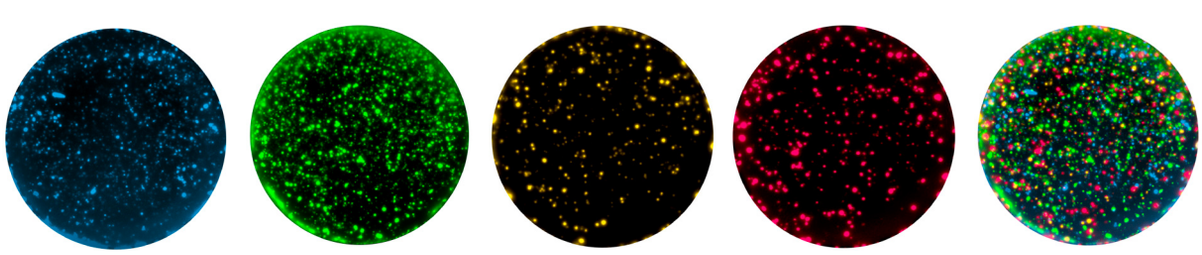
Capture | Detect | Discover

MABTECH

Discover new paths with **FluoroSpot**

FluoroSpot combines the sensitivity of ELISpot with the capacity to analyze secretion of several analytes simultaneously, enabling studies of cell populations with different functional profiles. This highly sensitive cellular assay is robust, easy to perform, and suitable for both single tests and large-scale screening.

Tell the story of every cell	FluoroSpot visualizes the secretory profile as a spot, which is the footprint of one responding cell
Study physiologically relevant secretion	Analytes with different kinetics can be combined without manipulating intracellular processes
World leaders	We have focused on spot analysis for over 30 years and know how to best design a FluoroSpot assay



IL-5 IFN-γ IL-22 IL-17A Overlay

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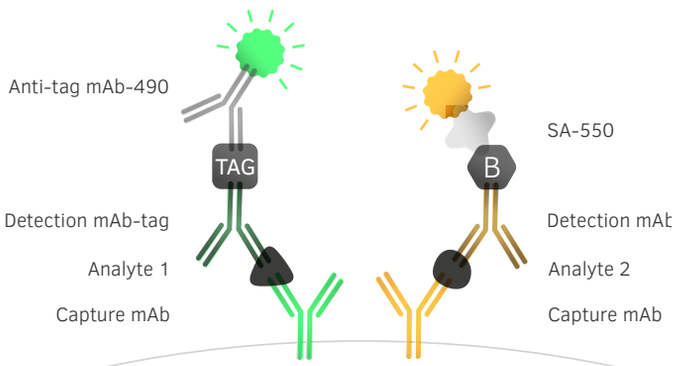
Discover more with FluoroSpot

FluoroSpot can be used in numerous applications and is equally well suitable for single tests and large-scale screening. The sandwich assay design combined with fluorophore-labeled detection reagents enables analysis of two or more analytes in the same well.

A sandwich assay principle is applied in FluoroSpot in which a mixture of monoclonal capture antibodies with different specificities is coated onto PVDF membranes in a 96-well plate. Detection of dual-, triple- or quadruple-secreting cells is made possible by the use of a biotinylated detection antibody for one analyte and a tag-labeled detection antibody for the other analyte(s). The detection step is visualized and amplified by specific fluorophore-conjugated reagents and the resulting spots are analyzed in an automated reader.



Fluorophore	Filter
380	DAPI equivalent
490	FITC equivalent
550	Cy3 equivalent
640	Cy5 equivalent

FluoroSpot assay principle showing dual FluoroSpot. The use of fluorophores with maximum excitation at 380, 490, 550, and 640 nm requires a FluoroSpot reader equipped with DAPI, FITC, Cy3 and Cy5 equivalent filters, respectively.

