Capture | Detect | Discover

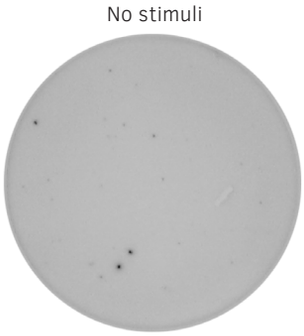
MABTECH

Find 1 cell in 100,000 with **ELISpot**

ELISpot is a sensitive assay used to quantify cytokine- or immunoglobulin-secreting cells at the single-cell level. ELISpot has been widely applied to investigate specific immune responses in infections, cancer, allergies and autoimmune diseases. With detection levels as low as one cell in 100,000, ELISpot is **one of the most sensitive cellular assays available.**

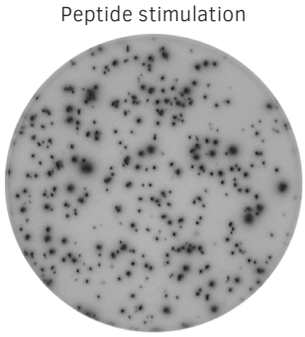
- Sensitive detection of antigen-specific cytokine responses
- Investigation of vaccine efficacy and evaluation of novel vaccine adjuvants
- Characterization of cytotoxic T-cell activity by IFN-γ, granzyme B, and perforin analysis
- Quantification of antigen-specific memory B cells and vaccine-induced antibody responses

No stimuli



4 spots

Peptide stimulation



283 spots

ELISpot analysis of IFN-γ secretion by PBMCs incubated overnight in the absence or presence of the CEF peptide pool. The CEF peptide pool consists of 23 viral peptides and stimulates human CD8+ T cells to produce cytokines (predominantly IFN-γ). Each spot represents a single T cell that has responded to the CEF peptide pool by secreting IFN-γ.

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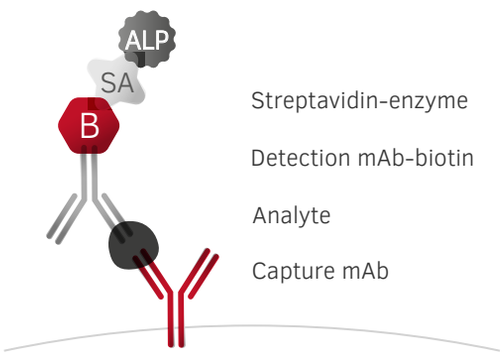
The ELISpot assay

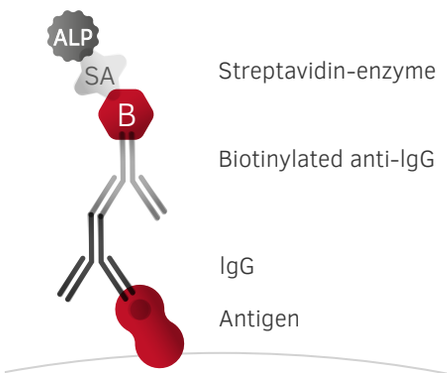
The ELISpot assay is a highly sensitive immunoassay that measures the frequency of cytokine-secreting cells at the single-cell level.

In ELISpot, cells are cultured on an antibody-coated surface in the presence or absence of stimuli. Proteins, for example cytokines, secreted by the cells will be captured by the specific antibodies. After an incubation period, the cells are removed by washing and the secreted cytokines are detected by biotinylated or enzyme-conjugated detection antibodies. By using a precipitating enzyme substrate, the end result is visible as a spot, where each spot corresponds to a single secreting cell.

The ELISpot assay is carried out in a 96-well plate and an automated ELISpot reader is used for analysis. The assay is robust and easy to perform, making it suitable for both large-scale trials and field studies. We have optimized the ELISpot protocol over more than 20 years, and today ELISpot kits and reagents are available for a multitude of analytes in many different species.

