

# ELISpot

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

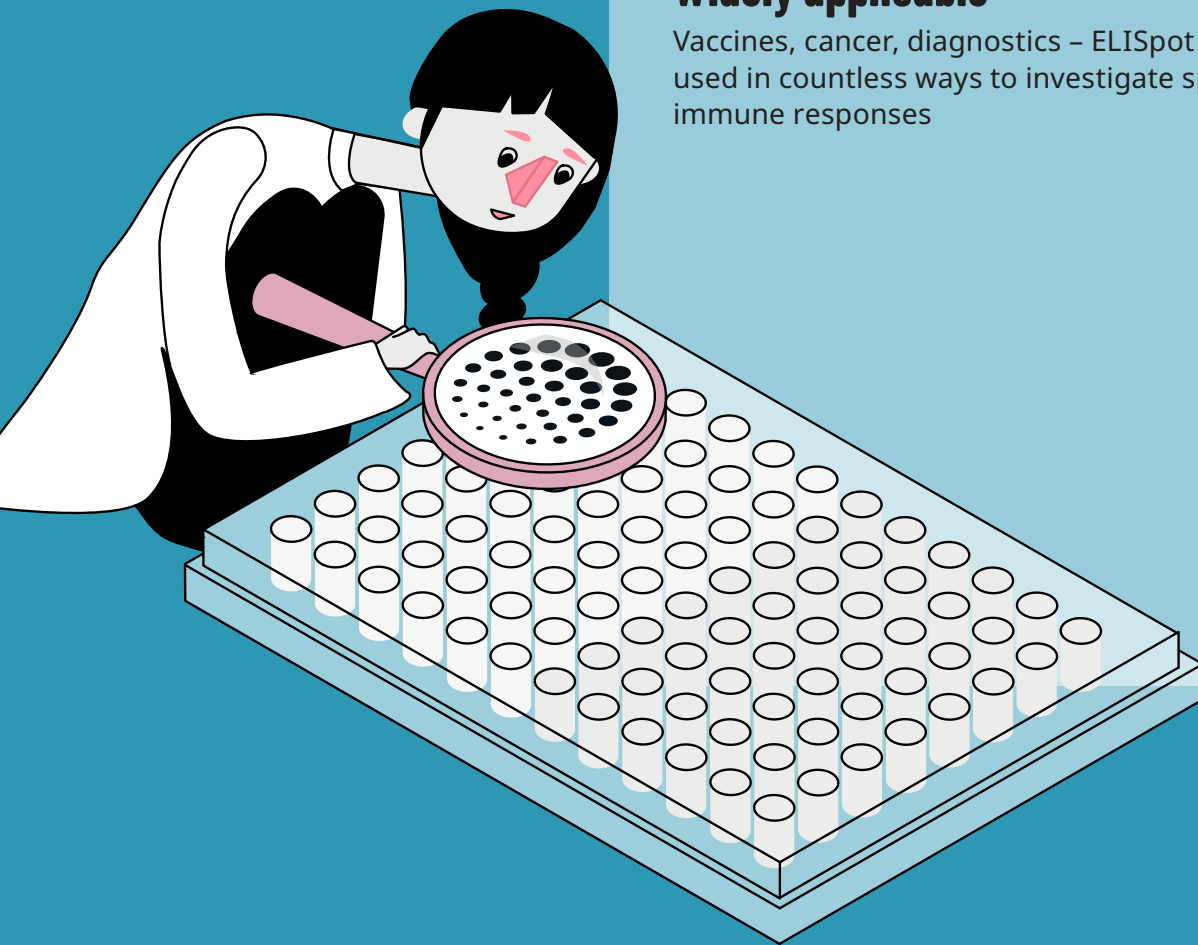
ELISpot is a cell-based immunoassay with detection levels as low as one cell in a million

## **Study analyte secretion**

Analytes are captured immediately and throughout the stimulation process

## **Widely applicable**

Vaccines, cancer, diagnostics – ELISpot can be used in countless ways to investigate specific immune responses



