abcam

Product datasheet

Human Interferon Gamma + Granzyme B FLUOROSPOT Kit ab48468

Overview

Product name Human Interferon Gamma + Granzyme B FLUOROSPOT Kit

Detection method Fluorescent

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, 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

After cell stimulation, locally produced cytokines are captured by IFN gamma and Granzyme B specific monoclonal antibodies. After cell lysis, trapped cytokine molecules are revealed by a secondary anti- IFN gamma FITC conjugated antibody and a biotinylated anti-Granzyme B 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 Granzyme B is revealed by red spots. Yellow spots will indicate dual cytokine producing cells.

Tested applications Suitable for: FluroSpot

Platform Microplate

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Properties

Storage instructions

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

Components	5 x 96 tests	10 x 96 tests
96 PVDF-bottomed-well plates.	5 units	10 units
Anti-FITC antibody green fluorescence conjugate.	1 vial	2 vials
Biotinylated detection antibody for Granzyme B (resuspend in 0.55mL).	1 vial	2 vials
Dry Skimmed milk	1 vial	2 vials
Bovine Serum Albumin	1 x 1g	2 x 1g
Capture antibody for Granzyme B	1 x 500µl	2 x 500µl
Human IFN Gamma Capture antibody	1 x 500µl	2 x 500µl
FITC conjugated detection antibody for IFNg (resuspend in 0.55mL)	1 vial	2 vials
Streptavidin-phycoerythrin conjugate.	1 vial	2 vials

Relevance

Interferon Gamma is produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons. Granzyme B is necessary for target cell lysis in cell-mediated immune responses. It cleaves after Asp. Seems to be linked to an activation cascade of caspases (aspartate-specific cysteine proteases) responsible for apoptosis execution. Cleaves caspase-3, -7, -9 and 10 to give rise to active enzymes mediating apoptosis.

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab48468 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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