FluoroSpot

Tell the story of every cell

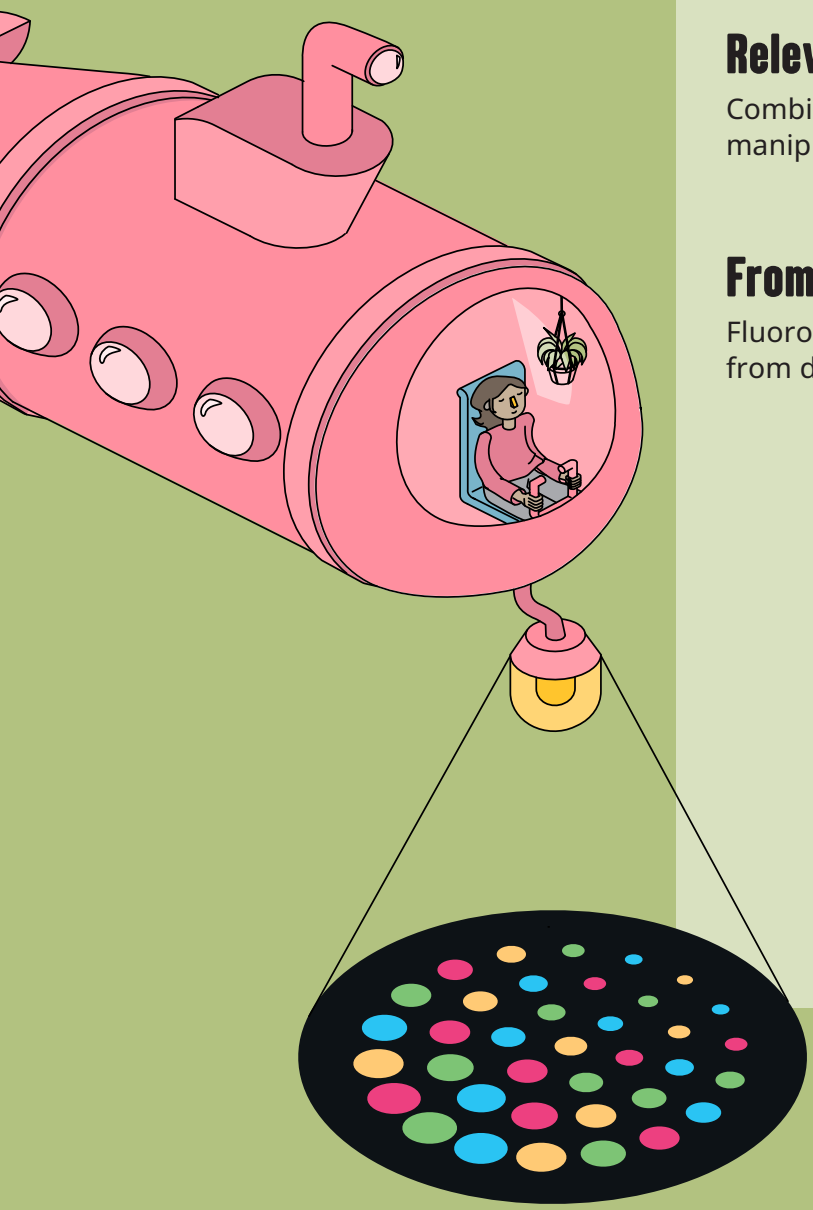
FluoroSpot is the multiplex version of ELISpot

Relevant secretion

Combine analytes with different kinetics without manipulating intracellular processes

From research to clinical trials

FluoroSpot is robust and therefore easy to scale up from discovery phase to larger studies



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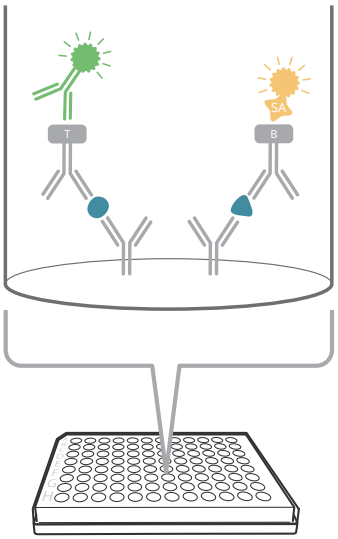
How does FluoroSpot work?

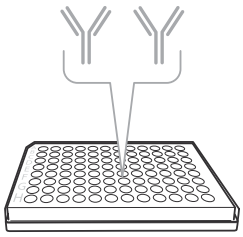
FluoroSpot is used to detect and quantify protein-secreting cells. This immunoassay combines the sensitivity of ELISpot with the capacity to study the secretion of several proteins simultaneously.

In FluoroSpot, a cell suspension is added to the wells of a plate coated with one or more protein-specific capture antibodies. Proteins secreted by the cells are immediately captured by these antibodies during the entire stimulation period. The cells are removed and then tagged or biotinylated protein-specific detection antibodies are added, followed by fluorophore-labeled secondary reagents.