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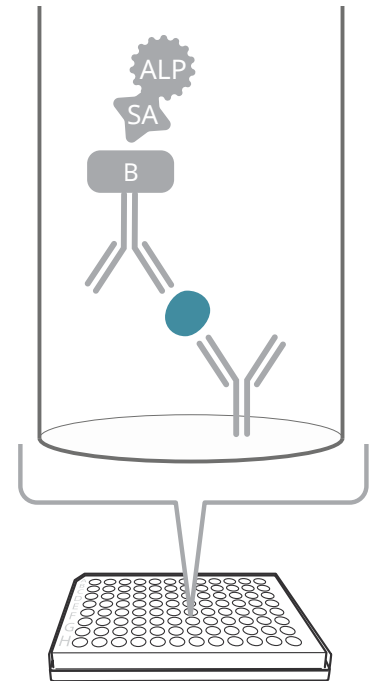


# How does ELISpot work?

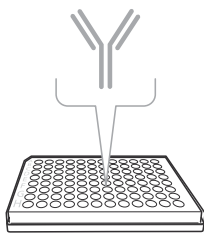
ELISpot is used to quantify protein-secreting cells.

In ELISpot, a cell suspension is added to the wells of a plate coated with protein-specific capture antibody. Protein secreted by the cells is immediately captured by these antibodies during the entire stimulation period. The cells are removed and then biotinylated protein-specific detection antibody is added followed by a streptavidin-enzyme conjugate. Visible spots are formed after adding a precipitating substrate.

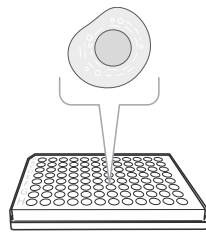
The plate is then read using an ELISpot reader where each spot corresponds to a responding analyte-secreting cell.



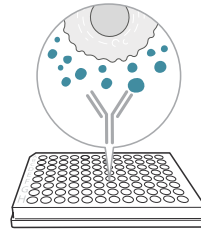
## Step-by-step



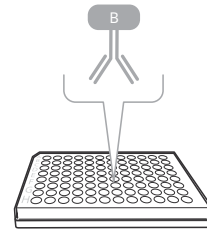
**1. Coating**  
Capture antibody is added to a plate with a PVDF membrane.



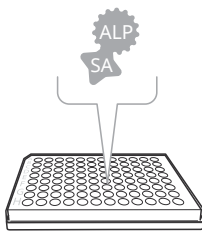
**2. Cell incubation**  
Cells and specific stimuli are added to each well and allowed to incubate for optimal analyte secretion.



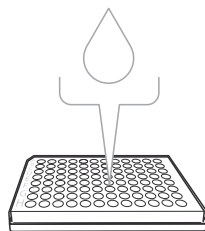
**3. Analyte capture**  
Secreted analyte binds to the capture antibodies surround the activated cell.



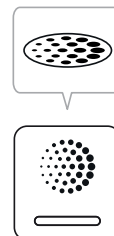
**4. Detection antibodies**  
The cells are removed, and biotinylated detection antibodies are added.



**5. Secondary detection**  
Addition of a streptavidin-enzyme conjugate enables enzymatic detection.



**6. Substrate addition**  
A substrate forms an insoluble precipitate when catalyzed by the enzyme, resulting in spot formation.



**7. Analysis**  
The plate is analyzed with an ELISpot reader. Each spot corresponds to an analyte-secreting cell.

# What are the benefits?

## Identify rare cells

ELISpot is a cell-based immunoassay used to quantify analyte-secreting cells at the **single-cell level**. Due to capacity to find one cell in a million, ELISpot is considered one of the most sensitive cellular assays available. This makes the method particularly valuable for studies of rare cell populations.

