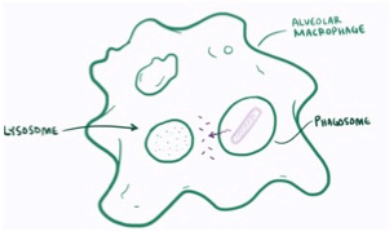
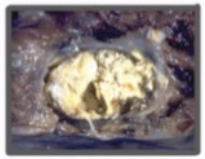
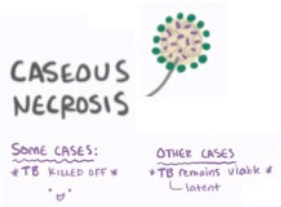
Application : Tuberculosis Diagnostics



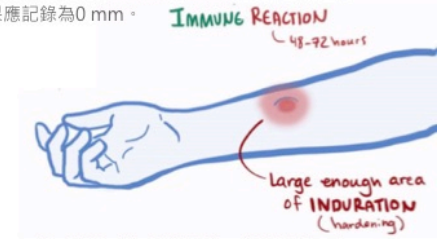






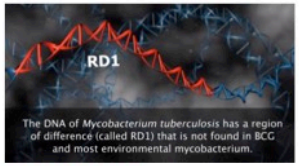
Application : Tuberculosis Diagnostics

- 方法是於皮下PPD (Purifiedprotein Derivative - a mixture of protein derided from dead Mycobacterium Bovis cultures) 引發記憶性T cell 之延遲性過敏反應
- BUT施打的Bacillus Calmette-Guerin (BCG-卡介苗)或曾經受到非結核分歧桿菌感染，易造成偽陽性；免疫機能不全（如愛滋病）或受損（使用免疫抑制劑者）及受到某些病毒感染（如麻疹病毒）的人，或肺結核已發作時，TST有可能呈現偽陰性反應或甚至出現無反應之情況
- TST的缺點還包括病人至少需要兩次就診、不同觀察者一致性低、免疫不全者(other disease：HIV... etc)偽陽性高；同時對於潛伏期的偵測準確度也不好，也無法區別是潛伏期或是發病期。
- 進行皮內注射後，並在48至72小時後觀察結果；這個測試結果的判定由明顯突起的硬化區的直徑（垂直於手臂）來反映。如果不存在任何硬結，結果應記錄為0 mm。
- 第一次注射後48至72小時檢查結果
  - 呈陽性，考慮感染者
  - 呈陰性，在1至3周後給予第二次測試
- 第二次注射後48至72小時檢查結果
  - 呈陽性，考慮以前的感染
  - 呈陰性，考慮未感染



Tuberculin Skin Test (TST)：1907 Mautoux

Application : Tuberculosis Diagnostics



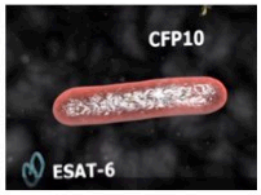
Unrivalled Clinical Performance

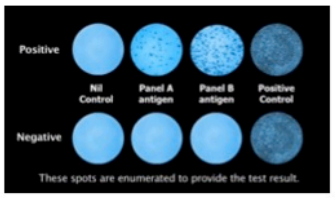
Oxford Immunotec

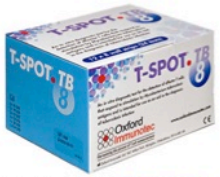
- Very few false negative results (sensitivity of 98.8%)
- No patient exclusions - Can be used in HIV, very young children, screening before anti-TNFα treatment, transplant renal dialysis, and other immunocompromised patient groups, as well as in pregnancy

T-SPOT.*TB*

The T-SPOT.*TB* test sets new clinical standards of sensitivity and reliability. The product was licensed across Europe in July 2004, received FDA premarket approval in July 2008, was approved in China in 2010 and in Japan in 2012. It has been designed to replace the tuberculin skin test (Mantoux test), bringing effective TB testing to many new patient groups where the skin test gives poor or unreliable results.







Application :Cancer Research

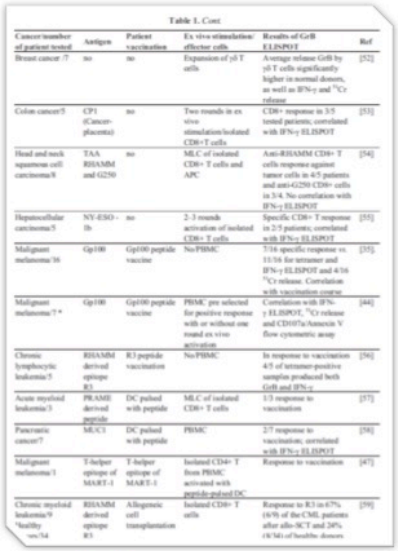


Table 1. Cont.

