

Granzyme B / Perforin Pathway

One mechanism used by cytotoxic T lymphocytes and NK cells to kill tumor cells and virus-infected cells is the release of granzyme and perforin (a membrane pore- protein) proteins from cytotoxic granules. Following adhesion of the cytotoxic cell to the target, granzyme B is directly exocytosed and enters the target cell, assisted by perforin. Perforin polymerizes into transmembrane pores facilitating the intracellular delivery of granzymes. Granzyme B is a serine protease and induces cleavage of procaspase-8 which activates a caspase cascade and cleavage of factors like ICAD that results in the apoptotic death of the cell.

Since granzymes and perforin constitute the primary effectors of the granule exocytose pathway, identification of this phenomena by development of assays to assess the involvement of activated CTL or NK in pathological processes is of high interest for the scientific community.

The chromium release assay or the use of MHC: peptide tetramer staining constitute conventional methods to measure antigen/peptide-specific CD8+ T cell immune response. However, because of their low sensitivity and specific experimental conditions requirements, these assays are not suitable for screening large number of peptides.

Thus, Diacclone has developed Granzyme B and Perforin Elispot assays which offer the advantages of being rapid, technically straightforward requiring little sophisticated equipment and permit measurement of low-frequency T cells from a relatively small number of cells.

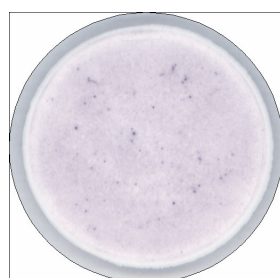
Principle

After cell stimulation, locally produced Granzyme B or Perforin is captured by a specific monoclonal antibody. After cell lysis, trapped Granzyme B or Perforin molecules are revealed by a secondary biotinylated detection antibody, which is in turn recognised by streptavidin conjugated to alkaline phosphatase. PVDF-bottomed-well plates are then incubated with BCIP/NBT substrate. Colored "purple" spots indicate Granzyme B or Perforin production by individual cells.

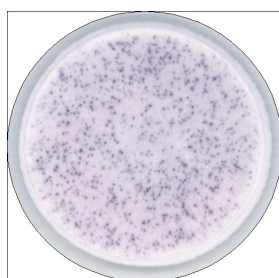
Application

- Vaccine development
- Viral infection monitoring and treatment
- Cancerology
- Autoimmune diseases

Human Perforin ELISpot Assay



Non-stimulated PBMC
(10^5 cells)



PMA/Ionomycin stimulated
PBMC ($1.25 \cdot 10^4$ cells)

Human Granzyme B ELISpot Assay



Non-stimulated PBMC
($5 \cdot 10^4$ cells)



PMA/Ionomycin stimulated
PBMC ($1.25 \cdot 10^4$ cells)

Related product

Host	Clone	Isotype	Format	Size	Cat N°
			Azide free	200 µg	854 950 000
Mouse	B-D48	IgG1	Purified	100 tests	854 953 020
			PE	100 tests	854 952 010

Human IFN- γ / Granzyme B & IFN- γ / Perforin ELISpot

Kits



October 2007

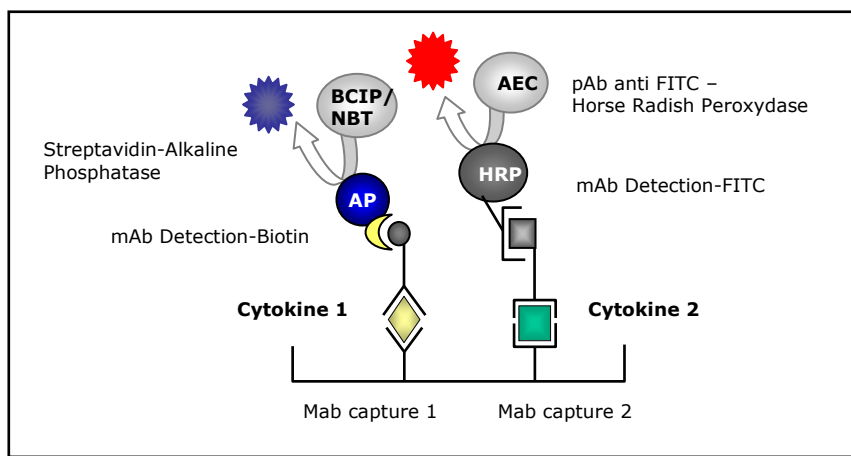
Most likely Dual ELISpot will be required in future analysis. Evidence is available that information on more than one protein secreted by the same cell gives supplementary information (IFN γ and IL-2, (1)) or IFN γ and perforin, (2)). With other cells like Th17 cells, producing multiple cytokines and other factors the analysis of the different proteins produced by the same cells may provide information on the severity and character of inflammation.