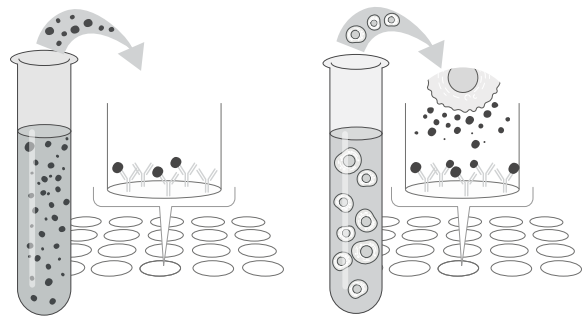
## Easy to scale up

ELISpot is robust and easy to perform, making it suitable for analyzing many samples in parallel or at different timepoints during a clinical trial. The reproducibility of the method has been repeatedly shown in numerous proficiency tests.

The assay is carried out in a 96-well plate and analyzed with an automated ELISpot reader, enabling rapid analysis of several plates in a row.

## Capture transient analytes

As ELISpot captures target proteins immediately after secretion, the method can detect cytokines that otherwise are too diluted or disappear from samples. For example, ELISpot can detect analytes that are rapidly degraded by proteases (IL-2), are quickly taken up by bystander cells (IL-4), or bind to soluble receptors (TNF- $\alpha$ ).



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

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

## 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 thanks to the fixed camera and an automatic XY table. In addition, the spot-counting algorithm RAWspot™ is based on signal processing and therefore able to identify spots reliably up to 3,000 spots per well.

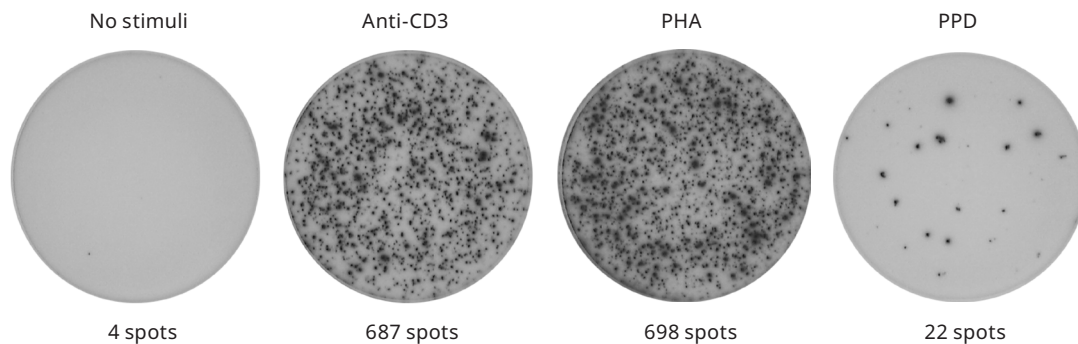


# How is ELISpot used?

## To detect cytokine-secreting cells

ELISpot is commonly used to investigate **antigen-specific immune responses** and to discriminate between subsets of activated T cells. This is applied in studies of infectious diseases, cancer, allergies, and autoimmune diseases. In vaccine research, ELISpot is the gold standard to define vaccine efficacy by

measuring the capacity to elicit potent T cell responses, for example by assessing IFN- $\gamma$  secretion. Diagnostic assays based on ELISpot are available, including tests to detect patients with tuberculosis or SARS-CoV-2 infection by measuring IFN- $\gamma$  secretion from T cells responding to defined antigens.