Cancer/number of patient tested	Antigen	Patient vaccination	Ex vivo stimulation/ effector cells	Results of GrB ELISPOT	Ref
Breast cancer /7	no	no	Expansion of γδ T cells	Average release GrB by γδ T cells significantly higher in normal donors, as well as IFN-γ and ^{51}Cr release	[52]
Colon cancer/5	CP1 (Cancer-placenta)	no	Two rounds in ex vivo stimulation/isolated CD8+T cells	CD8+ response in 3/5 tested patients, correlated with IFN-γ ELISPOT	[53]
Head and neck squamous cell carcinoma/8	TAA RHAMM and G250	no	MLC of isolated CD8+ T cells and APC	Anti-RHAMM CD8+ T cells response against tumor cells in 4/5 patients and anti-G250 CD8+ cells in 3/4. No correlation with IFN-γ ELISPOT	[54]
Hepatocellular carcinoma/5	NY-ESO-1b	no	2-3 rounds activation of isolated CD8+ T cells	Specific CD8+ T response in 2/5 patients, correlated with IFN-γ ELISPOT	[55]
Malignant melanoma/36	Gp100	Gp100 peptide vaccine	No/PBMC	T/16 specific response vs. 11/16 for tetramer and IFN-γ ELISPOT and 4/16 ^{51}Cr release. Correlation with vaccination course	[35]
Malignant melanoma/7 *	Gp100	Gp100 peptide vaccine	PBMC pre selected for positive response with or without one round ex vivo activation	Correlation with IFN-γ ELISPOT, ^{51}Cr release and CD107a/Annexin V flow cytometric assay	[44]
Chronic lymphocytic leukemia/5	RHAMM derived epitope R3	R3 peptide vaccination	No/PBMC	In response to vaccination 4/5 of tetramer-positive samples produced both GrB and IFN-γ	[56]
Acute myeloid leukemia/3	PRAME derived peptide	DC pulsed with peptide	MLC of isolated CD8+ T cells	1/3 response to vaccination	[57]
Pancreatic cancer/7	MUC1	DC pulsed with peptide	PBMC	2/7 response to vaccination; correlated with IFN-γ ELISPOT	[58]
Malignant melanoma/1	T-helper epitope of MART-1	T-helper epitope of MART-1	Isolated CD4+ T from PBMC activated with peptide-pulsed DC	Response to vaccination	[47]
Chronic myeloid leukemia/9 *healthy vs/54	RHAMM derived epitope R3	Allogeneic cell transplantation	Isolated CD8+ T cells	Response to R3 in 67% (6/9) of the CML patients after allo-SCT and 26% (8/34) of healthy donors	[59]

ELISPOT Assay for Monitoring Cytotoxic T Lymphocytes (CTL) Activity in Cancer Vaccine Clinical Trials

Anatoli M. Malyguine [1,*], Susan Strobl [1], Kimberly Dunham [1], Michael R. Shurin [2] and Thomas J. Savers [1]

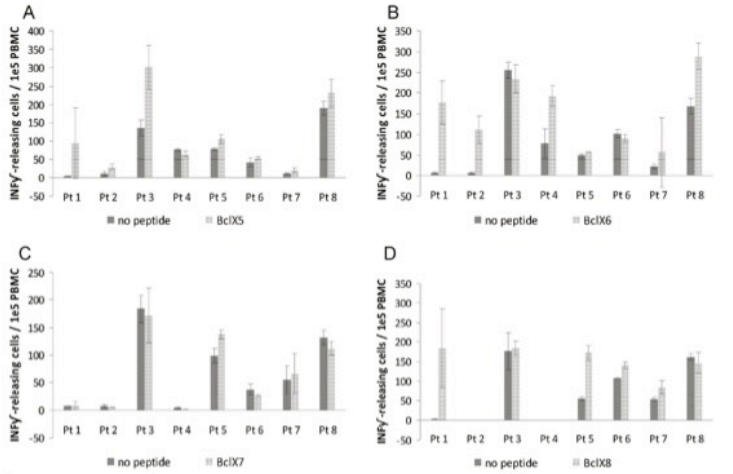
Table 1. Application of GrB ELISPOT assay for monitoring antitumor response in cancer patients.

Cancer/number of patient tested	Antigen	Patient vaccination	Ex vivo stimulation/ effector cells	Results of GrB ELISPOT	Ref
Breast cancer/3	Bcl-x_L	no	One round/PBMC	CTL responses against Bcl-$x_{L176-184}$ in 2/3 patients, correlated with IFN-γ ELISPOT and ^{51}Cr release assay	[51]

Monitoring T cell responses in the course of clinical trials is widely used to assess the efficacy of cancer immunotherapy. Selection of an *ex vivo* monitoring technique that provides the best measure of immune reactivity is important in determining potential correlations between clinical and immunologic responsiveness to specific immunotherapy. Standard immunological assays, such as cytokine induction, cell proliferation, and ^{51}Cr-release, can detect the overall immune responses in vaccinated patients but are not suitable for efficient evaluation of individual effector cell reactivity. For instance, the tetramer assay identifies the number of epitope-specific cytotoxic T lymphocytes (CTL) [1] but does not necessarily equate to their functional activity [2,3]. To quantitate the functionally active cells, this assay should be combined with intracellular cytokine staining. Assays that can monitor both CTL frequency and function, such as the IFN-γ ELISPOT assay, have gained growing popularity for the immunomonitoring of clinical trials [4–6]. However, since Granzyme B and perforin are key mediators of targeted cell death via the granule-mediated pathway [7], the Granzyme B (GrB) and Perforin (Pfp) ELISPOT assays may represent more direct methods for the analysis of cell-mediated cytotoxicity compared to the IFN-γ ELISPOT.