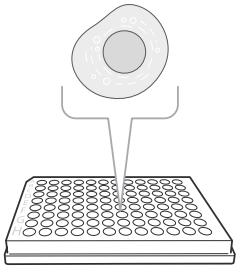
The plate is then read using a FluoroSpot reader, where each fluorescent spot corresponds to a responding protein-secreting cell.



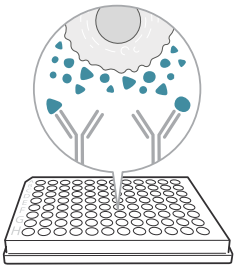
Step-by-step



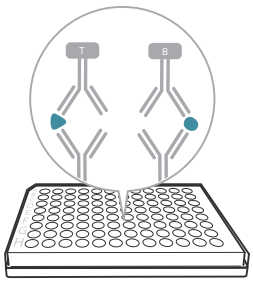
1. Coating
Several capture antibody clones are added to a plate with a low-fluorescent PVDF membrane.



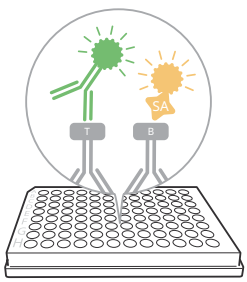
2. Cell incubation
Cells and specific stimuli are added to each well and allowed to incubate for optimal analyte secretion.



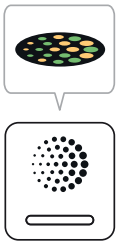
3. Protein capture
Secreted proteins bind to the capture antibodies around the activated cells.



4. Detection antibodies
The cells are removed, and a mixture of tagged and biotinylated detection antibodies is added.



5. Secondary detection
Addition of fluorophore-labeled secondary detection reagents enables fluorescent detection.

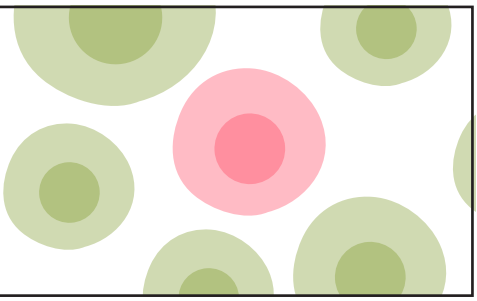


6. Analysis
The plate is analyzed in a FluoroSpot reader with separate filters for the different fluorophores.

What are the benefits?

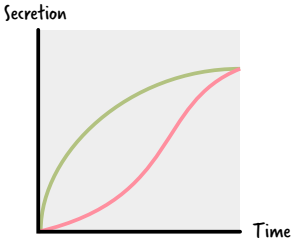
Identify rare cells

FluoroSpot is one of the most sensitive cellular assays available, up to 500 times more sensitive than intracellular cytokine staining. If one cell secretes the protein, it's detected and visualized as a single spot.



Study analytes with different kinetics

With FluoroSpot, the release of multiple cytokines or immunoglubulins can be studied at the single-cell level. Cytokines released directly after activation can be analyzed together with cytokines that have slower kinetics without manipulating intracellular processes.

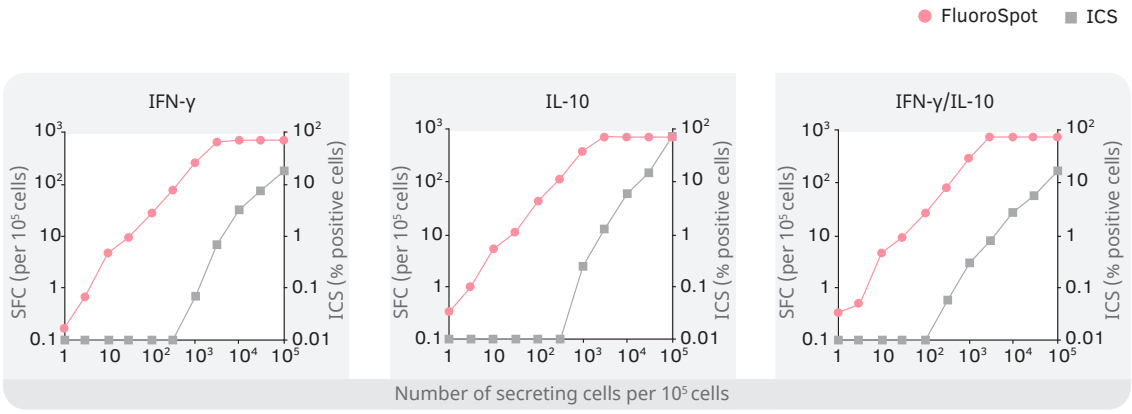


Case study showing sensitivity

To compare the sensitivity of FluoroSpot with flow cytometry, increasing numbers of transfected CHO cells constitutively secreting IFN-γ and IL-10 were mixed with 10^5 non-transfected cells, shown on the x-axis.

The number of spot forming cells (SFC) are depicted on the left y-axis, and the frequency of cells stained intracellularly for cytokine (ICS) on the right y-axis.

FluoroSpot could detect cytokine secretion when only 10 transfected cells were added. By contrast, at least 5,000 transfected cells were required to detect the cytokines by flow cytometry. (Figure adapted from Chauvat et al., Hum Vaccin Immunother. 2014).

