Human IFNγ / IL-10
Dual ELISpot

Instructions for use

Catalogue Numbers:

	Without Plates	With non-Sterile Plates	With Sterile Plates
1x96 tests	874.000.001	874.000.001P	874.000.001S
5x96 tests	874.000.005	874.000.005P	874.000.005S

For research use only

As a material condition to Diaclone providing its Products to Purchaser, Purchaser agrees that the end user shall not, directly or indirectly, attempt to reverse engineer, disassemble, or otherwise perform any compositional, structural, functional or other analyses directed to learning the methodology, components, formulae, processes, make-up, or production of any Product or any portion thereof.

Fast Track Your Research…….

Table of Contents

Human IFNγ / IL-10 Dual ELISpot

1. Intended use

Diaclone **ELISpot** is a highly specific immunoassay for the analysis of cytokine and other soluble molecule production and secretion from T-cells at a single cell level in conditions closely comparable to the *in-vivo* environment with minimal cell manipulation. This technique is designed to determine the frequency of cytokine producing cells under a given stimulation and the comparison of such frequency against a specific treatment or pathological state. The ELISpot assay constitutes an ideal tool in the investigation of Th1 / Th2 responses, vaccine development, viral infection monitoring and treatment, cancerology, infectious disease, autoimmune diseases and transplantation.

Utilising sandwich immuno-enzyme technology, Diaclone ELISpot assays can detect both secreted cytokines and single cells that simultaneously produce multiple cytokines. Cell secreted cytokines or soluble molecules are captured by coated antibodies avoiding diffusion in supernatant, protease degradation or binding on soluble membrane receptors. After cell removal, the captured cytokines are revealed by tracer antibodies and appropriate conjugates.

This Dual Colour ELISpot kit allows you to analyze the production of two cytokines simultaneously in the same well.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

2. Introduction

2.1. Summary

IFNγ (1-21)

IFNγ, also called Type II interferon, is a homodimeric glycoprotein containing approximately 21 to 24 kD subunits. The human IFNγ gene, situated on chromosome 12, contains three introns; the four exons code for a polypeptide of 166 amino acids, 20 of which constitute the signal peptide (11). In contrast to IFNα and IFNβ synthesis, which can occur in any cell, production of IFNγ is a function of T cells and NK cells. All IFNγ inducers activate T cells either in a polyclonal (mitogens or antibodies) or in a clonally restricted, antigen-specific, manner. IFNγ is produced during infection by T cells of the cytotoxic/suppressor phenotype (CD8) and by a subtype of helper T cells, the Th1 cells. Th1 cells secrete IL-2, IL-3, TNFα and IFNγ, whereas Th2 cells main produce IL-3, IL-4, IL-5, and IL-10, but little or no IFNγ (9). IFNγ preferentially inhibits the proliferation of Th2 but not Th1 cells, indicating that the presence of IFNγ during an immune response will result in the preferential proliferation of Th1 cells (7).

Type II IFN or IFNγ is a lymphokine that displays no molecular homology with type I IFN, but shares some important biologic activities. Specifically, IFNγ induces an anti-viral state and is anti-proliferative. In addition, IFNγ has several properties related to immunoregulation. **1)** IFNγ is a potent activator of mononuclear phagocytes, e.g. IFNγ stimulates the expression of Mac-1, augments endocytosis and phagocytosis by monocytes (15), and activates macrophages to kill tumor cells by releasing reactive oxygen intermediates and TNFα (21). **2)** IFNγ induces or augments the expression of MHC antigens on macrophages, T and B cells and some tumor cell lines (3). **3)** On T and B cells IFNγ promotes differentiation. It enhances proliferation of activated B cells and can act synergistically with IL-2 to increase immunoglobulin light-chain synthesis (8, 13). IFNγ is one of the natural B-cell differentiation factors (17). **4)** Finally, IFNγ activates neutrophils, NK cells and vascular endothelial cells (6).

