

Cytokine ELI-spot/PVDF Procedure

Cytokines are soluble (glyco)proteins produced by leukocytes and other cell types which act as chemical communicators between cells. Most are secreted but some can be expressed on the cell membrane as well (juxtacrine). Most cytokines are growth and/or differentiation factors and act in general on cells within the haematopoietic system but interaction with the brain, muscles and endocrine organs has also been described. Apoptosis or programmed cell death is another activity concerted by several cytokines. There is now enough evidence that cytokines do not act alone but synergistically at different time points on target cells. This complex network of agonistic and antagonistic factors with such a broad spectrum of targets needs a permanent and extremely tight balanced control. Perturbation of cytokine release has been shown to lead to improper and sometime deleterious responses in several disease states including septic shock, parasitaemia, cancer and autoimmune diseases.

Measurement of cytokines in biologic fluids is one way to monitor cytokine production. However many cytokines are only found and act in the micro-environment of producing cells (paracrine). Despite its biological importance few methods are currently available for the analysis of cytokine production at the single cell level.

The ELI-spot assay is based on immuno-enzyme technology originally developed for the enumeration of antibody-secreting cells (1) and subsequently this assay was adapted to measure cytokine production at the single cell level. The ELI-spot assay is easy to perform and it requires, compared to the limiting dilution approach, a minimum *in-vitro* cell manipulation, allowing analysis as close as possible to the *in-vivo* situation. The principle is depicted in figure 1 (page 2) and involves the following steps:

- a) Single cell suspensions are distributed in PVDF bottomed wells previously coated with an anti-cytokine antibody. These cells may upon stimulation with the proper antigen produce cytokine molecules in their close environment which are caught by the coated antibody.
- b) After cell removal, the captured cytokine is revealed by a secondary biotinylated anti-cytokine antibody, which is in turn
- c) detected by streptavidin conjugated to alkaline phosphatase.
- d) The enzymatic reaction produces a precipitate
- e) The cytokine produced by individual cells is visualised by sharp blue spots that can be counted on an inverted microscope, allowing the numeration of cytokine producing cells under a given stimuli. The total length of the assay depends mainly on incubation steps and requires a minimum handling time.

Monitoring cytokine production at the single cell level has proven to be a sensitive and unique method to monitor cytokine production closely comparable to the « in situ » environment. This provides an attractive way to follow disease development and allows the design of appropriate clinical strategies to prevent a progressive or fatal outcome. Several studies have indicated that alterations in the frequency of cytokine producing cells in different compartments of the body adequately reflects changes in immune functions.

As shown with the ELI-spot assay in experimental autoimmune encephalomyelitis, the treatment of rats with IFN β significantly decrease the frequency of IFN γ producing cells in the spinal cord tissue (2). The ELI-spot assay is also useful to evaluate the vaccinating potency of immunogenic peptides (3) or the influence of vaccinating routes (4). Cytokine producing patterns of TH1/TH2 T cells and their shift toward one or the other subpopulation can be closely analysed by ELI-spot (5, 6). This method has also been successfully used to determine the frequency of tumour reactive T cells in melanoma patients (7).

(1) Sedwick, J.D. and Holt, P.G. , 1983. J. Immuno. Methods 57 : 301.

(2) Ruuls, S. R. et al., 1996. J. Immunol. 157 : 5721.

(3) Kulane, A. et al., 1997. Acta Tropica. 68 : 37.

(4) Ibsen, M.W. et al., 1997. Scand. J. Immunol. 46 : 274.

(5) Gabrielsson, S. et al., 1997. Allergy. 52 : 860.

(6) Elghazali, G. et al., 1997. Clin. Exp. Immunol. 109 : 84.

(7) Scheibenbogen, C. et al. 1997 Int. J. Cancer 71 : 932.

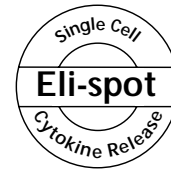
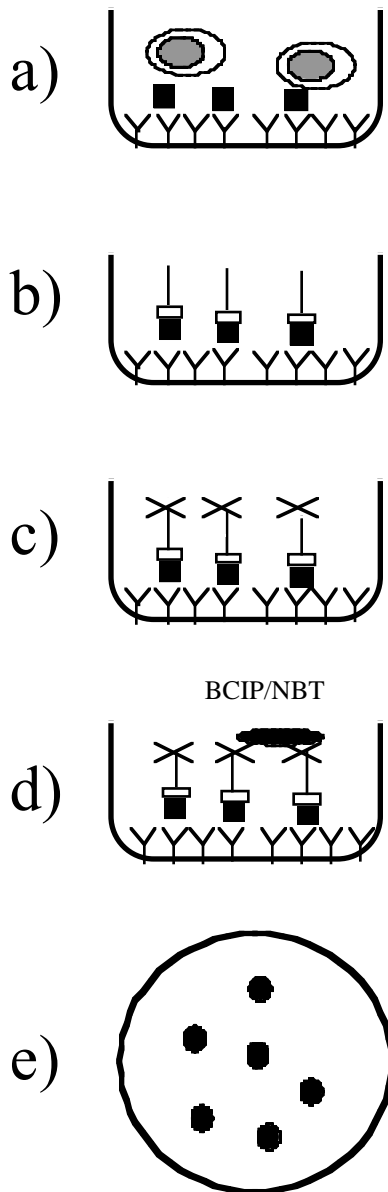


Figure 1) Principe of Cell Sciences Cytokine ELI-spot



- ELI-spot PVDF kit contents:**
- Capture and Detection antibodies
 - streptavidin-alkaline phosphatase conjugate
 - BSA
 - Ready-to-use substrate buffer