Cells, 2012, 1, 111-126; doi:10.3390/cells1020111

Application :Cancer Research



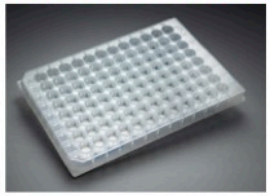
BclX"n" family mapping and immune response

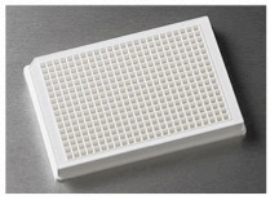
Andersen et al. *Cells*, **2012**

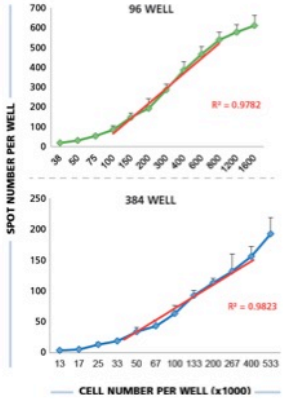
Current State ELISPOT , more Throughput and Content

- Typical assays using PBMC are done with $1x10^5$ – $2.5x10^4$ cells per test condition for 96-well (45mL 的全血就可以分析超過600種抗原或peptides)

High-throughput Suitability







PBMC were plated in serial dilution, and CMV-PP65 was used to elicit IFN-γ production by the specific CD8 cells.

For 96 well plates, a linear relationship was seen between $1x10^5$ and $8x10^5$ PBMC per well.

For 384 well plates, this range was shifted by approximately one third of the cell number, between 0.3×10^5 and 3×10^5 PBMC per well.

High-throughput Suitability

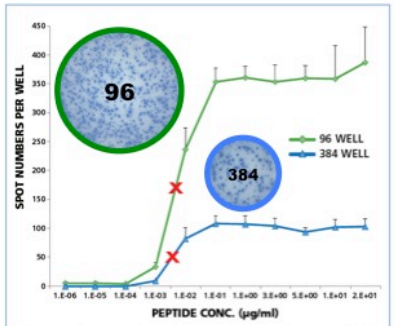


Figure 4: **Identical antigen dose response curves for 96- and 384-well plates.** HCMV peptide pp65-induced IFN-γ production was measured in both plate types in parallel with one-third of the numbers of PBMC plated per well for 384-well plates. While the maximal spot counts induced by the peptide were approximately 3x higher for 96-well plates, the 50% maximally stimulatory peptide dose (marked by the red X) was identical for the two plate types. Thus, the 384-well format is equally suited for T cell affinity measurements.

Cells **2015**, 4, 71-83; doi:10.3390/cells4010071

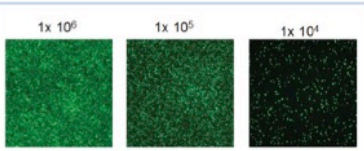


Figure 3. **PBMC number-dependent monolayer formation in 96-well plate format.** PBMC were stained with calcein and the specified numbers of cells were plated per well. Note, cell crowding starts at $1x10^6$ cells per well, and cells do not form a monolayer any more under $1x10^6$ cells per well corresponding to the range in which PBMC numbers and spot counts are linear in the ELISPOT assay (See Figure 2).

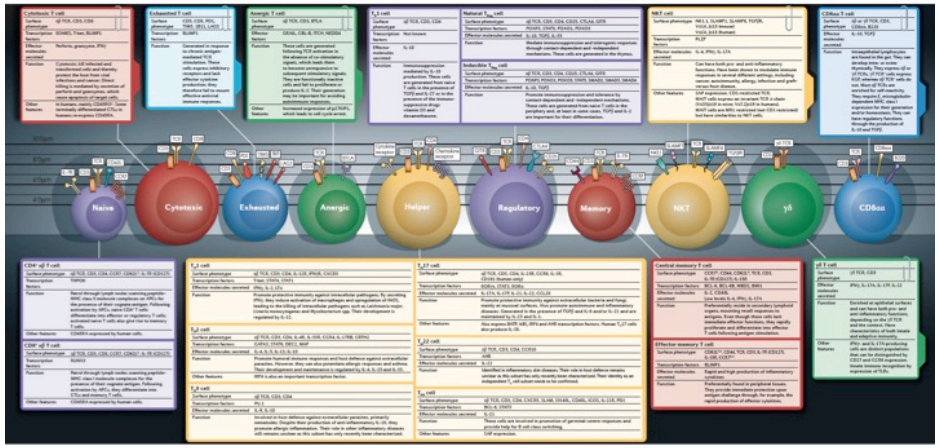
Test	96 W	384 W	96W/384W
1	670.0	177.5	**3.8**
2	577.5	177.3	**3.3**
3	547.3	173.8	**3.1**
4	499.3	164.0	**3.0**
5	423.0	118.5	**3.6**
6	344.5	102.8	**3.4**
7	260.0	77.8	**3.3**
8	140.3	52.3	**2.7**
9	126.0	35.5	**3.5**
10	66.8	21.8	**3.1**
11	26.0	7.5	**3.5**
			MEAN 3.3 ±0.3

