

# Human Interferon Gamma + Granzyme B ELISPOT Set ab48463

### Overview

<b>Product name</b>	Human Interferon Gamma + Granzyme B ELISPOT Set
<b>Sample type</b>	Suspension cells
<b>Assay type</b>	Sandwich (qualitative)
<b>Assay duration</b>	Multiple steps standard assay
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Product overview</b>	<p>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.</p> <p>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.</p> <p>The dual colour ELISPOT allows you to monitor the production of two cytokines simultaneously in the same well.</p> <p><b>Principle of the Method</b></p> <p>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 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 Granzyme B is revealed by blue/purple spots.</p>
<b>Tested applications</b>	<b>Suitable for:</b> ELISpot

## Platform

## Reagents

## Properties

### Storage instructions

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

Components	5 x 96 tests	10 x 96 tests	15 x 96 tests	20 x 96 tests
10 x concentrate buffer for the preparation of AEC buffer	1 x 5ml	2 x 5ml	3 x 5ml	4 x 5ml
50 x concentrate AEC substrate buffer	1 x 1ml	2 x 1ml	3 x 1ml	4 x 1ml
Anti-FITC antibody HRP conjugate	1 x 100µl	2 x 100µl	3 x 100µl	4 x 100µl
Bovine Serum Albumin	1 x 1g	2 x 1g	3 x 1g	4 x 1g
Dry Skimmed milk	1 x 1g	2 x 1g	3 x 1g	4 x 1g
Granzyme B Biotinylated detection antibody	1 vial	2 vials	3 vials	4 vials
Granzyme B Capture Antibody	1 x 500µl	2 x 500µl	3 x 500µl	4 x 500µl
Human IFN Gamma Capture antibody	1 x 500µl	2 x 500µl	3 x 500µl	4 x 500µl
IFNγ FITC conjugated detection antibody	1 vial	2 vials	3 vials	4 vials
Ready-to-use BCIP/NBT substrate buffer	1 x 50ml	2 x 50ml	3 x 50ml	4 x 50ml
Streptavidin - Alkaline Phosphatase conjugated	1 x 50µl	2 x 50µl	3 x 50µl	4 x 50µl

### 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 ab48463 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.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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