References:

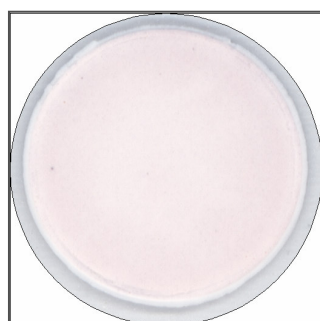
(1) A. Gazagne, E. Claret, J. Wijdenes, H. Yssel, F. Bousquet, E. Levy, P. Vielh, F. Scotte, T. Le Goupil, W. H. Fridman, E. Tartour *Journal of Immunological Methods*, 2003, 283, 91-98. A fluospot assay to detect single T lymphocytes simultaneously producing multiple cytokines

(2) E. Tartour and all. In preparation.

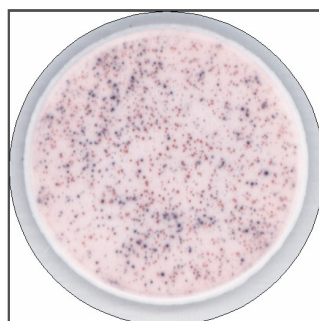
DUAL COLOR ENZYMATIC



h IFN- γ / h Granzyme B

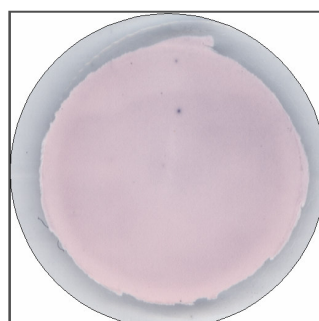


Non-stimulated PBMC
(5.10⁴ cells)

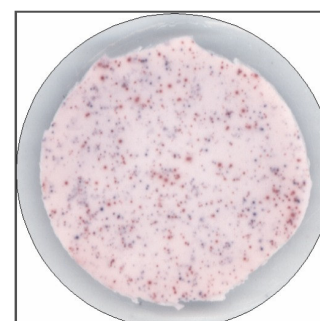


PMA/Ionomycin stimulated
PBMC (5.10⁴ cells)

h IFN- γ / h Perforin



Non-stimulated PBMC
(2,5.10⁴ cells)



PMA/Ionomycin stimulated
PBMC (2,5.10⁴ cells)

Description	Without plates		With PVDF plates	
			Non sterile	Sterile
Human Granzyme B ELISpot Kit				
1 x 96 tests			856 131 001	856 131 111
5 x 96 tests	856 131 005		856 131 005P	856 131 005S
10 x 96 tests	856 131 010		856 131 010P	856 131 010S
15 x 96 tests	856 131 015		856 131 015P	856 131 015S
20 x 96 tests	856 131 020		856 131 020P	856 131 020S
Human Perforin ELISpot Kit				
1 x 96 tests			856 141 001	856 141 111
5 x 96 tests	856 141 005		856 141 005P	856 141 005 S
10 x 96 tests	856 141 010		856 141 010P	856 141 010 S
15 x 96 tests	856 141 015		856 141 015P	856 141 015 S
20 x 96 tests	856 141 020		856 141 020P	856 141 020 S
Human IFN-γ / Granzyme B ELISpot Kit				
1 x 96 tests			874 060 001	874 060 111
5 x 96 tests	874 060 005		874 060 005P	874 060 005S
10 x 96 tests	874 060 010		874 060 010P	874 060 010S
15 x 96 tests	874 060 015		874 060 015P	874 060 015S
20 x 96 tests	874 060 020		874 060 020P	874 060 020S
Human IFN-γ / Perforin ELISpot Kit				
1 x 96 tests			874 070 001	874 070 111
5 x 96 tests	874 070 005		874 070 005P	874 070 005S
10 x 96 tests	874 070 010		874 070 010P	874 070 010S
15 x 96 tests	874 070 015		874 070 015P	874 070 015S
20 x 96 tests	874 070 020		874 070 020P	874 070 020S