The role of IFNγ as a disease marker has been demonstrated for a number of different pathological situations:

- *Infections*: IFNγ is produced during viral infections (4). IFNγ is a diagnostic tool for distinguishing tuberculous from other nontuberculous ascites (5, 18). IFNγ values in pleural fluid are significantly higher in tuberculous pleuritis patients than those in non- tuberculous pleuritis patients, with a sensitivity and a specificity of 100% (1, 2). Therapy-induced (treatment with thalidomide) alleviation of clinical symptoms of erythema nodosum leprosum correlates with IFNγ and TNFα levels (14). Tuberculoid leprosy patients show increased lymphocyte proliferation and IFNγ production in response to stimulation with Mycobacterium leprae as compared to lepromatous leprosy patients and healthy individuals (16).
- *Autoimmune diseases*: Accurate measurements of cellular production of cytokines, e.g. IFNγ is important in the design and monitoring of immunotherapy of multiple sclerosis (12).
- *Transplant rejection*: Intragraft IFNγ mRNA expression occurs in active acute transplant rejection preceding clinical transplant rejection, thus offering an early diagnostic tool for detection of transplant rejection (10).
- *Allergy*: IFNγ production by isolated lymphocytes is not detectable in patients with cow's milk allergy as compared to control individuals (19). Infants who develop atopy produce significantly less IFNγ at birth compared to infants who do not develop atopy (20).
- *Diabetes*: Peripheral blood lymphomononuclear cells from newly diagnosed type I diabetes produce significantly less IFNγ in comparison to controls and long standing diabetes (4).

IL-10 (22-36)

Interleukin-10 is a pleiotropic cytokine playing an important role as a regulator of lymphoid and myeloid cell function. Due to the ability of IL-10 to block cytokine synthesis and several accessory cell functions of macrophages this cytokine is a potent suppressor of the effector functions of macrophages, T-cells and NK cells. In addition, IL-10 participates in regulating proliferation and differentiation of B-cells, mast cells and thymocytes (30). The primary structure of human IL-10 has been determined by cloning the cDNA encoding the cytokine (36). The corresponding protein exists at 160 amino acids with a predicted molecular mass of 18.5 kDa (29, 36). Based on its primary structure, IL-10 is a member of the four -helix bundle family of cytokines (33). In solution human IL-10 is a homodimer with an apparent molecular mass of 39 kDa (35). Although it contains an N-linked glycosylation site, it lacks detectable carbohydrates (36). Recombinant protein expressed in E. coli thus retains all known biological activities. The human IL-10 gene is located on chromosome 1 and is present as a single copy in the genome (27). The human IL-10 exhibits strong DNA and amino acid sequence homology to the murine IL-10 and an open reading frame in the Epstein- Barr virus genome, BCRF1 (22, 29, 36) which shares many of the cellular cytokine's biological activities and may therefore play a role in the host-virus interaction. The immunosuppressive properties of IL-10 (25) suggest a possible clinical use of IL-10 in suppressing rejections of grafts after organ transplantations. IL-10 can furthermore exert strong anti-inflammatory activities (25).

IL-10 in disease

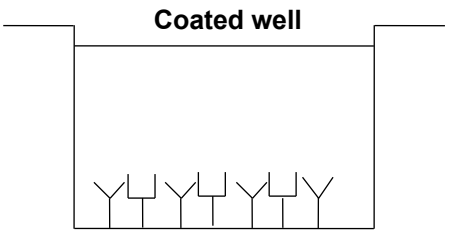
IL-10 expression was shown to be elevated in parasite infections like in Schistosoma mansoni (28), Leishmania (26), Toxoplasma gondii (33) and Trypanosoma (34) infection.
Furthermore, high IL-10 expression was detected in mycobacterial infections as shown for Mycobacterium leprae (24), Mycobacterium tuberculosis (23) and Mycobacterium avium infections.
High expression levels of IL-10 are also found in retroviral infections inducing immunodeficiency (31).

2.2. Principle of the method

Capture antibodies highly specific for the analytes of interest are coated to the wells of a PVDF bottomed 96 well microtiter plate either during kit manufacture or in the laboratory. The plate is then blocked to minimise any non-antibody dependent unspecific binding and finally washed before adding the cells to be investigated. Cell suspension and stimulant are added to the coated and blocked microtiter plate and the plate incubated allowing the specific antibodies to bind any analytes produced. Biotinylated and FITC detection antibodies are then added which bind to the previously captured analyte. HRP conjugated anti-FITC antibodies and Streptavidin Alkaline Phosphatase are added binding to the detection antibodies. Any excess unbound analyte and antibodies are removed by careful washing. Colour substrate is then applied to the wells resulting in coloured spots which can be quantified using appropriate analysis software or manually using microscopes.