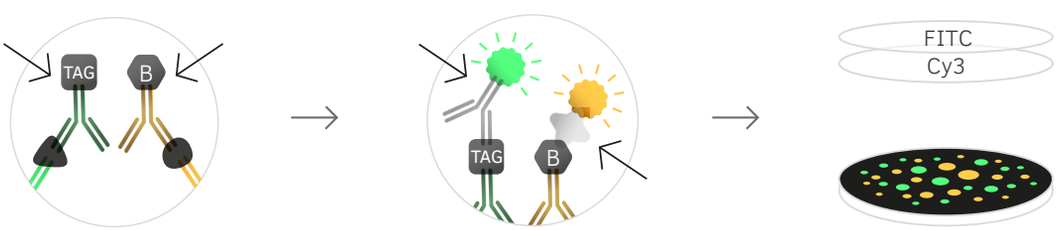
FluoroSpot step-by-step guide

Step-by-step guide to the FluoroSpot assay, demonstrated here by a dual FluoroSpot setup.

Coating
A mixture of capture antibodies is added to all wells of an ethanol-treated PVDF membrane plate.

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

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



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

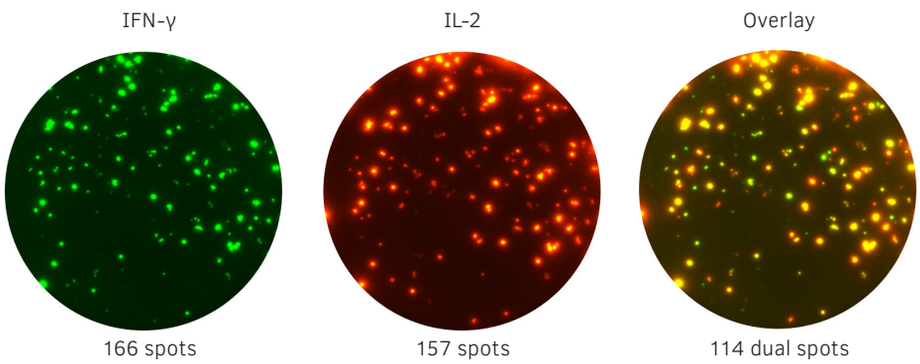
Fluorophore-labeled conjugates
A mixture of fluorophore-labeled anti-tag antibody and streptavidin-fluorophore conjugate is added.

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

T-cell FluoroSpot

The FluoroSpot assay is ideal for analysis of antigen-specific T-cell responses.

Delineating functional T-cell subsets by cytokine profiling is of great interest in many different research fields. FluoroSpot can be used in vaccine development to detect and analyze vaccine-specific immmue responses by polyfunctional T cells. In cancer research, FluoroSpot can be used, for example, to monitor tumor-infiltrating lymphocytes or to provide a more direct assessment of cytotoxic T-cell responses by simultaneous analysis of IFN-γ and granzyme B secretion.

