

**The ELISpot (enzyme-linked immunoassay)** was originally described for the detection of individual B cells secreting antibody. However, this method has since been modified to **detect** and **enumerate individual cells producing** and **secreting** effector molecules such as **cytokines**. It is the **most appropriate method** to **analyse cytokine production** in conditions closely comparable to the **in situ environment** in a highly specific way. ELISpot is the ideal tool to follow disease regression under appropriate treatment.

Diaclone ELISpot assays are based on sandwich immuno-enzyme technology. Cell secreted cytokines or soluble molecules are captured by coated antibodies avoiding diffusion in supernatant, protease degradation or fixation on (soluble) membrane receptor. After cell removal, the captured cytokines are revealed by tracer antibodies and appropriate conjugates.

Each spot represents a single secreting cell. The sensitivity of the ELISpot allows the detection of a single antigen specific cell amongst **10 000 to 100 000 cells**.

Therefore the ELISpot technique may be considered as being **semi-quantitative** providing information both qualitative (secreted molecule) and quantitative (number of secreting cells).

Monitoring immune response of patients treated with immunotherapy is essential to evaluate the efficacy of treatment and correlate clinical responses to Cytotoxic T Lymphocyte (CTL) responses. ELISpot assays are **specific, accurate, sensitive, precise and robust**.

For these reasons, this bioassay is now recognized as the **reference method** to evaluate **vaccine efficacy** in clinical trials. The use of ELISpot assay as a predictive tool in immunotherapy emerges especially in cancer<sup>1,2,3</sup> and infectious disease<sup>4</sup>.

**Cancerology:** The identification of tumour antigens as target structures of CD8+ CTL has led to the development of vaccination strategies. Many studies have been made on melanoma. The detection of melanoma-specific T-cells spots in IFN $\gamma$  Elispot assay could be predictive of anti-tumoral clinical responses<sup>2,3</sup>.

**Vaccination:** It is proven that the Elispot assay is a good approach to evaluate vaccination strategies in many infectious diseases : HIV<sup>4</sup>, EBV<sup>6</sup>, Hepatitis C virus.

**Transplantation:** The Elispot assay could also be useful in the prediction of infectious risk after transplantation<sup>7</sup>.

However, these fields of application are not exhaustive, the Elispot assay takes an important place in **Autoimmunity, Allergy, TH1 / TH2 analysis, Pharmacology ...**

## Advantages of Diaclone ELISpot

### Wide range

**Several species:** human, mouse and rat

**Human Dual ELISpot or Dual FLUOROSpot range:** to monitor the production of two cytokines simultaneously in the same well

### High Performance

**Fast Procedure:** only 3 1/2 hours after cell stimulation

**High sensitivity**

### Flexibility

**Enzymatic or fluorescent detection**

**Mono or Dual ELISpot**

### Iso Quality

**Accuracy and reliability guaranteed:** all of our reagents have been extensively validated according to the ISO 9001 quality system

### Convenience

**Supplied with pre-coated plates or non pre-coated plates or without plates**

**Demo kit** in a 1 x 96 well-plate format

**Storage box**

	ELISpot Kits	Dual ELISpot	Dual FLUOROSpot	Matched Ab Pairs
Enzymatic detection	●	●		●
Fluorescent detection			●	
With non stérile plates	●	●	●	
With sterile plates	●	●	●	
Without plates	●	●	●	
With pre-coated plates	●			