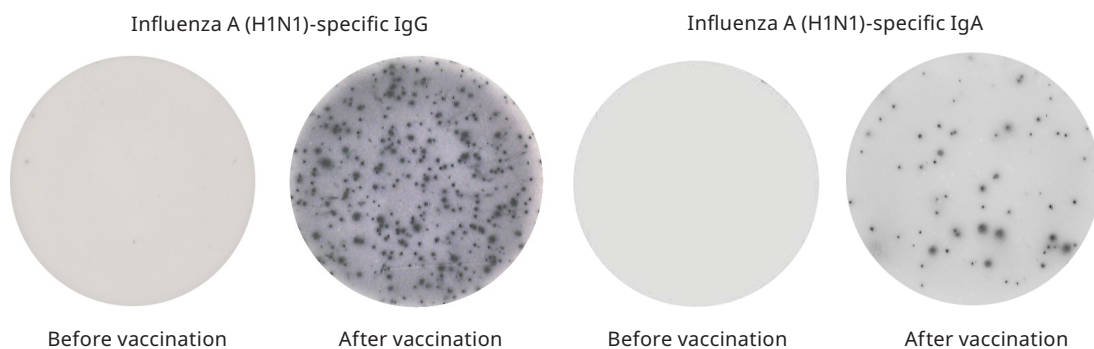
### Human IFN- $\gamma$ ELISpot

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

## To detect antibody-secreting cells

The B cell ELISpot assay is one of few assays measuring **immunoglobulins directly upon secretion**. There are two strategies: First, B cell ELISpot can be used to assess antibody-secreting cells (ASCs). Due to its sensitivity, the method enables identification of rare ASCs

to a specific antigen. Second, you can evaluate circulating antigen-specific memory B cells after polyclonal activation. B cell ELISpot is thus regularly used to detect B cell responses elicited by infection or vaccination.

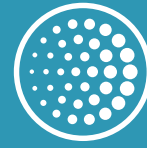


### Human IgG and IgA ELISpot

PBMCs were collected before and after vaccination against influenza and secretion of influenza A (H1N1)-specific IgG and IgA was analyzed by ELISpot

# Which kit format to choose?

We offer ELISpot kits in different formats for different research needs: from flexible kits that include the core reagents to complete pre-coated kits made for studying the immune response to specific pathogens.



	<b>ELISpot Flex</b> <i>Adaptable</i>	<b>Recommended</b> <b>ELISpot Plus</b> <i>Reproducible</i>	<b>ELISpot Pro</b> <i>One-step detection</i>	<b>ELISpot Path</b> <i>Antigen-specific</i>
ELISpot plate	-	Pre-coated	Pre-coated	Pre-coated
Capture mAb	√	Coated on plate	Coated on plate	Coated on plate
Detection mAb ALP/HRP	-	-	√	√
Detection mAb, biotinylated	√	√	-	√
Streptavin-ALP/HRP	√	√	-	√
Substrate	-	√	√	√
Anti-CD3 mAb (positive control)*	-	√	√	√
Anti-CD28 mAb (for co-stimulation)*	-	-	-	√
R848+IL-2 (polyclonal activators)**	√	-	-	√
Peptide pool or antigen	-	-	-	√
Size	Reagents for 4 plates	2, 10, and 100 plates	2, 10, and 100 plates	1 plate

\*Included for certain cytokine analytes

\*\*Included for certain immunoglobulin analytes

# Check out all of our kits

We have kits for numerous analytes in a number of different species, and we're regularly expanding our range of products. Please visit [www.mabtech.com](http://www.mabtech.com) or scan the QR to see all of our products.



## Selected references

**Our ELISpot kits appear in numerous publications ranging from vaccine development to cancer research and diagnostics. Scan the QR code for a full list of references.**

Gebre et al., *Optimization of non-coding regions for a non-modified mRNA COVID-19 vaccine*, Nature 2022

Marsh et al., *ChAdOx1 nCoV-19 (AZD1222) vaccine candidate significantly reduces SARS-CoV-2 shedding in ferrets*, NPJ Vaccines 2021

Te Kamp et al., *Comparable Long-Term Rabies Immunity in Foxes after IntraMuscular and Oral Application Using a Third-Generation Oral Rabies Virus Vaccine*, Vaccines (Basel) 2021

Gasser et al., *The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab*, Nat Commun. 2020

Sicard et al., *Donor-specific chimeric antigen receptor Tregs limit rejection in naive but not sensitized allograft recipients*, Am J Transplant. 2020



# MABTECH

## About Mabtech

Mabtech is a Swedish biotech company that was founded in 1986. Our mission is to aid scientists to reach new frontiers through optimal immunoassays and instruments.