Current State ELISPOT , more Throughput and Content

More Content of ELISpot for T cell immune monitoring

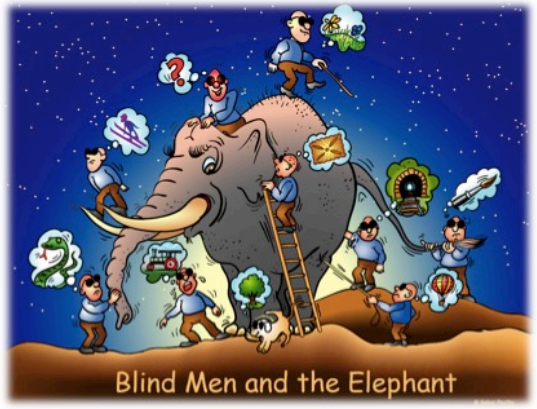
- **A brief history of T-cell mediated immunity**
  - In 80s proliferation and killing assays – one type of T cell
  - In 90s Th1/Th2
  - Past few decades：70% of people -> IFNγ
  - NOW：multitude of effectors function and still not totally understand
- **Effector / memory T cell classes：the need for multiplexing T cell measurements, and how different ELISPOT assays can contribute…**
- Functional T-cell master regulators of many immune response
  - Th1 – IFNγ
  - Th2 – IL4
  - Th17 – IL17
  - Th5 – IL5
  - Tfh – IL21
  - Treg – IL10
  - …etc

Current State ELISPOT , more Throughput and Content



C.T.L. IMMUNOSPOT

Current State ELISPOT , more Throughput and Content



The need for Comprehensive Immune Monitoring

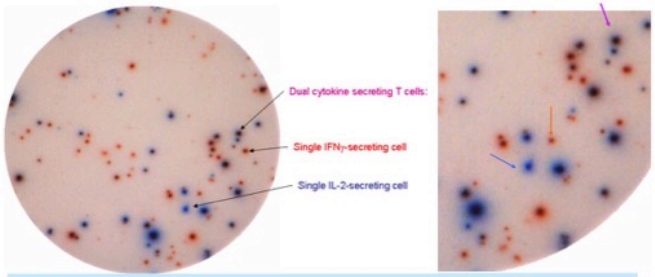
This push up the content of current state ELISPOT

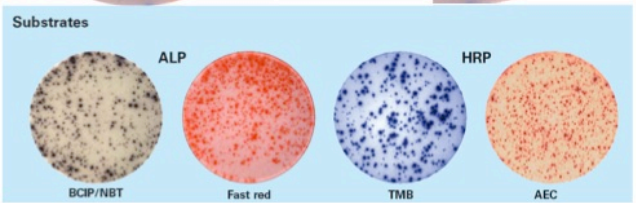
- Dr. Alexey Karulin (co-founder and vice president of R&D at CTL, USA) in 2001 : **FIRST** Dual Color ELISpot

C.T.L. IMMUNOSPOT

Current State ELISPOT , more Throughput and Content

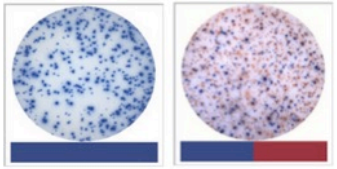
Dual Color ELISpot





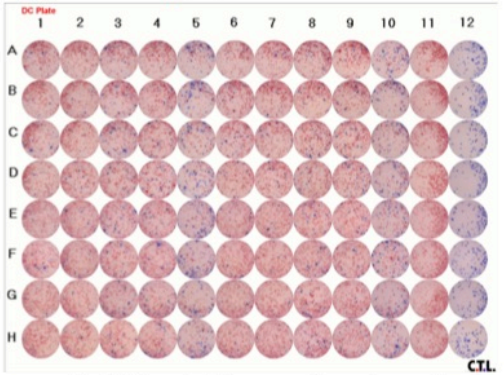
C.T.L. IMMUNOSPOT

Current State ELISPOT , more Throughput and Content



SINGLE-COLOR ENZYMATIC	DOUBLE-COLOR ENZYMATIC
hu-IFN-γ	hu-IFN-γ/GzB
hu-GzB	hu-IFN-γ/TNFα
hu-TNF-α	hu-IFN-γ/IL-2
hu-IL-1B	hu-IFN-γ/IL-4
hu-IL-2	hu-IFN-γ/IL-5
hu-IL-4	hu-IFN-γ/IL10
hu-IL-5	hu-IFN-γ/IL12
hu-IL-10	hu-IFN-γ/IL-17
hu-IL-12	hu-IL-10/IL-12
hu-IL-13	hu-IL-10/IL-17
hu-IL-17	