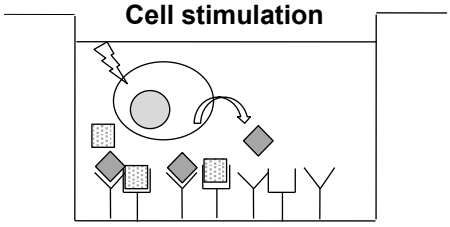
1. 96 PVDF-bottomed-well plates are first treated with 35% ethanol and then coated with anti-IFNγ and anti-IL-10 capture antibodies.

Figure 1



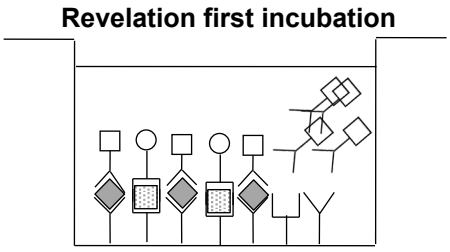
2. Cells are incubated in the presence of the antigen. Upon stimulation they release cytokines which bind to the capture antibodies.

Figure 2



3. Cells are removed by washing. Anti- IFNγ-FITC and anti-IL-10-biotin detection antibodies are added and bind to the captured cytokines

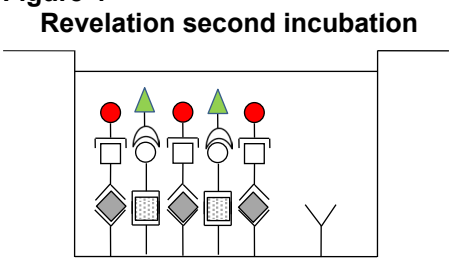
Figure 3

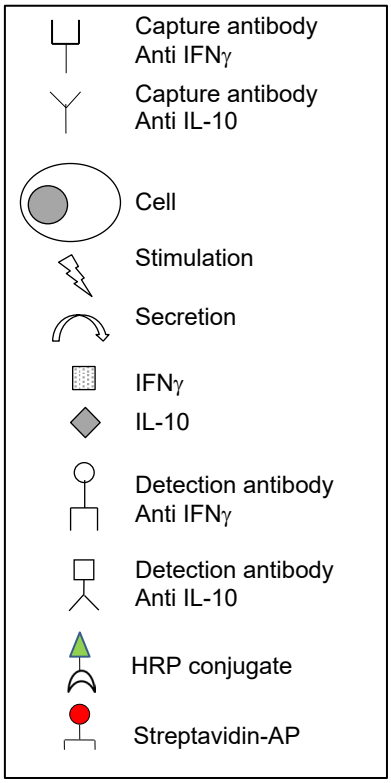


4. Any excess unbound detection antibodies is removed by washing.

Detection antibodies are in turn bound by anti-FITC-HRP for IFNγ and Streptavidin-AP for IL-10.

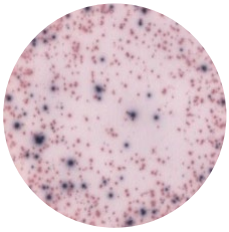
Figure 4





5. Any excess of AP and HRP conjugate is removed by washing.

Finally coloured spots are developed by separate incubations with first AEC and then BCIP/NBT substrate buffers. Cells producing IFNγ give red/brownish spots while those producing IL-10 give blue/purple spots. Double producing cells corresponding to violet spots (preferably identified by a computerised overlay of blue and red spots).



