

Company History by Dr John WIJDENES



Welcome to the first Diaclone Newsletter. In this edition we hope to provide you with a brief history of the development of Diaclone, an introduction to the people at Diaclone & information on some of the products available.

Although the name Diaclone has only existed for four years, the laboratories were created some 12 years ago as Section 38 in the Blood Bank of Besançon, France, directed by Professor Peters. Our name has changed over the years but we have maintained our commitment as an innovator in therapeutic, diagnostic and research products.

Monoclonal antibodies (mAb's) have the ability to recognise only four or five amino acids yet still bind with affinities as high as 10^{10} , which makes each mAb unique in its binding capability and therefore, immunological and biological activity. This epitope recognition combined with binding affinity is an area of expertise at Diaclone which is used when developing mAb's. We raise a number of mAb's against the same antigen but with different activities, and the screening assays we use are tailored to the desired activity of the monoclonal antibody.

For example, therapeutic mAb's are screened using bioassays closely related to the clinical situation, mAb's for ELISA's are selected using screening assays in which the soluble antigen is detected, and those developed for recognising membrane

bound structures are screened using flow cytometry. This ensures that Diaclone manufactured monoclonal antibodies, all starting with B- (for Besançon), are optimised for the conditions in which they will be used.

Over the years we have developed many unique therapeutic mAb's such as B-E2 (CD2), B-F5 (CD4), B-B10 (CD25 / Leukotac), B-C7 (anti-TNF- α) and B-E8 (anti-IL-6). For our own continued research programme and clinical trial work we specifically select mAb's for flow cytometry and our own ELISA's. Diaclone is in an almost unique position of having developed and manufactured most of the monoclonal antibodies in our extensive product catalogue and used in our own products (ELISA, Eli-pair, Eli-spot). We have the knowledge and expertise in the Diaclone laboratories.

Our collaboration with researchers using Diaclone products has resulted in co-authorship of more than 150 scientific publications. This relationship with leading research laboratories keeps Diaclone at the forefront of scientific research and has helped us to develop a series of pioneering and unique products such as IL-10 ELISA, a wide range of soluble adhesion molecule ELISA's, IgG's inducing apoptosis plus a first and unique plasmocyte marker, B-B4 resulting in CD138 (syndecan-1), and an ELISA to measure soluble syndecan-1.





In addition to our scientific innovation, Diaclone is also committed to quality. We have achieved ISO9001 accreditation and all Diaclone Research products are manufactured under these guidelines. We undertake research and development of mAb's for other organisations, and we can manufacture mAb's with our or your cell line in serum free conditions in a continuous bio reactor system, yielding up to 5 grams of purified antibody per day. We can take care of cell adaptation, creating Master, and Working Banks and all the necessary (viral) testing on cells and final products to meet the required specifications. More about this topic will be explained in further detail in our next Newsletter. Should you require any further information on our manufacturing services, please contact myself, John Wijdenes, directly.



Many laboratories have used Diaclone B-clone products to their satisfaction, without knowing their origin. So, to make sure you recognise the guarantee of quality, all our products will now be distributed world-wide under the name of Diaclone Research. All the latest information and a full Diaclone product catalogue is available from your local distributor (see back page), and we will be expanding our network of international suppliers over the next few years.

New Eli-spot Assays by Dr Emmanuel Claret

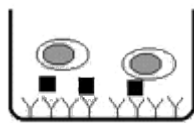


Cytokines are soluble (glyco)proteins produced by leucocytes and other cell types, which act as chemical communicators between cells. Most are secreted but some can also be expressed on the cell membrane (juxtacrine). Most cytokines are growth and/or differentiation factors and act in general on cells within the haematopoietic system but interaction with the brain, muscles and endocrine organs has also been described. Induction of Apoptosis or programmed cell death is another activity of some cytokines. It is now clearly established 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 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.>

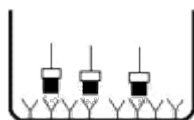
Measurement of cytokines in biologic fluids is one way to monitor cytokine production. However, many cytokines are only found and act in the micro-environment of producing cells (paracrine). Despite its biological importance few methods are currently available for the analysis of cytokine production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

The new Diaclone Eli-spot assays are based on immuno-enzyme technology originally developed for the enumeration of antibody-secreting cells (1) and subsequently these assays were adapted to measure cytokine production at the single cell level. The Eli-spot assay is easy to perform and it requires minimum in-vitro cell manipulation, allowing analysis as close as possible to their-in-vivo situation.

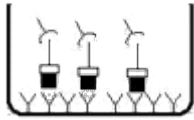
The principal is depicted in figure 1 and involves the following steps:



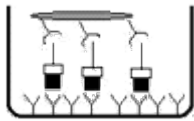
a) Single cell suspensions are distributed in wells previously coated with an anti-cytokine antibody. Upon stimulation these cells produce cytokine molecules in their close environment which are captured by the coated antibody.



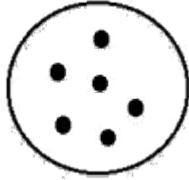
b) After cell removal, the captured cytokine is revealed by a secondary biotinylated anti-cytokine antibody.



c) Detection is by an anti-biotin antibody conjugated to alkaline phosphatase.



d) The chromogenic reaction is performed in an agarose overlay to prevent spreading of the coloured product.



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.

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 allowing 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 (fig. 2), 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.

Each Eli-spot kit contains sufficient reagents for 5, 10, 15 or 20 x 96 wells, including:

- Capture & Detection antibodies
- Enzyme conjugate
- Low gelling temperature Agarose
- Stabilisers 1 and 2
- 20% BSA in PBS
- Wash Buffer