IFNγ and IL-2

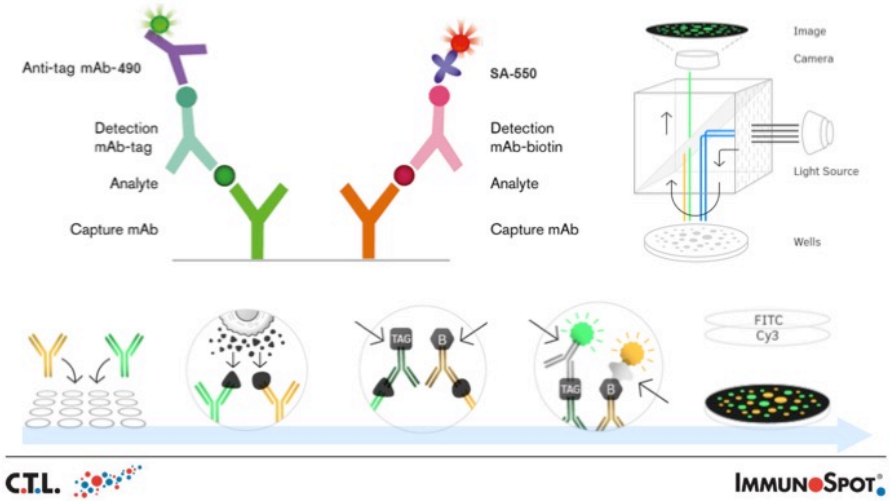


- Multiple cytokine monitor at one time
- Complex immune response study
- Time and Cost Saving

C.T.L Dual Color ELISPOT

Current State ELISPOT , more Throughput and Content

From ELISpot to FluoroSpot

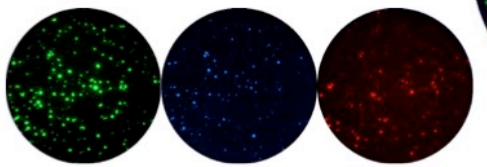


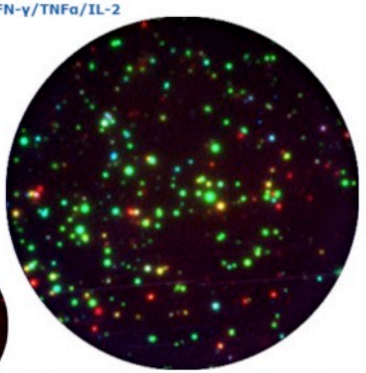
C.T.L.

IMMUNOSPOT

Current State ELISPOT , more Throughput and Content

SINGLE-COLOR FLUOROSPOT	DOUBLE-COLOR FLUOROSPOT	THREE-COLOR FLUOROSPOT
IFN-γ	IFN-γ/GzB	IFN-γ/TNFα/Granzyme B
GzB	IFN-γ/TNFα	IFN-γ/TNFα/IL-2
TNFα	IFN-γ/IL-2	
IL-2	IFN-γ/IL-4	
IL-4	IFN-γ/IL-5	
IL-5	IFN-γ/IL10	
IL-10	IFN-γ/IL-17	
IL-12	IL-10/IL-12	
IL-17		



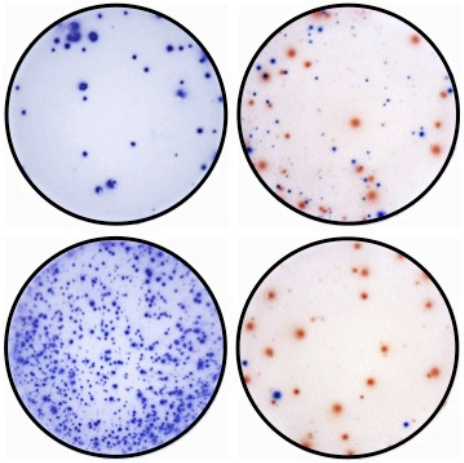


More cytokine detection at once

C.T.L FluoroSpot

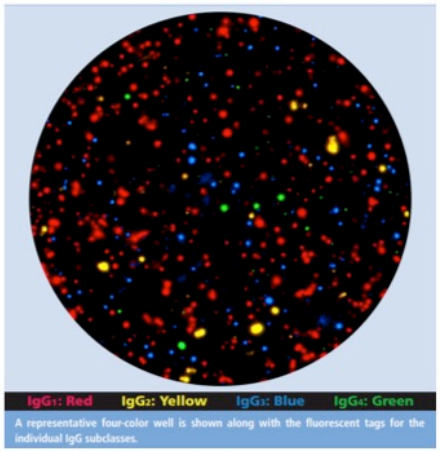
Current State ELISPOT , more Throughput and Content

- Single Color
  - IgA
  - IgM
  - IgE
  - IgG
  - Sub-Isotype
    - IgG1
    - IgG2
    - IgG3
    - IgG4
- Double Color
  - Any pair from above

