

## Detect single-cell secretion with **ELISpot**

### **If one cell responds, you will find it**

Analytes are captured immediately after secretion, ensuring high sensitivity

### **Scale up easily**

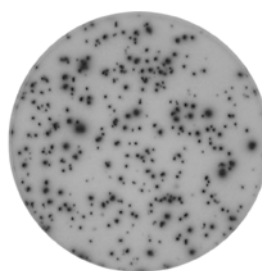
Assays in 96-well plates allow rapid analysis of large numbers of samples

### **Trusted supplier**

Today, more than 2,700 scientific publications feature our ELISpot kits



No stimuli



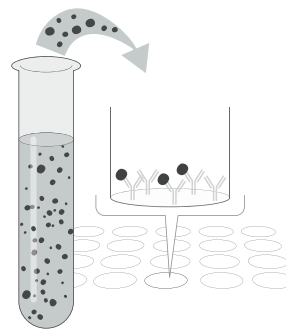
Peptide stimulation

## The ELISpot assay

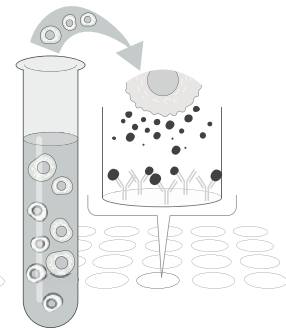
ELISpot is a sensitive assay used to quantify analyte secreting cells at the single-cell level. Cytokines, immunoglobulins, or other target proteins secreted by the cells are **captured immediately after secretion and throughout the stimulation process** by specific antibodies. With detection levels as low as one cell in 250,000, ELISpot is one of the most sensitive cellular assays.

We have worked to optimize the ELISpot protocol for more than 30 years, and today, our ELISpot kits and reagents are available for numerous analytes in many different species.

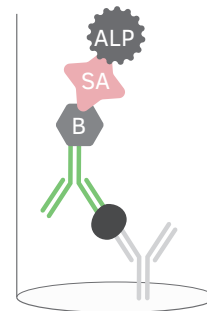
The assay is robust and easy to perform, making it suitable for both large-scale trials and basic research. ELISpot has been widely applied to investigate specific immune responses in infectious diseases, cancer, allergies, autoimmune diseases, and vaccine development.



ELISA: A sample containing the analyte is added to the wells

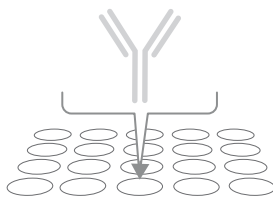


ELISpot: A cell suspension is added to the wells and the cells secrete the analyte



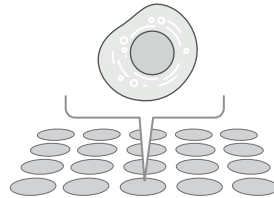
Schematic illustration of the ELISpot assay

## ELISpot step-by-step guide



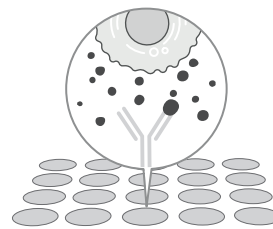
### 1. Coating

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



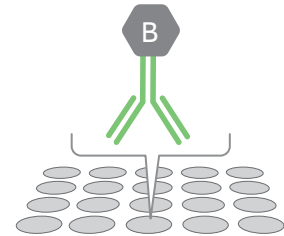
### 2. Cell incubation

Cells are added in the presence of stimuli, inducing cytokine secretion during incubation



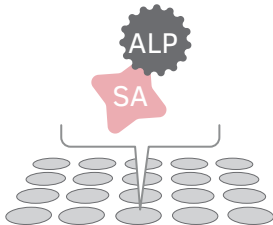
### 3. Cytokine capture

Secreted cytokines bind to capture antibodies in close proximity of the activated cell



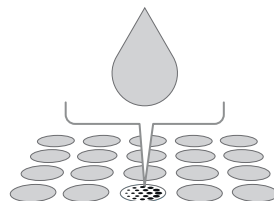
### 4. Detection antibodies

Cells are washed away before biotinylated detection antibodies are added



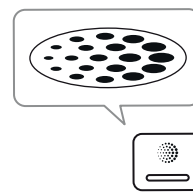
### 5. Streptavidin-enzyme conjugate

Addition of a streptavidin-conjugate enables the formation of spots on the membrane



