abcam

Product datasheet

Human Interferon gamma + IL4 ELISPOT Kit ab46649

Overview

Product name	Human Interferon gamma + IL4 ELISPOT Kit
Detection method	Colorimetric
Sample type	Suspension cells
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.

This 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.

The dual colour Elispot allows you to monitor the production of two cytokines simultaneously in the same well.

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 HRP and streptavidin-AP conjugates. PVDF-bottomed-well plates are

then incubated first with AEC substrate buffer, washed and subsequently incubated with BCIP/NBT. Coloured red/brownish spots indicate IFN gamma production while IL4 is revealed by blue/purple spots.

Notes Store plates at room temperature.

Tested applications **Suitable for:** ELISpot

Platform Microplate

Properties

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

Components	5 x 96 tests	10 x 96 tests
10 x concentrate buffer for the preparation of AEC buffer	1 x 5ml	2 x 5ml
50 x concentrate AEC substrate buffer	1 x 1ml	2 x 1ml
96 PVDF-bottomed-well plates.	5 units	10 units
Anti-FITC antibody HRP conjugate	1 x 100µl	2 x 100µl
Bovine Serum Albumin	1 x 1g	2 x 1g
Human IFN Gamma Capture antibody	1 x 500µl	2 x 500µl
IFNγ FITC conjugated detection antibody	1 vial	2 vials
IL-4 Biotinylated detection antibody	1 vial	2 vials
IL-4 Capture antibody	1 x 500µl	2 x 500µl
Ready-to-use BCIP/NBT substrate buffer	1 x 50ml	2 x 50ml
Streptavidin - Alkaline Phosphatase conjugated	1 x 50µl	2 x 50µl

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

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab46649 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
ELISpot		Use at an assay dependent dilution.

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