3. Reagents provided

Reagents	SET 001*	SET 005	Reconstitution
96-well PVDF bottomed plates (if ordered)	2	5	Ethanol treatment (see section 9)
Capture Antibody anti hIFNγ	1 (0.1 ml)	1 (0.5 ml)	Sterile, dilute prior to use (see Capture Antibodies, section 7.6)
Capture Antibody anti hIL-10	1 (0.1 ml)	1 (0.5 ml)	
FITC conjugated detection antibody anti hIFNγ	1 (100 µl)	1	Reconstitute with 0.55 ml of distilled water Dilute prior to use (see Detection Antibodies, section 7.7)
Biotinylated detection antibody anti hIL-10	1 (100 µl)	1	
Anti-FITC HRP conjugate	1	1	Dilute prior to use As indicated on the vials (see diluted AP and HRP conjugates, section 7.8)
Streptavidin-Alkaline Phosphatase conjugate	1	1	
Bovine Serum Albumin (BSA) – 2 g	1	1	Dissolve to prepare dilution buffer (see 1%BSA PBS solution, section 7.4)
AEC Buffer A 10X - (Concentrate Buffer)	1 (1 ml)	1 (5 ml)	Dilute prior to use (see AEC substrate, section 7.9)
AEC Buffer B 50X - (Concentrate Substrate)	1 (200 µl)	1 (1 ml)	
Ready to use BCIP/NBT - (Substrate buffer)	1 (11 ml)	2 (25 ml)	Ready to use

*** Please note for discovery set 001 : detection antibody is provided in liquid form. Volume of reagents are sufficient for a total of 96 tests but 2 plates are provided to allow to run 2*48 tests.**

4. Materials/Reagents required but not provided

- Miscellaneous laboratory plastic and/or glass, if possible sterile
- Ethanol
- Cell culture reagents (e.g. RPMI-1640, L-glutamine, FCS)
- Cell stimulation reagents (e.g. PMA and Ionomycin)
- CO_2 incubator
- Phosphate Buffered Saline (PBS)
- 96 well PVDF bottomed plates if not ordered (we recommended Millipore plates catalogue # MSIPN4510, MSIPS4510 and M8IPS4510)

5. Storage Instructions

Store kit reagents between 2 and 8°C except uncoated plates which should be stored at RT. Immediately after use remaining reagents should be returned to cold storage (2 to 8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if in the case of repeated use of one component, the reagent is not contaminated by the first handling.

6. Safety & Precautions for use

- For **research use only** not to be used as a diagnostic test
- Handling of reagents, blood specimens, PBMC, human cell lines should be in accordance with local safety procedures, e.g. CDC/NIH Health manual : "Biosafety in Microbiological and Biomedical Laboratories" 1984
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used
- Do not pipette by mouth
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels
- All reagents should be warmed to room temperature before use
- Cover or cap all reagents when not in use
- Do not mix or interchange reagents between different lots
- Do not use reagents beyond the expiration date of the kit
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination
- Use a clean plastic container to prepare the washing solution
- Thoroughly mix the reagents and samples before use by agitation or swirling
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells
- **AEC and BCIP/NBT substrates** are potentially carcinogenic and should be disposed of appropriately, caution should be taken when handling these reagents, always wear gloves
- Follow incubation times described in the assay procedure

7. Reagent Preparation

7.1. 1X Phosphate Buffered Saline (PBS) (Coating & Wash Buffer)

For 1 litre of 10X PBS, weigh-out: 80g NaCl
2g KH_2PO_4
14.4g Na_2HPO_4 ; $2H_2O$.

Add distilled water to 1 litre.

Dilute the solution to 1X before use.
Check the pH of the 1X solution and adjust to required pH : 7.4 +/- 0.1.

7.2. 35% Ethanol (PVDF Membrane Activation Buffer)

For one plate, mix 3.5 ml of ethanol with 6.5 ml of distilled water.

7.3. Cell culture medium + 10% Serum (Blocking Buffer)

For one plate, add 1 ml of Serum (e.g. FCS) to 9 ml of culture medium.
Use same cell culture medium as used to derive the cell suspension.

7.4. 1% BSA PBS Solution (Dilution Buffer)

For one plate, dissolve 0.2 g of BSA in 20 ml of PBS 1X.

7.5. Capture Antibodies

These reagents are supplied sterile, once opened keep the vials sterile or aliquot and store at -20°C.
For optimal performance prepare the Capture Antibodies dilution immediately before use.

For one plate, add 100 µl of each capture antibody in a same tube in 10 ml of PBS 1X. Mix well.

7.6. Detection Antibodies

Reconstitute each lyophilised antibody with 0.55 ml of distilled water. Gently mix the solutions and wait until all the lyophilised material is back into solution.
Please note for 1x96 demo kits, detection antibodies are provided in liquid form.