### 6. Substrate addition

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



### 7. Analysis

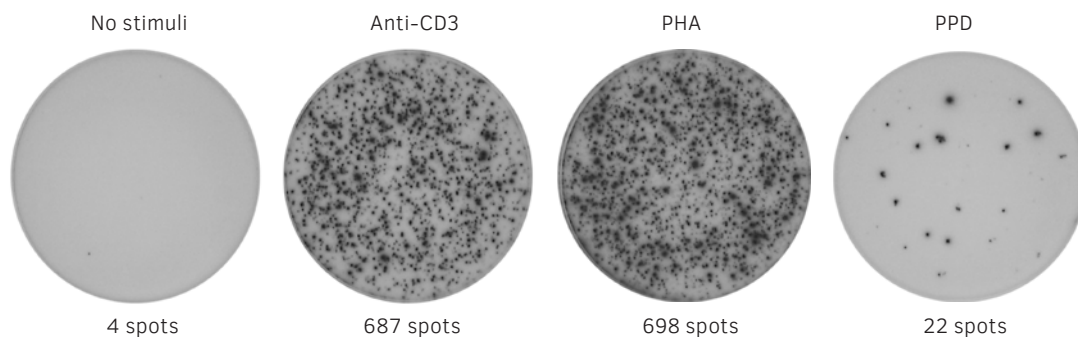
The result is analyzed in an automated spot reader. Each spot corresponds to a single analyte-secreting cell



## T cell ELISpot

ELISpot is a sensitive method for investigating specific immune responses and is able to discriminate between subsets of activated T cells. For example, Th1 cells are characterized by their production of **IFN- $\gamma$** , **IL-2**, and **TNF- $\alpha$** , whereas Th2 cells produce other cytokines such as **IL-4**, **IL-5**, and **IL-13**. This is widely applied for example in studies of infectious diseases, cancer, allergies, and autoimmune diseases.

In vaccine research, ELISpot is a standard tool that is used to define vaccine efficacy by measuring the capacity to elicit potent T cell responses, for example IFN- $\gamma$  secretion. Today, diagnostic assays based on ELISpot are available, including a test to detect patients with tuberculosis infection by measuring IFN- $\gamma$  secretion from T cells responding to defined antigens from *Mycobacterium tuberculosis*.



### Human IFN- $\gamma$ ELISpot

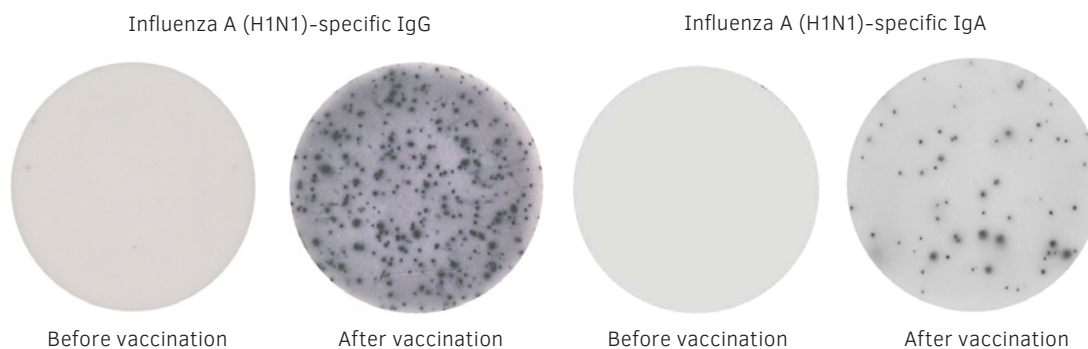
IFN- $\gamma$  secretion by peripheral blood mononuclear cells (PBMCs) incubated overnight without stimuli or with anti-CD3, phyto-haemagglutinin (PHA) or purified protein derivative (PPD)

## B cell ELISpot

The B cell ELISpot assay is a powerful tool to analyze antibody immune responses and is one of few assays directly measuring immunoglobulins upon secretion.

Commonly, the B cell ELISpot is used to directly assess antibody-secreting cells (ASCs). Because of its extreme sensitivity, the method enables

identification of ASCs to a specific antigen. Additionally, with B cell ELISpot, it is possible to evaluate the number of long-term memory B cells in the blood, which is difficult to assess by other methods. The main applications of B cell ELISpot include detection of B cell responses to infections and responses elicited by vaccination.



### 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



## Analysis

It is possible to analyze ELISpot plates with the naked eye, but to save (a considerable amount of) time and minimize errors, an automated reader is highly recommended.

Mabtech ASTOR™ is tailor made for ELISpot analysis. It allows for a plug-and-play workflow: No calibration is needed because of a fixed camera and an automatic XY table. In addition, the spot-counting algorithm RAWspot™ is based on signal processing and therefore is able to identify spots reliably up to 3,000 spots per well.

## Mabtech ELISpot kits

We offer ELISpot kits in different formats for different research needs, from ELISpot<sup>BASIC</sup> to complete ELISpot<sup>PRO</sup> kits with pre-coated

plates as well as ELISpot Path kits with antigen included.

