

Product datasheet

Interferon gamma + IL4 Human Fluorospot Kit (Reagents for 10 x 96 tests, without plates) ab46646

Overview

Product name	Interferon gamma + IL4 Human Fluorospot Kit (Reagents for 10 x 96 tests, without plates)
Sample type	Cell culture supernatant, Cell culture extracts
Assay type	Sandwich (qualitative)
Assay duration	Multiple steps standard assay
Species reactivity	Reacts with: Human
Product overview	Intended use

The ELISPOT assay is designed to enumerate cytokine producing cells in a single cell suspension. This method has the advantage of requiring a minimum of in-vitro manipulations allowing cytokine production analysis as close as possible to in-vivo conditions in a highly specific way. This technique is designed to determine the frequency of cytokine producing cells under a given stimulation, and the follow-up of such frequency during a treatment and/or a pathological state. Elispot assay constitutes an ideal tool in the TH1 / TH2 response, vaccine development, viral infection monitoring and treatment, cancerology, infectious diseases, autoimmune diseases and transplantation.

The Elispot assay is based on sandwich immuno-enzyme technology. 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.

Principle of the method

After cell stimulation, locally produced cytokines are captured by IFN gamma and IL4-specific monoclonal antibodies. After cell lysis, trapped cytokine molecules are revealed by a secondary anti-IFN gamma FITC-conjugated antibody and a biotinylated anti-IL4 antibody. Those are in turn recognised by anti-FITC green fluorescent dye and streptavidin-phycoerythrin conjugates. PVDF-bottomed-well plates are then read under a UV light beam. Green fluorescent spots indicate IFN gamma production while IL4 is revealed by red spots. Yellow spots will indicate dual cytokine producing cells.

Tested applications	Suitable for: FluroSpot
Platform	Reagents

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	
Capture antibody for IFN gamma	2 x 0.5ml
Capture antibody for IL-4	2 x 0.5ml
FITC conjugated detection antibody for IFN gamma (resuspend in 0.55mL).	2 x 0.55ml
Biotinylated detection antibody for IL-4 (resuspend in 0.55mL).	2 x 0.55ml
Anti-FITC antibody green fluorescence conjugate.	2 x unit
Streptavidin-phycoerythrin conjugate.	2 x unit
Bovine Serum albumin.	2 x 1g
Fluorescence buffer	2 x 2.5ml

Relevance Mammalian Interferon gamma is mainly produced by T lymphocytes and NK cells. It is a pleiotropic cytokine involved in the regulation of nearly all phases of immune and inflammatory responses, including the activation, growth and differentiation of T cell, B cells, macrophages, NK cells and other cell types such as endothelial cells and fibroblasts. It has weak antiviral and antiproliferative activity, and potentiates the antiviral and anti tumor effects of IFN alpha/beta (type I interferon). It is upregulated by IL2, FGF basic, EGF and downregulated by vitamin D3 or DMN. Labile at pH 2. IL4 is a pleiotropic cytokine produced by activated T cells, mast cells and basophils. It is a ligand for Interleukin 4 receptor. The Interleukin 4 receptor also binds to IL13, which may contribute to many overlapping functions of this cytokine and IL13. IL4 elicits many different biological responses, but has two dominant functions. The first is regulating differentiation of naïve CD4+ T cell to the Th2 type. Th2 cells produce IL4, IL5, IL10 and IL13, which tend to favor a humoral immune response while suppressing a cell mediated immune response controlled by Th1 cells. STAT6, a signal transducer and activator of transcription, has been shown to play a central role in mediating the immune regulatory signal of this cytokine. The second is regulating IgE and IgG1 production by B cells. Two alternatively spliced transcript variants of this gene encoding distinct isoforms have been reported.

Cellular localization Secreted

Applications

Our [Abpromise guarantee](#) covers the use of **ab46646** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
FluroSpot		Use at an assay dependent dilution.

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