

Product datasheet

Human Interferon gamma + IL-10 ELISPOT Set ab48722

Overview

Product name Human Interferon gamma + IL-10 ELISPOT Set

Sample type Suspension cells

Assay type Sandwich (qualitative)

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Human

Product overview 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, oncology, 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.

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

Principle

After cell stimulation, locally produced cytokines are captured by IFN gamma and IL10 specific monoclonal antibodies. After cell lysis, trapped cytokine molecules are revealed by a secondary anti-IFN gamma FITC conjugated antibody and a biotinylated anti-IL-10 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 IL-10 is revealed by blue/purple spots.

Tested applications

Suitable for: ELISpot

Platform

Reagents

Properties

Storage instructions

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

Components	20 x 96 tests
10 x concentrate buffer for the preparation of AEC buffer	4 x 5ml
50 x concentrate AEC substrate buffer	4 x 1ml
Anti-FITC antibody HRP conjugate	4 x 100µl
Biotinylated detection antibody, clone B-T10	4 vials
Bovine Serum Albumin	4 x 1g
Human IFN Gamma Capture antibody	4 x 0.5ml
Human IL-10 Capture antibody	4 x 0.5ml
IFN γ FITC conjugated detection antibody	4 x 0.55ml
Ready-to-use BCIP/NBT substrate buffer	4 x 50ml
Streptavidin - Alkaline Phosphatase conjugated	4 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. Interleukins (ILs) are a large group of cytokines that are produced mainly by leukocytes, although some are produced by certain phagocytes and auxiliary cells. ILs have a variety of functions, but most function to direct other immune cells to divide and differentiate. Each IL acts on a specific, limited group of cells through a receptor specific for that IL. Human IL10 is a non glycosylated polypeptide consisting of 160 amino acids. There is 73% homology between the human and mouse IL10 proteins, however, the human IL10 acts on both human and mouse target cells, while the mouse IL10 has species specific activity. The cellular sources of IL10 are CD4+ T cells and T cell clones, thymocytes, B cells and B cell lymphomas, macrophages, mast cell lines and keratinocytes. IL10 will stimulate the growth of stem cells, mast cells and thymocytes. IL10 enhances cytotoxic T cell development, and costimulates B cell differentiation and immunoglobulin secretion. IL10 inhibits cytokine production by macrophages and suppresses macrophage class II MHC expression. The human IL10 gene is on human chromosome 1.

Cellular localization

Secreted

Applications

The Abpromise guarantee

Our [Abpromise guarantee](#) covers the use of ab48722 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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