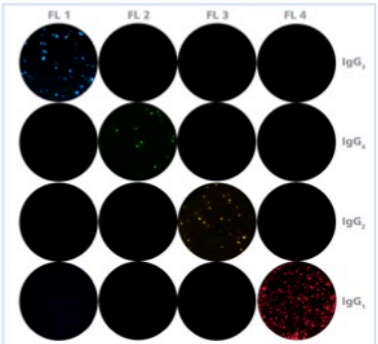


C.T.L B cells ELISpot

Current State ELISPOT , more Throughput and Content



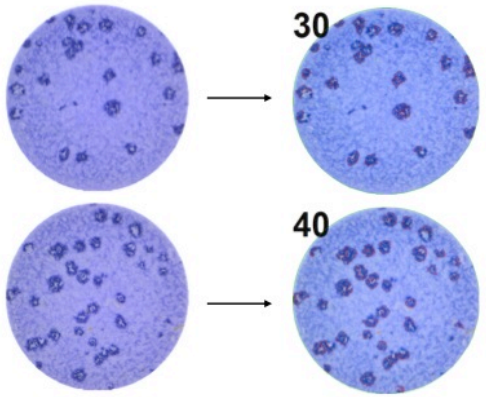
A representative four-color well is shown along with the fluorescent tags for the individual IgG subclasses.



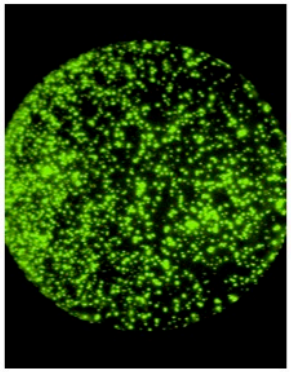
In a 4-Color B cell FluoroSpot assay, each sub-class specific detection antibody is labeled with a fluorochrome that has an emission wavelength that clearly distinguishes it from the other fluorochromes it is used in combination with. Using an ImmunoSpot® Analyzer allows clear, unambiguous detection of all 4 wavelengths, and thus all 4 IgG subclasses can be visualized simultaneously.

C.T.L 4 Color Human B cell FluoroSpot

Current State ELISPOT :
A New Approach of Reader : BioSpot®



Viral Plaque Assay



Stem Cell CFU

Why C.T.L ?

We Provide Total Solution for your ELISPOT Assay !

Why C.T.L ?

CTL's Serum-Free Media

Standardization Your ELISPOT Assay

Serum is the largest variable in ELISPOT assay performance. Therefore CTL has a fine tuned serum-free media portfolio for standardized, high-performance T cell monitoring with PBMC

CTL-Test™ Medium – Serum Free Media
提供充足營養的無血清培養基。

CTL-Wash™ Medium
T 細胞和抗原呈現細胞在清洗過程中如果沒有血清中的營養物質，細胞的功能性會受到很大損害，CTL-Wash™在去除血清影響的同時，在清洗過程中提供充足的營養，保證細胞的功能不受損害。

CTL-Cryo™:
無血清凍存液；按照CTL的細胞凍存解凍操作流程，解凍後的細胞能保持完整的功能性，並且死亡和凋亡的比例小於10%。

CTL Anti-Aggregate Wash™:
協助細胞解凍過程穩定並防止細胞聚集而死亡。

"Performs better than serum"

- Low Background
- High Signal
- Standardization and Quality Control
- Cost effective

Why C.T.L ?

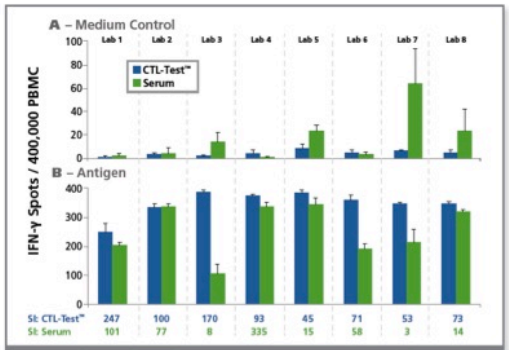


Figure 1: **Performance of serum-free CTL Test™ Medium compared with eight different qualified sera.** Cryopreserved PBMC of the same batch were thawed and tested in an IFN-γ ELISPOT assay in eight different laboratories using either CTL-Test™ (blue), or, in parallel, in the serum that the respective laboratory has selected for T cell work (green). Spot counts obtained in the medium control are shown in **Panel A.** The antigen- (CEF peptide)- induced spot counts are shown in **Panel B.** The stimulation index (SI: antigen-induced spots/medium background) defining the strength of signal measured is also shown for each of the laboratories for the results obtained with CTL-Test™ (SI: CTL-Test™), and with the respective laboratory's serum (SI: Serum). In all cases, the maximal spot counts with CTL-Test™ were equal to better than the sera, and four of the eight sera induced an elevated background (Zhang et al., *J. Immunotoxicology*, 2009, 6:227).