# Diaclone ELISpot reagents

ELISpot Kits	ELISpot Matched Ab pairs	Dual ELISpot	Dual FLUOROSpot
<b>Human</b>	<b>Human</b>	<b>Human</b>	<b>Human</b>
IFN- $\gamma$	IFN- $\gamma$	IFN- $\gamma$ / IL-2	IFN- $\gamma$ / IL-2
IL-1 $\beta$	IL-1 $\beta$	IFN- $\gamma$ / IL-4	IFN- $\gamma$ / IL-4
IL-2	IL-2	IFN- $\gamma$ / IL-5	IFN- $\gamma$ / IL-5
IL-4	IL-4	IFN- $\gamma$ / IL-10	IFN- $\gamma$ / IL-10
IL-5	IL-5	IL-10 / IL-2	IL-10 / IL-2
IL-6	IL-6	IL-10 / IL-4	IL-10 / IL-4
IL-10	IL-10	IFN- $\gamma$ / Granzyme B	IFN- $\gamma$ / Granzyme B
IL-12	IL-12	IFN- $\gamma$ / Perforin	
IL-13	IL-13		
IL-17A	IL-17A		
TNF- $\alpha$	TNF- $\alpha$		
Granzyme B	Granzyme B		
Perforin	Perforin		
CD178 / Fas-Ligand	CD178 / Fas-Ligand		
<b>Mouse</b>	<b>Mouse</b>		
IFN- $\gamma$	IFN- $\gamma$		
IL-2	IL-2		
<b>Rat</b>	<b>Rat</b>		
IFN- $\gamma$	IFN- $\gamma$		
TNF- $\alpha$	TNF- $\alpha$		

## Human Elispot Enzymatic references:

**IFN $\gamma$  ELISpot** : Chen A. et al., *J. Virol.*, 2005, 5568-5576; Li H. et al. *J. Immunol.*, 2005; 174:195-204; Speiser D.E. et al., *J. Clin. Invest.*, 2005; 115:739-746; Gazagne A. et al., *J. Immunol. Methods.*, 2003; 283: 91-98 ; Sun Y. et al., *J. Immunol. Methods*, 2003; 272: 23-34 ; Sauce D. et al., *Blood*, 2003; 102: 1241-1244 ; Hudak S. et al., *J. Immuno.*, 2002; 169: 1189 -1196 ; Alatrakchi N. et al., *AIDS* 2002; 16: 713-717 ; Ayyoub M. et al., *J. Immunol.*, 2002; 168: 1717-1722 ; Sauce D. et al., *Blood*, 2002; 99(4) : 1165-1173 ; Pittet M. J. et al., *J. Immunol.*, 2001;166 : 7634-7640 / **IL-10 ELISpot** : Hudak S. et al., *J. Immuno.*, 2002; 169 : 1189-1196 / **IL- 4 ELISpot** : Hudak S. et al., *J. Immuno.* 2002; 169 : 1189-1196 ; Sauce D. et al., *Blood*, 2002; 99(4): 1165-1173 ; **Granzyme B ELISpot** : Godard B. et al., *Hum. Immunol.*, 2004; 65 (11):1307-1318

## Kit contents

ELISpot Kits	ELISpot Matched Ab Pairs
Available in 1 x 96 / 5 x 96 / 10 x 96 / 15 x 96 / 20 x 96 tests	Available in 10 x 96 tests
Capture antibody	Capture antibody
Biotinylated detection antibody	Biotinylated detection antibody
Streptavidin - Alkaline Phosphatase conjugate	
BSA	
Dry skimmed milk	
BCIP / NTB	
With or without sterile or non sterile PVDF plates	
Dual ELISpot	Dual FLUOROSpot
Available in 1 x 96 / 5 x 96 / 10 x 96 / 15 x 96 / 20 x 96 tests	
Capture antibody for first cytokine	Capture antibody for first cytokine
Capture antibody for second cytokine	Capture antibody for second cytokine
FITC conjugated detection antibody for first cytokine	FITC conjugated detection antibody for first cytokine
Biotinylated detection antibody for second cytokine	Biotinylated detection antibody for second cytokine
Anti-FITC antibody HRP conjugate	Anti-FITC antibody green fluorescence conjugate
Streptavidin Alkaline Phosphatase conjugate	Streptavidin-phycoerythrin conjugate
BSA	BSA
Dry skimmed milk	Dry skimmed milk
BCIP / NBT and AEC	With or without sterile or non sterile PVDF plates
With or without sterile or non sterile PVDF plates	

## References

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- Hebart H. *et al.*, 2002 Sensitive detection of human cytomegalovirus peptide-specific cytotoxic T-lymphocyte responses by interferon-gamma-enzyme-linked immunospot assay and flow cytometry in healthy individuals and in patients after allogeneic stem cell transplantation. *Blood* 99 (10): 3830-7