If not used within a short period of time, reconstituted Detection Antibodies should be aliquoted and stored at -20°C. In these conditions the reagent are stable for at least one year. For optimal performance prepare the reconstituted antibodies dilution immediately prior to use.

For one plate, add 100 µl of each antibody in a same tube in 10 ml of Dilution Buffer. Mix well.

To avoid nonspecific background, it is recommended to filter the working solution using a disposable syringe and a 0.2µm filter disc.

7.7. Diluted AP and HRP conjugates

For optimal performance prepare the dilution immediately prior to use.
It is recommended to centrifuge the vials for a few seconds to collect all the volume at the bottom.

For one plate, add in a same tube Streptavidin-AP conjugate and anti-FITC antibody HRP conjugate at the volume indicated on each vial in 10 ml of Dilution Buffer. Mix well.

To avoid nonspecific background, it is recommended to filter the working solution using a disposable syringe and a 0.2µm filter disc.

DO NOT KEEP THE DILUTIONS FOR FURTHER EXPERIMENTS.

7.8. AEC Substrate

For optimal performance prepare the dilution immediately prior to use.

For one plate, dilute 1 ml of AEC buffer A 10X with 9 ml of distilled water.
Then add 200 µl of AEC buffer B 50X.

7.9. BCIP/NBT

The reagent is ready-to-use.

It should be clear to pale yellow. If precipitates occur, filter the solution using a disposable syringe and a 0.2µm filter disc.

8. Sample and Control Preparation

8.1. Cell Stimulation

Cells can either be stimulated directly in the antibody coated wells (Direct) or, first stimulated in 24 well plates or flask, harvested, and then plated into the coated wells (Indirect).

The method used is dependent on 1) the type of cell assayed 2) the expected cell frequency. When a low number of cytokine producing cells are expected it is also advised to test them with the direct method, however, when this number is particularly high it is better to use the indirect ELISpot method.

All the method steps following stimulation of the cells are the same whatever the method (direct/indirect) chosen.

8.2. Positive Assay Control, IFNγ / IL-10 production

We recommend using the following polyclonal activation as a positive control in your assay.

Dilute CD4+ T cells in culture medium (e.g. RPMI 1640 supplemented with 2mM L-glutamine and 10% heat inactivated fetal calf serum) containing 1 ng/ml PMA and 500 ng/ml Ionomycin (Sigma, Saint Louis, MO). Distribute $2.5x10^4$ to $1x10^5$ cells per 100 µl in required wells of an antibody coated 96-well PVDF plates and incubate for 15-20 hours in an incubator.

For other stimulators, incubation times may vary, depending on the frequency of cytokine producing cells, and should be optimised in each situation.

8.3. Negative Assay Control

Dilute CD4+ T cells in culture medium to give an appropriate cell number (same number of unstimulated cells as stimulated sample cells) per 100 µl with no stimulation.

8.4. Sample

Dilute CD4+ T cells in culture medium and stimulator of interest (i.e. Sample, Vaccine, Peptide pool or infected cells) to give an appropriate cell number per 100 µl.

Optimal assay performances are observed between $1x10^5$ and $2.5x10^5$ cells per 100 µl.

Stimulators and incubation times can be varied depending on the frequency of cytokine producing cells and therefore should be optimised by the testing laboratory.

9. Method

Prepare all reagents as shown in section 7 and 8.