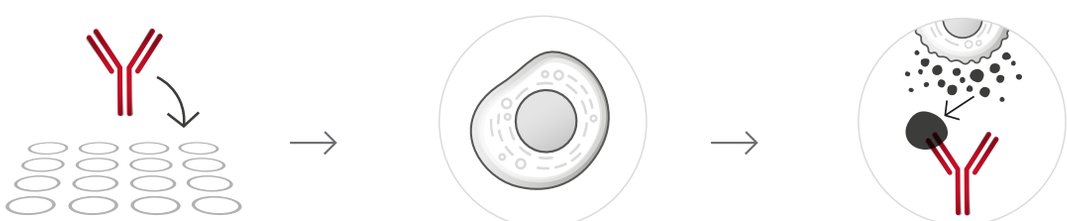


Schematic illustration of the ELISpot assay



Antigen-specific B-cell ELISpot

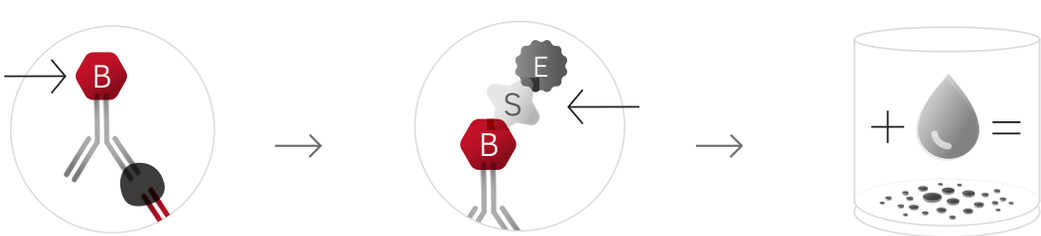
ELISpot step-by-step guide



Coating
Monoclonal capture antibodies are added to an ethanol-treated PVDF membrane plate.

Cell incubation
Cells are added in the presence or absence of stimuli and the plate is incubated to allow cytokine secretion.

Cytokine capture
Secreted cytokines bind to capture antibodies surrounding the activated cells.



Detection antibodies
Cells are removed and the plate is washed before biotinylated detection antibodies are added.

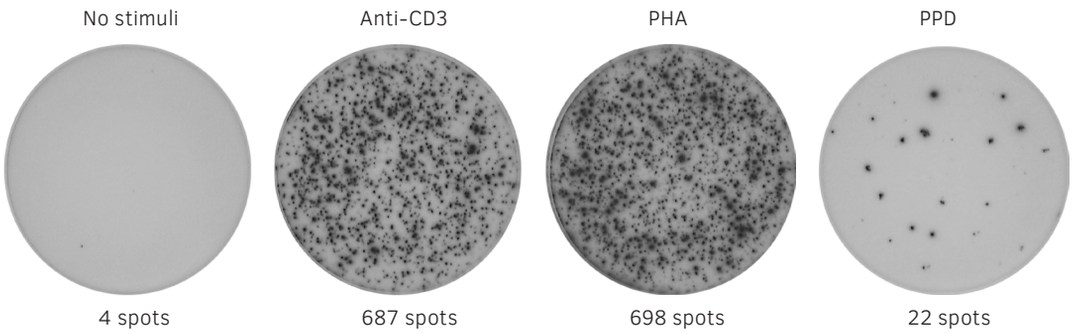
Streptavidin–enzyme conjugate
Addition of a streptavidin–conjugate enables the formation of spots on the membrane.

Addition of substrate
A colorimetric substrate forms an insoluble precipitate when catalyzed by the enzyme.

T-cell ELISpot

T-cell ELISpot is widely applied in investigations of specific immune responses in e.g infectious diseases, cancer, allergies, and in autoimmune diseases. T-cell ELISpot is also considered a standard tool in the development and monitoring of new vaccines and vaccine candidates.

ELISpot can be used to discriminate between subsets of activated T cells. For example, Th1 cells are characterized by their production of **IFN-γ**, **IL-2,** and **TNF-α**, whereas Th2 cells produce other cytokines such as **IL-4**, **IL-5**, and **IL-13**. In vaccine research and development, ELISpot can be used to define vaccine efficacy by measuring the capacity to elicit potent T-cell responses. Here, IFN-γ is often used as an immunocorrelate for CD8+ cytotoxic T-cell responses, but other mediators such as **granzyme B** or **perforin** may be analyzed as well. Today, diagnostic assays based on ELISpot are available, including a test to detect patients with tuberculosis infection by measuring IFN-γ secretion from T cells responding to defined antigens from Mycobacterium tuberculosis.