	<b>ELISpot<sup>BASIC</sup></b> Flexible	<b>ELISpot<sup>PLUS</sup></b> Pre-coated	<b>ELISpot<sup>PRO</sup></b> One-step	<b>ELISpot Path</b> Antigen-specific
ELISpot plate(s)	–	Pre-coated	Pre-coated	Pre-coated
Capture mAb(s)	✓	Inside plate	Inside plate	Inside plate
Detection mAb ALP/HRP	–	–	✓	✓
Biotinylated detection mAb	✓	✓	–	✓
Streptavidin-ALP/HRP	✓	✓	–	✓
Substrate	–	✓	✓	✓
Positive control stimuli*	–	Anti-CD3 mAb	Anti-CD3 mAb	Anti-CD3 mAb
Polyclonal activator	For B cell ELISpot	–	–	✓
Size	Reagents for 4 plates	2 and 10 plates	2 and 10 plates	1 plate

\*Included for certain cytokine analytes

## FluoroSpot: An alternative to ELISpot

FluoroSpot utilizes fluorescence instead of an enzymatic reaction for detection. Consequently, this method enables multiplex assays of several analytes simultaneously. Read more at [www.mabtech.com](http://www.mabtech.com).

## Mabtech ELISpot kits

● BASIC

○ PLUS

● PRO

● PATH

### Human

ApoE	● ○
EBI3	● ○
GM-CSF	● ○
Granzyme B	● ○ ●
IFN-α2	● ○
IFN-α pan	● ○
IFN-γ	● ○ ● ●
IgA	●
IgE	●
IgG	● ●
IgG1	●
IgG2	●
IgG3	●
IgG4	●
IgM	●
IL-1α	● ○
IL-1β	● ○
IL-2	● ○
IL-3	● ○
IL-4	● ○ ●
IL-5	● ○
IL-6	● ○
IL-8 (CXCL8)	● ○
IL-10	● ○
IL-12 (p70)	● ○
IL-12/-23 (p40)	● ○
IL-13	● ○
IL-17A	● ○
IL-21	● ○
IL-22	● ○
IL-23	● ○
IL-27	● ○
IL-31	● ○
Perforin	● ○
TNF-α	● ○

### Monkey

GM-CSF	● ○
IFN-α pan	● ○
IFN-γ	● ○ ●
IgA	●
IgG	●
IgM	●
IL-2	● ○
IL-4	● ○ ●
IL-5	● ○
IL-6	● ○
IL-8 (CXCL8)	● ○
IL-12 (p70)	● ○
IL-12/-23 (p40)	● ○
IL-13	● ○
IL-17A	● ○
IL-21	● ○
IL-23	● ○
Perforin	● ○
TNF-α	● ○

### Cow

IFN-γ	● ○
IgG	●
IL-2	● ○
IL-4	● ○
IL-5	●
IL-8 (CXCL8)	● ○
IL-17A	● ○

### Pig

IFN-γ	● ○
IgG	●
IL-2	●
TNF-α	● ○

### Mouse

IFN-α	●
IFN-γ	● ○
IgA	●
IgE	●
IgE <sup>a</sup>	●
IgG	●
IgG1	●
IgG2a	●
IgG2c	●
IgG3	●
IgM	●
IL-1α	● ○
IL-2	● ○
IL-4	● ○
IL-5	● ○
IL-6	● ○
IL-10	● ○
IL-12 (p70)	● ○
IL-12/-23 (p40)	● ○
IL-17A	● ○
IL-22	● ○
TNF-α	● ○

### Sheep

IFN-γ	● ○
IL-4	● ○
IL-17A	● ○
IL-5	●

### Goat

IL-5	●
IL-17A	● ○

### Rat

IFN-γ	● ○
IL-22	● ○

### Hamster

IFN-γ	● ○
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### Cotton rat

IFN-γ	● ○
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### Woodchuck

IFN-γ	●
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### Rabbit

IFN-γ	● ○
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### Horse

IFN-γ	● ○
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### Dog

IFN-γ	● ○
IL-8 (CXCL8)	● ○

### Ferret

IFN-γ	● ○
IL-2	●

### Cat

IFN-γ	● ○
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### Chicken

IFN-γ	● ○
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### Salmon

IFN-γ	●
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### Rhinoceros

IFN-γ	● ○
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## ● ELISpot Path

SARS-CoV-2 kits with peptide pools or antigen,  
CMV and Adv5 kits with peptide pool,  
Mtb kits with ESAT-6, CFP-10, and EspC peptide pools

We are continually expanding our product portfolio. Please visit [www.mabtech.com](http://www.mabtech.com) for a current list of products and prices.



## About Mabtech

Mabtech AB is a Swedish biotech company that was founded in 1986. Our mission is to aid researchers to reach new frontiers and develop novel drugs, by supplying optimal immunoassays based on high-quality monoclonal antibodies and instruments.