Assay Step		Details
1.	Addition	Add 25 µl of 35% ethanol to every well
2.	Incubation	Incubate plate at room temperature (RT) for 30 seconds
3.	Wash	Empty the wells by flicking the plate over a sink & gently tapping on absorbent paper. Thoroughly wash the plate 3x with 200 µl of PBS 1X per well
4.	Addition	Add 100 µl of the diluted mixture of **capture antibodies** to every well
5.	Incubation	Cover the plate and incubate at 4°C overnight
6.	Wash	Empty the wells as previous and wash the plate once with 200 µl of PBS 1X per well
7.	Addition	Add 100 µl of **blocking buffer** to every well
8.	Incubation	Cover the plate and incubate at RT for 2 hours
9.	Wash	Empty the wells as previous and thoroughly wash 3x with 200 µl of PBS 1X per well
10.	Addition	Add 100 µl of **sample, positive and negative controls** cell suspension to appropriate wells providing the required concentration of cells and stimulant (cells may have been previously stimulated see section 8.)
11.	Incubation	Cover the plate and incubate at 37°C in a CO_2 incubator for an appropriate length of time (15-20 hours). ***Note: do not agitate or move the plate during this incubation***
12.	Addition	Empty the wells and remove excess solution then add 200 µl of PBS 1X to every well
13.	Incubation	Incubate the plate at 4°C for 10 min
14.	Wash	Empty the wells as previous and wash the plate 3x with 200 µl of PBS 1X
15.	Addition	Add 100 µl of the diluted mixture of **detection antibodies** to every well
16.	Incubation	Cover the plate and incubate at RT for 1 hour 30 min
17.	Wash	Empty the wells as previous and wash the plate 3x with 200 µl of PBS 1X
18.	Addition	Add 100 µl per wells of diluted **HRP and AP conjugates**
19.	Incubation	Cover the plate and incubate at RT for 1 hour
20.	Wash	Empty the wells and wash the plate 3x with 200 µl of PBS 1X
21.	Wash	Peel off the plate bottom and wash both sides of the membrane 3x under running distilled water, once washing complete remove any excess solution by repeated tapping on absorbent paper.
22.	Addition	Add 100 µl of **prepared AEC substrate** to every well
23.	Development	Incubate the plate for **5-20 min** protected from light, monitoring spot formation visually throughout the incubation period to assess sufficient colour development
24.	Wash	Empty the wells and rinse both sides of the membrane 3x under running distilled water. Completely remove any excess solution by gentle repeated tapping on absorbent paper
25.	Addition	Add 100 µl of **ready to use BCIP/NBT** buffer to every well
26.	Development	Incubate the plate for **5-15 min** protected from light, monitoring spot formation visually throughout the incubation period to assess sufficient colour development
27.	Wash	Empty the wells and rinse both sides of the membrane 3x under running distilled water. Completely remove any excess solution by gentle repeated tapping on absorbent paper
Read Spots: allow the wells to dry and then read results. The frequency of the resulting coloured spots corresponding to the cytokine producing cells can be determined using an appropriate ELISpot reader and analysis software or manually using a microscope. *Note: spots may become sharper after overnight incubation at 4°C in the dark*		

Plate should be stored at RT away from direct light, but please note that colour may fade over prolonged periods so read results within 24 hours.

10. Performance Characteristics

10.1. Specificity

The assay recognizes natural human IFNγ and human IL-10.

To define specificity of the IFNγ antibody pair, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested: IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, TNFα and IL-13. This testing was performed using the equivalent human IFNγ antibody pair in an ELISA assay.

To define specificity of the IL-10 antibody pair, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested: IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, IL-13, IL-17A, IFNγ and TNFα. This testing was performed using the equivalent human IL-10 antibody pair in an ELISA assay.