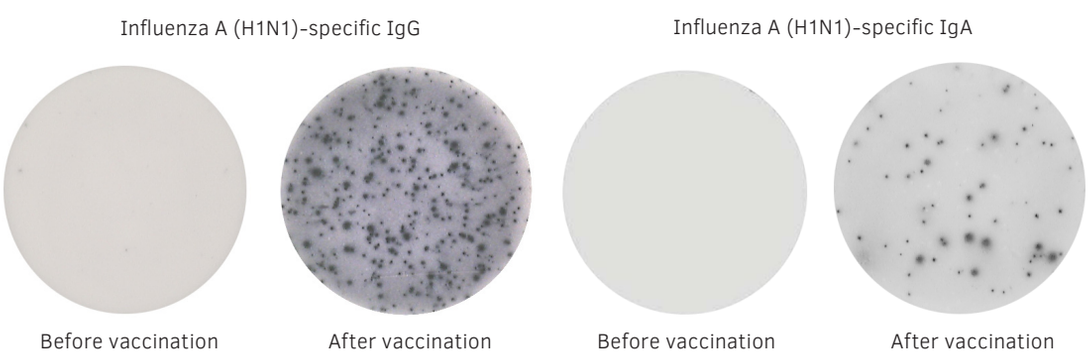


Human IFN-γ ELISpot
IFN-γ secretion by PBMCs incubated overnight without stimuli or with anti-CD3, phytohaem-agglutinin (PHA) or purified protein derivative (PPD).

B-cell ELISpot

The B-cell ELISpot assay is a powerful tool used to analyze antibody immune responses. Principal applications include detection of B-cell responses to infections and responses elicited by vaccination.

B-cell ELISpot is one of only a few assays that focuses directly on antibody-secreting cells (ASCs), in contrast to assays designed to measure antibody reactivity in solution. This extremely sentitive method allows researchers to identify ASCs in a sample, and measure the total number as well as those secreting antibodies to a specific antigen. With B-cell ELISpot, it is possible, for example, to demonstrate the presence and the frequencies of long-term memory B cells in the blood, which are difficult to assess by other methods. Mabtech supplies ELISpot kits for detection of B cells secreting **IgG**, **IgA**, **IgM**, and **IgE**.

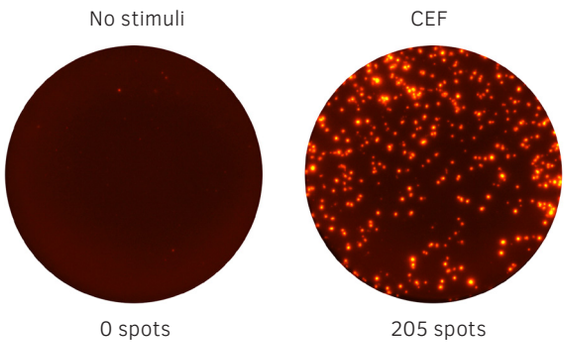


Human IgG and IgA ELISpot
PBMCs were collected before and after vaccination with Pandemrix® and secretion of influenza A (H1N1)-specific IgG (left) and IgA (right) was analyzed by ELISpot.

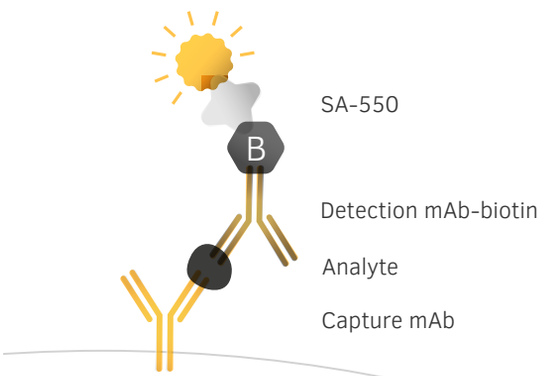
Single FluoroSpot: an alternative to ELISpot

Single-color FluoroSpot is similar to the standard enzymatic ELISpot assay but utilizes fluorescence instead of an enzymatic reaction for detection.

Therefore, there is no need for substrate addition and spot development, thus making single FluoroSpot less subjective than regular ELISpot. Furthermore, the dynamic range of spots in single FluoroSpot is greater. For those used to ELISpot, FluoroSpot is an excellent methodological improvement, which will also facilitate the transition into dual or triple FluoroSpot. Analyzing two or more analytes simultaneously not only saves time, and enables the use of fewer cells and less antigen compared to ELISpot but also provides data on dual- and triple-secreting cells; such data are unobtainable by separate ELISpot assays. Mabtech provides single FluoroSpot kits for analysis of several different cytokines in human, monkey, and mouse research. More information is available at www.mabtech.com.



PBMCs were incubated overnight with or without CEF peptide pool, and IFN-γ-secreting cells were analyzed by **single FluoroSpot**.