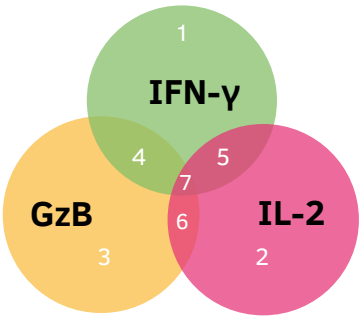


How is FluoroSpot used?

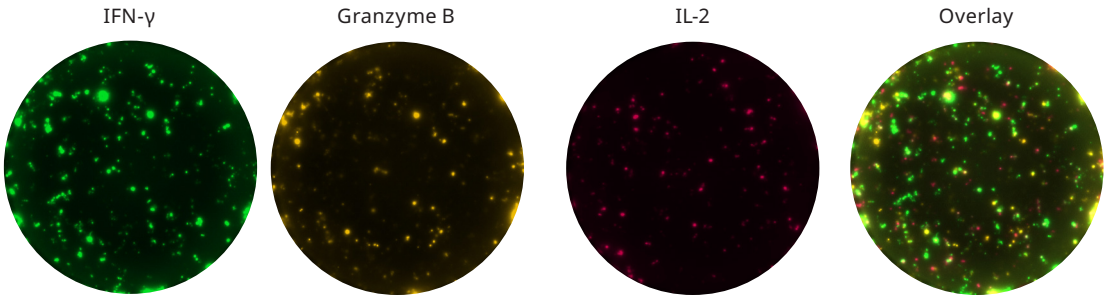
Due to its high sensitivity, FluoroSpot is ideal to identify **polyfunctional cells** in vaccine or cancer research.

The detection of different secretory profiles separates the responding cells into different functional groups. Looking at several proteins in parallel increases the possibility of identifying distinct cell populations in the same well.

IFN-γ is often combined with IL-2, TNF-α, or Granzyme B in analyses investigating cytotoxic T cell responses against selected antigens. Stimulation is usually achieved with peptide pools covering the vaccine antigen.



A 3-color FluoroSpot assay can be used to identify three cell populations secreting one analyte (1-3), three populations secreting two (4-6), and one population secreting all three analytes (7).



FluoroSpot analysis with three images from the same well and a corresponding overlay of the three filters.

Analysis

The analysis of our FluoroSpot kits requires a FluoroSpot reader equipped with filters for the following wavelengths (excitation/emission):

- 380 nm/430 nm (LED380, DAPI)
- 490 nm/510 nm (LED490, FITC)
- 550 nm/570 nm (LED550, Cy3)
- 640 nm/660 nm (LED640, Cy5)

All of our FluoroSpot kits are easily read on our FluoroSpot reader, **Mabtech IRIS™ 2**. The user-friendly software and patented RAWSpot™ algorithm is optimized for precise spot center detection ensuring accurate multiplexing and also introduces the newest FluoroSpot data, **relative spot volume (RSV).**

Which kit format to choose?

Flexible, validated, or down a charted path – it's your choice. With **FluoroSpot Flex** you can select analytes and create over 100,000 possible combinations. **FluoroSpot Plus** kits, have validated analyte combinations and pre-coated plates to save time and minimize variability. Finally, **FluoroSpot Path** kits include specific antigens and are designed to study immune responses to a specific pathogen.

		Recommended	
	FluoroSpot Flex *Build your own kit*	**FluoroSpot Plus** *Reproducible*	**FluoroSpot Path** *Antigen-specific*
FluoroSpot plate	Non-coated	Pre-coated	Pre-coated
Capture mAb(s)	√	Coated on plate	Coated on plate
Detection mAb(s)	√	√	√
Secondary detection reagents conjugated to fluorophores	√	√	√
Anti-CD3 mAb (positive control)*	–	√	√
Anti-CD28 mAb (for co-stimulation)*	√	√	√
R848+IL-2 (polyclonal activators)**	√	√	√
FluoroSpot enhancer	√	√	√
Peptide pool or antigen	–	–	√
Size	1 and 10 plates	2 and 10 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 or scan the QR to see all of our products.

Selected references

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

Janetzki et al., *Stepping up ELISpot: Multi-Level Analysis in FluoroSpot Assays,* Cells. 2014

Jahnmatz et al., *Memory B-Cell Responses Against Merozoite Antigens After Acute Plasmodium falciparum Malaria, Assessed Over One Year Using a Novel Multiplexed FluoroSpot Assay,* Front Immunol. 2020

Singhania et al., *The TCR repertoire of alpha-synuclein-specific T cells in Parkinson's disease is surprisingly diverse,* Sci Rep. 2021

Bronge et al., *Myelin oligodendrocyte glycoprotein revisited-sensitive detection of MOG-specific T-cells in multiple sclerosis*, Journal of autoimmunity 2019

Mateus et al., *Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans,* Science 2020

Ishigaki et al., *No Tumorigenicity of Allogeneic Induced Pluripotent Stem Cells in Major Histocompatibility Complex-matched Cynomolgus Macaques,* Cell Transplant. 2021

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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.

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