11. Bibliography

IFNγ

1. Aoki, Y., Katoh, O., Nakanishi, Y., Kuroki, S., and Yamada, H. (1994). A comparison study of IFN-gamma, ADA, and CA125 as the diagnostic parameters in tuberculous pleuritis. Respir. Med. 88, 139-143.
2. Capobianchi, M. R., Ameglio, F., Tosi, R., and Dolei, A. (1985). Differences in the expression and release of DR, BR, and DQ molecules in human cells treated with recombinant interferon gamma: comparison to other interferons. Hum. Immunol. 13, 1-11.
3. Ciampolillo, A., Guastamacchia, E., Caragiulo, L., Lollino, G., De Robertis, O., Lattanzi, V., and Giorgino, R. (1993). In vitro secretion of interleukin-1 beta and interferon-gamma by peripheral blood lymphomononuclear cells in diabetic patients. Diabetes Res. Clin. Pract. 21, 87-93.
4. Cunningham, A. L., Nelson, P. A., Fathman, C. G., and Merigan, T. C. (1985). Interferon gamma production by herpes simplex virus antigen-specific T cell clones from patients with recurrent herpes labialis. J. Gen. Virol. 66, 249-258.
5. Davidson, P. M., Creati, L., Wood, P. R., Roberton, D. M., and Hosking, C. S. (1993). Lymphocyte production of gamma-interferon as a test for non-tuberculous mycobacterial lymphadenitis in childhood. Eur. J. Pediatr. 152, 31-35.
6. Edwards, B. S., Merritt, J. A., Fuhlbrigge, R. C., and Borden, E. C. (1985). Low doses of interferon alpha result in more effective clinical natural killer cell activation. J. Clin. Invest. 75, 1908-1913.
7. Gajewski, T. F., and Fitch, F. W. (1993). Anti-proliferative effect of IFN-gamma in immune regulation. I. IFNgamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. J. Immunol. 140, 4245-4252.
8. Le thi Bich Thuy, Queen, C., and Fauci, A. S. (1986). Interferon- gamma induces light chain synthesis in interleukin 2 stimulated human B cells. Eur. J. Immunol. 16, 547-550.
9. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., and Coffman, R. L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136, 2348-2357.
10. Nast, C. C., Zuo, X. J., Prehn, J., Danovitch, G. M., Wilkinson, A., and Jordan, S. C. (1994). Gammainterferon gene expression in human renal allograft fine-needle aspirates.Transplantation 57,498-502.
11. Naylor, S. L., Sakaguchi, A. Y., Shows, T. B., Law, M. L., Goeddel, D. V., and Gray, P. W. (1983). Human immune interferon gene is located on chromosome 12. J. Exp. Med. 157, 1020-1027.
12. Olsson, T. Multiple sclerosis,cerebrospinal fluid. (1994). Ann. Neurol. 36 Suppl, 100-102.
13. Romagnani, S., Giudizi, M. G., Biagiotti, R., Almerigogna, F., Mingari, C., Maggi, E., Liang, C. M., and Moretta, L. (1986). B cell growth factor activity of interferon-gamma. Recombinant human interferon-gamma promotes proliferation of anti-mu-activated human B lymphocytes. J. Immunol. 136, 3513-3516.
14. Sampaio, E. P., Kaplan, G., Miranda, A., Nery, J. A., Miguel, C. P., Viana, S. M., and Sarno, E. N. (1993). The influence of thalidomide on the clinical and immunologic manifestation of erythema nodosum leprosum. J. Infect. Dis. 168, 408-414.
15. Sastre, L., Roman, J. M., Teplow, D. B., Dreyer, W. J., Gee, C. E., Larson, R. S., Roberts, T. M., and Springer, T. A. (1986). A partial genomic DNA clone for the alpha subunit of the mouse complement receptor type 3 and cellular adhesion molecule Mac-1. Proc. Natl. Acad. Sci. U. S. A. 83, 5644-5648.
16. Shinde, S. R., Chiplunkar, S. V., Butlin, R., Samson, P. D., Deo, M. G., and Gangal, S. G. (1993). Lymphocyte proliferation, IFN-gamma production and limiting dilution analysis of T-cell responses to ICRC and Mycobacterium leprae antigens in leprosy patients. Int. J. Lepr. Other Mycobact. Dis. 61, 51-58.

17. Sidman, C. L., Marshall, J. D., Shultz, L. D., Gray, P. W., and Johnson, H. M. (1984). Gamma-interferon is one of several direct B cell-maturing lymphokines. Nature 309, 801-804.
18. Soliman, A. A., el Aggan, H. A., el Hefnawy, A. M., Mahmoud, S. A., and Abo Deya, S. H. (1994). The value of ascites adenosine deaminase activity and interferon gamma level in discriminating tuberculous from nontuberculous ascites. J. Egypt. Soc. Parasitol. 24, 93-105.
19. Suomalainen, H., Soppi, E., Laine, S., and Isolauri, E. (1993). Immunologic disturbances in cow's milk allergy, 2: Evidence for defective interferon-gamma generation. Pediatr. Allergy Immunol. 4, 203-207.
20. Tang, M. L. K., Kemp, A. S., Thorburn, J., and Hill, D. J. (1994). Reduced interferon-gamma secretion in neonates and subsequent atopy. Lancet 344, 983-985.
21. Urban, J. L., Shepard, H. M., Rothstein, J. L., Sugarman, B. J., and Schreiber, H. (1986). Tumor necrosis factor: a potent effector molecule for tumor cell killing by activated macrophages. Proc. Natl. Acad. Sci. U. S. A. 83, 5233-5237.