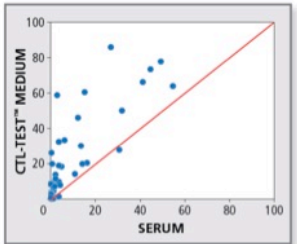


Figure 2. **IFN-γ ELISPOT assay performance with serum-containing complete RPMI medium vs. the serum-free CTL-Test™ Medium.** Freshly isolated PBMC of three healthy donors were tested in parallel under both conditions; the number of spots obtained using CTL-Test™ (Y axis) or in serum (X axis) after stimulation with antigens that stimulate CD4 cells, Mumps, PPD and Candida, is represented by the dots. The red line indicates equal performance. The medium background was zero for all conditions. Note, several recall responses that were not detectable in serum became clearly positive in CTL Test™ Medium.

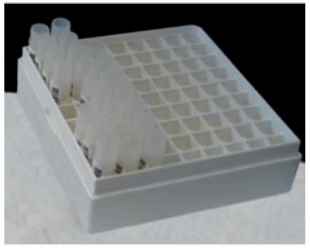
Why C.T.L ?

CTL's Cryopreserve PBMC

Standardization Your ELISPOT Assay

- PBMC frozen serum-free for >90% viability at thawing, with >95% functionality in ELISpot and cytokine recall assays compare to the fresh PBMC.
  - Characterized PBMC
  - HLA-typed: at low and high resolution* Immunotyped - characterized for antigen reactivity*
  - Custom characterization available*

C.T.L公司大規模的凍存PBMC，成立PBMC資料庫，提供經過測試(characterized)與標準化(quality control)的PBMC，這些保持完整功能性的凍存PBMC，現在您可以像購買試劑一樣輕鬆獲得!



- 大規模的樣本庫為您提供持續穩定的樣本來源，為您的PBMC實驗提供標準校驗。
- 免除您獲得樣本的法律和道德麻煩。
- Characterized PBMC極大 節省您篩選樣本的費用和精力，只需在庫中選取就能立即獲得符合您需求特性的樣本

C.T.L. IMMUNOSPOT

Why C.T.L ?

CTL's CEF Peptide Pools

Standardization Your ELISPOT Assay

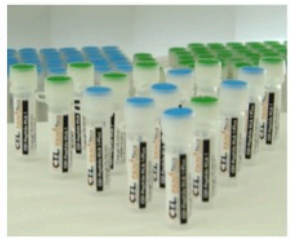
CTL's CEF Peptide Pools:
Peptides of CMV, EBV and Flu virus to stimulate cytokine release in peptide-specific CD8 memory cells.

CEF Class I Peptides and Peptide Pools

Reference Standards for Detecting CD8 Memory T Cells in PBMC

CEF Class II Peptide Pools

Reference Standards for Detecting CD4 Memory T Cells in PBMC



23 CEF Class I Peptide Pool
The "classic" ***23 peptide*** pool that covers determinants restricted by ***11 HLA class I alleles.***

32 CEF Class I Peptide Pool
The extended peptide pool consisting of ***32 peptides*** that cover ***15 HLA class I alleles.***

"The ultimate positive control for CD8 cell function in PBMC"

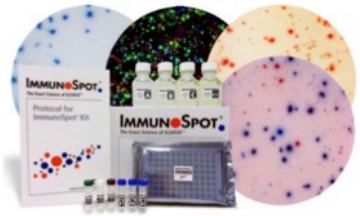
C.T.L. IMMUNOSPOT

Why C.T.L ?

CTL's ImmunoSPOT Kits

Standardization Your ELISPOT Assay

- High Specificity
- No Prewetting
- **No Blocking**
- Sterile Plates
- **Serum-free medium included**
- **Fix substrate development time**
- **All buffer included**
- Less Washing Steps
- Exquisite Sensitivity
- **Ease of standardization**



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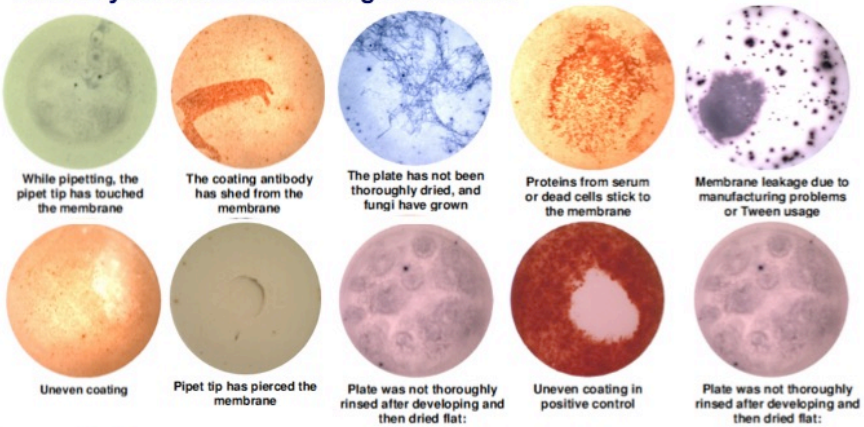
Why C.T.L ?