Single FluoroSpot assay principle

Mabtech ELISpot kits

All researchers have different needs, therefore we offer ELISpot kits in different formats; from ELISpot^{BASIC} to complete ELISpot^{PRO} kits with pre-coated strip plates, tests are available for a wide variety of requirements. ELISpot plates and substrates can also be purchased separately.

ELISpot^{BASIC}

Set of basic ELISpot reagents

- Capture mAb
- Biotinylated detection mAb
- Streptavidin-enzyme
- Stimuli (B-cell ELISpot)

ELISpot^{PLUS}

Kits with pre-coated plates, two-step detection and substrate

- Pre-coated PVDF plates
- Biotinylated detection mAb
- Streptavidin-enzyme
- ELISpot substrate
- Anti-CD3 mAb (certain analytes)

ELISpot^{PRO}

Kits with pre-coated plates, one-step detection and substrate

- Pre-coated PVDF plates
- One-step detection mAb
- ELISpot substrate
- Anti-CD3 mAb (certain analytes)

Mabtech ELISpot kits

Human

ANALYTE	AVAILABLE ELISPOT KIT FORMATS
ApoE	Basic, Plus
GM-CSF	Basic, Plus
Granzyme B	Basic, Plus
IFN-α2	Basic, Plus
IFN-α pan	Basic, Plus
IFN-γ	Basic, Plus*, Pro*
IgA	Basic
IgE	Basic
IgG	Basic
IgM	Basic
IL-1β	Basic, Plus
IL-2	Basic, Plus
IL-4	Basic, Plus, Pro
IL-5	Basic, Plus
IL-6	Basic, Plus
IL-8 (CXCL8)	Basic, Plus
IL-10	Basic, Plus
IL-12 (p70)	Basic, Plus
IL-12/-23 (p40)	Basic, Plus
IL-13	Basic, Plus
IL-17A	Basic, Plus
IL-21	Basic, Plus
IL-22	Basic, Plus
IL-23	Basic, Plus
IL-31	Basic, Plus
Perforin	Basic, Plus
TNF-α	Basic, Plus

Monkey

GM-CSF	Basic, Plus
IFN-α pan	Basic, Plus
IFN-γ	Basic, Plus*, Pro*
IgA	Basic
IgG	Basic
IgM	Basic
IL-2	Basic, Plus
IL-4	Basic, Plus, Pro
IL-5	Basic, Plus
IL-6	Basic, Plus
IL-8 (CXCL8)	Basic, Plus
IL-12/-23 (p40)	Basic, Plus
IL-13	Basic, Plus
IL-17A	Basic, Plus
IL-21	Basic, Plus
IL-23	Basic, Plus
Perforin	Basic, Plus
TNF-α	Basic, Plus

* also available with pre-coated strip plates

Mouse

ANALYTE	AVAILABLE ELISPOT KIT FORMATS
IFN-γ	Basic, Plus
IgA	Basic
IgG	Basic
IgM	Basic
IL-2	Basic, Plus
IL-4	Basic, Plus
IL-5	Basic, Plus
IL-6	Basic, Plus
IL-10	Basic, Plus
IL-12 (p70)	Basic, Plus
IL-12/-23 (p40)	Basic, Plus
IL-17A	Basic, Plus
TNF-α	Basic, Plus

Rat

IFN-γ	Basic, Plus

Rabbit

IFN-γ	Basic, Plus

Cow

IFN-γ	Basic, Plus
IL-2	Basic, Plus
IL-4	Basic, Plus
IL-8 (CXCL8)	Basic, Plus

Horse

IFN-γ	Basic, Plus

Sheep

IFN-γ	Basic, Plus
IL-4	Basic, Plus

Pig

IFN-γ	Basic, Plus

Dog

IFN-γ	Basic, Plus
IL-8 (CXCL8)	Basic, Plus

Ferret

IFN-γ	Basic, Plus

We continuously expand our product portfolio. Please visit **www.mabtech.com** for a complete pricing and product listing.

About Mabtech

Mabtech AB is a privately owned Swedish biotech company founded in 1986. We develop, manufacture, and market high quality monoclonal antibodies and kits suitable for ELISA, ELISpot, and FluoroSpot. For many years Mabtech has been a world leader in the field of ELISpot as a result of our strong research focus and continued effort to optimize this technique. Close international collaborations with both academia and industry are leading the way for future developments that help the research community achieve optimal results.

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