FluoroSpot

Tell the story of every cell

FluoroSpot is the multiplex version of ELISpot

Relevant secretion

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Combine analytes of 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 quantify protein-secreting cells. This immunoassay combines the sensitivity of ELISpot with the capacity to study the secretion of several analytes simultaneously.

In FluoroSpot, a cell suspension is added to the wells of a plate. Proteins, secreted by cells are captured immediately after secretion and throughout the stimulation process by specific antibodies. After cell removal, tagged or biotinylated detection antibodies are added, followed by fluorophorelabeled secondary reagents.

The captured proteins turn into fluorescent spots, where each spot corresponds to a single cell.



Step-by-step



1. Coating Several capture antibody clones are added to a plate with a lowfluorescent PVDF membrane.



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



2. Cell incubation Cells are added in the presence of stimuli and the plate is incubated to enable analyte secretion.



5. Secondary detection Addition of fluorophorelabeled secondary detection reagents enables fluorescent detection.



3. Analyte capture Different secreted analytes bind to the capture antibodies surrounding the activated cells.



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 analyte, it is detected and visualized as one 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, increasing numbers of transfected CHO cells constitutively secreting IFN-y and IL-10 were mixed with 10⁵ non-transfected cells, shown on the X-axis. Spot forming cells (SFC) are depicted on the left Y-axis, and 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;10(1):104-13)



How is FluoroSpot used?

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

The detection of different secretion 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 functional responses towards 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 showing three individual images from the same well and an image overlay, combining images from 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)

Our **Mabtech IRIS** matches our FluoroSpot kits. The unique software of Mabtech IRIS is optimized for spot center detection to ensure accurate multiplexing and analysis of the relative spot volume.



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, on the other hand, have validated analyte combinations and pre-coated plates to save time and minimize variability. Finally, **FluoroSpot Path** kits include 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)	\checkmark	Inside plate	Inside plate
Detection mAb(s)	\checkmark	\checkmark	\checkmark
Secondary detection reagents conjugated to fluorophores	\checkmark	\checkmark	\checkmark
Anti-CD3 mAb (positive control)*	_	\checkmark	\checkmark
Anti-CD28 mAb (for co-stimulation)*	\checkmark	\checkmark	\checkmark
R848+IL-2 (polyclonal activators)**	\checkmark	\checkmark	\checkmark
FluoroSpot enhancer	\checkmark	\checkmark	\checkmark
Peptide pool or antigen	_	-	\checkmark
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 many analytes and a lot of **different species**, and we are continually expanding our range of products. To keep you up to date, please visit our webshop on www.mabtech.com or scan the QR code.



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.

Jahnmatz, P. 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, A. et al. *The TCR repertoire of alphasynuclein-specific T cells in Parkinson's disease is surprisingly diverse*, Sci Rep. 2021

Bronge, M. et al., *Myelin oligodendrocyte glycoprotein revisited-sensitive detection of MOGspecific T-cells in multiple sclerosis*, Journal of autoimmunity 2019 Mateus, J. et al., *Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans*, Science 2020

Ishigaki, H. 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.