CTL is a group of ELISPOT Profession

Not only a technic provider ,
C.T.L is your trouble shooting consultant



C.T.L. IMMUNOSPOT

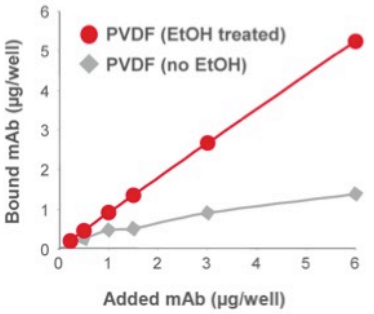
How to start ELISPOT assay ?

Detect INF-r Secretion of Antigen Specific CD8+ T Cell by ELISPOT Assay

C.T.L. IMMUNOSPOT

Pre-wet PVDF Membrane by EtOH

Don't need in CTL kit



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Coat well with capture antibody
(Pre-coated plate also provide)

Carefully empty the plate. Add 100 µl/well of coating antibody diluted in PBS. A concentration of 10-15 µg/ml coating antibody (1-1.5 µg antibody/well) is generally recommended. Leave the plate with coating antibody solution at +4°C over night.

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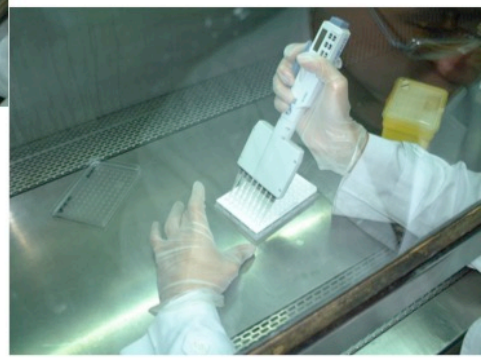
Blocking with Complete Medium

Don't need in CTL kit

Reactivate 2hr at RT



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Incubate ***1-5×10^5*** PBMC in Well with APC or Ag stimulus

(5 ng/ml PMA or 500 ng/ml Ionomycin or CEF peptide as positive control)

Setup plate layout

Plate Layout

	Stimulated, 1 X10E6 c/ml				Non-Stimulated							
	1	2	3	4	5	6	7	8	9	10	11	12
A	1:1				1:1							
B	1:3				1:3							
C	1:9				1:9							
D	1:27				1:27							
E	1:81				1:81							
F	1:243				1:243							
G	1:729				1:729							
H	No Cells	No Cells	No Cells	No Cells	No Cells	No Cells	No Cells	No Cells				

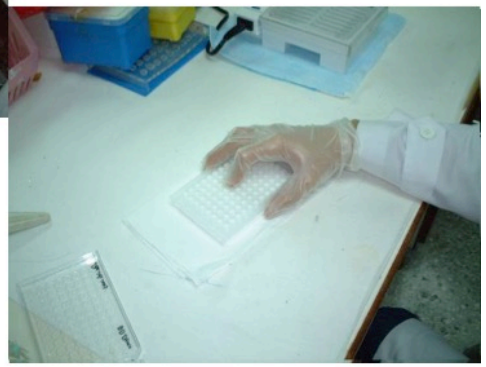
C.T.L. IMMUNOSPOT

Reactivate in incubator for 12~24hr

Note it! DO NOT MOVE PLATE WHEN STIMULATION

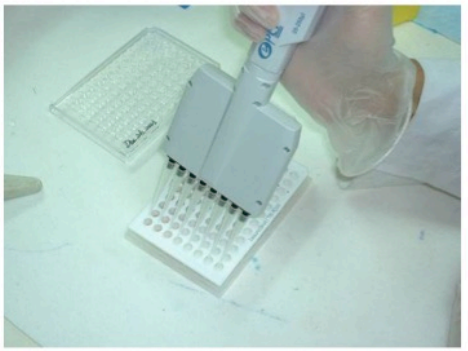
C.T.L. IMMUNOSPOT

Remove cell
(or collect for other experiment)



Rinse by ddH_2O several times
Remove Water by shaking.

C.T.L. IMMUNOSPOT

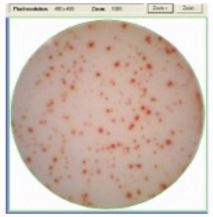


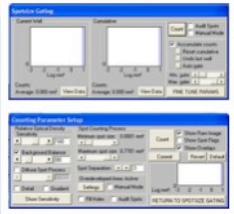
- Add biotinylated anti-INF-r detection antibody then wash
- Add Strapavdin-HRP then wash
- Add substrate and monitor formation of colored spots
- Analyze plate by CTL analyzer

Comparison of Substrates for ELISpot

Substrate	Format	Enzyme	Color Development	Sensitivity
TrueBlue	1-C	HRP	Blue	√√√
BCIP/NBT	1-C	AP	Dark Purple	√
AEC	2-C	HRP	Red	√

C= Component √=Least sensitive √√√=Most Sensitive





C.T.L. IMMUNOSPOT