IL-10

22. Baer R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, P. S. Tuffnell, and B. G. Barrell. (1984). DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature 310, 207-211.
23. Barnes P. F., D. Chatterjee, J. S. Abrams, S. Lu, E. Wang, M. Yamamura, P. J. Brennan, and R. L. Modlin. (1992). Cytokine production induced by Mycobacterium tuberculosis lipoarabinomannan. Relationship to chemical structure. J. Immunol. 149, 541-547.
24. Bloom B. R., and V. Mehra. (1984). Immunological unrespon-siveness in leprosy. Immunol. Rev. 80, 5-28.
25. De Waal Malefyt R., J. Abrams, B. Bennett, C. G. Figdor, and J. E. de Vries. (1991). Interleukin-10 inhibits cytokine synthesis by human monocytes - an autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 174, 1209-1220.
26. Heinzel F. P., M. D. Sadick, S. S. Mutha, and R. M. Locksley. (1991). Production of interferon gamma, interleukin 2, interleukin 4, and interleukin 10 by CD4 positive lymphocytes in-vivo during healing and progressive murine leishmaniasis. Proc. Natl. Acad. Sci., USA 88, 7011-7015.
27. Kim J. M., C. I. Brannan, N. G. Copeland, N. A. Jenkins, T. A. Khan, and K. W. Moore. (1992). Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. J. Immunol. 148, 3618-3623.
28. Kullberg M. C., E. J. Pearce, S. E. Hieny, A. Sher, and J. A. Berzofsky. (1992). Infection with Schistosoma mansoni alters Th1/Th2 cytokine responses to a non-parasite antigen. J. Immunol. 148, 3264-3270.
29. Moore K. W., P. Vieira, D. F. Fiorentino, M. L. Trounstine, T. A. Khan, and T. R. Mosmann. (1990). Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein Barr Virus gene BCRF1. Science 248, 1230-1234.
30. Moore K. W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T. R. Mosmann. (1993). Interleukin-10. Ann. Rev. Immunol. 11, 165-190.
31. Mosier D. E., R. A. Yetter, and H. C. Morse III. (1985). Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57 Bl/6 mice. J. Exp. Med. 161, 766-784.
32. Shanafelt A. B., A. Miyajima, T. Kitamura, and R. A. Katelein. (1991). The amino -terminal helix of GM-CSF and IL-5 governs high-affinity binding to their receptors. EMBO J. 10, 4105-4112.
33. Sher A., R. T. Gazzinelli, I. P. Oswald, M. Clerici, M. Kullberg, E. J. Pearce, J. A. Berzofsky, T. R. Mosmann, S. L. James, H. C. Morse III, and G. M. Shearer. (1992). Role of T-cell derived cytokines in the downregulation of immune responses in parasitic and retroviral infection. Immunol. Rev. 127, 183-204.
34. Silva, J. S., P. J. Morrissey, K. H. Grabstein, K. M. Mohler, D. Anderson, and S. G. Reed. (1992). Interleukin 10 and interferon gamma regulation of experimental trypanosoma cruzi infection. J. Exp. Med. 175, 169-174.
35. Spits H., and R. de Waal Malefyt. (1992). Functional characterization of human IL-10. Int. Arch. Allergy Immunol. 99, 8-15.
36. Vieira P., R. de Waal Malefyt, M. N. Dang, K. E. Johnson, R. Kastelein, D. F. Fiorentino, J. E. de Vries, M. G. Roncarolo, T. R. Mosmann, and K. W. Moore, (1991). Isolation and expression of human cytokine synthesis inhibitory factor (CSIF/IL-10) cDNA clones: homology to Epstein-Barr virus open reading frame BCRF1. Proc. Natl. Acad. Sci. USA 88, 1172-1176.

12. Diaclone IFNγ / IL-10 ELISpot References

Mfarrej, B. et al., J Transl Med.,2017; 15(1): 40.

Products Manufactured and Distributed by:

Diaclone SAS
6 Rue Dr Jean-François-Xavier Girod
25000 Besançon
France
Tel +33 (0)3 81 41 38 38
Email: techsupport@medixbiochemica.com