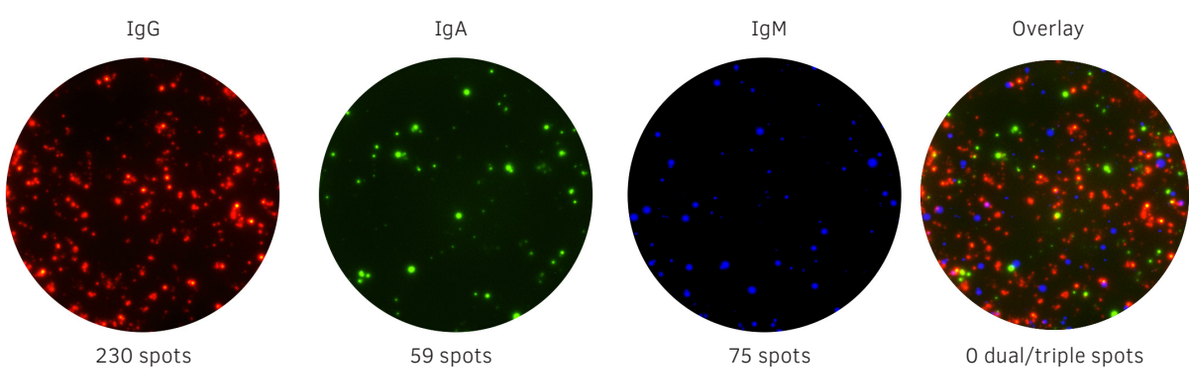


Human IFN-γ/IL-2 dual FluoroSpot
The images show a dual FluoroSpot analysis of cells secreting IFN-γ and/or IL-2 in response to PPD (purified protein derivative). Of the 166 IFN-γ-secreting T cells, 114 also secreted IL-2. Dual-secreting cells were determined as spots with the same position (center point) in an image overlay of the 490 (IFN-γ) and 550 (IL-2) images.

B-cell FluoroSpot

The B-cell FluoroSpot assay can identify the presence of and quantify both the total number of antibody-secreting cells and those secreting antigen-specific antibodies.

Detection based on fluorophores offers the possibility to analyze the secretion of different immunoglobulin isotypes or subclasses in the same well. Major application areas include analysis of B-cell responses in various diseases and those elicited by vaccination. For example, antigen-specific secretion of IgG, IgA, and IgM may be investigated before and after vaccination.

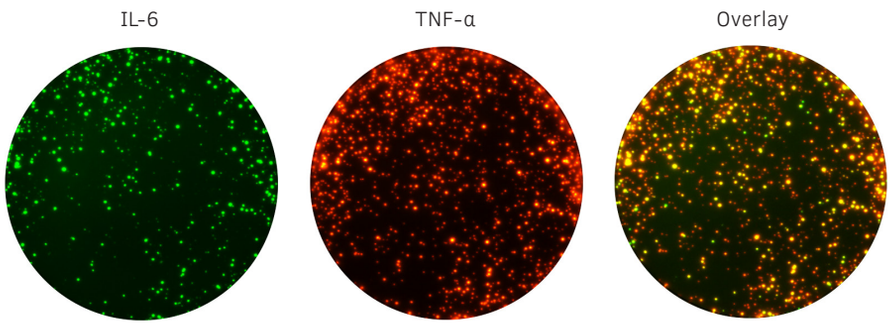


Human IgG/IgA/IgM triple FluoroSpot
In the images shown, PBMCs were pre-incubated in the presence of R848 and recombinant human IL-2, added to a FluoroSpot plate, and incubated overnight. The number of cells secreting IgG (550), IgA (490) and IgM (640, color substituted) was analyzed.

FluoroSpot for other cell types

FluoroSpot can be used to analyze dendritic cells, monocytes, and macrophages.

Cytokine secretion by innate immune cells such as dendritic cells, monocytes, and macrophages can easily be studied using FluoroSpot. For instance, the assay can be useful for **delineating distinct subpopulations** based on their cytokine profile.



Human IL-6/TNF-α dual FluoroSpot

The images show a dual FluoroSpot analysis of cells secreting IL-6 and/or TNF-α in response to the TLR7/8 ligand R848. Dual-secreting cells were confirmed as spots with the same position in an overlay of the 490 (IL-6) and 550 (TNF-α) images. Almost all IL-6-secreting cells also secreted TNF-α.

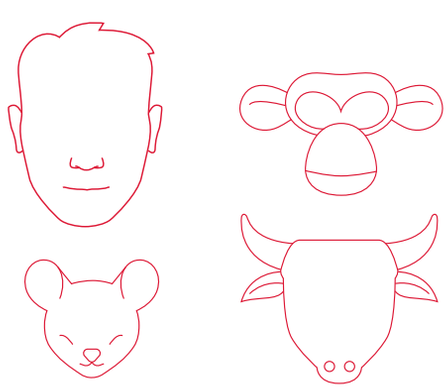
Analysis

An automated reader with separate filters is needed for analysis.

The reader should be equipped with filters for excitation (ex) 490 nm/emission (em) 510 nm (FITC), ex 550 nm/em 570 nm (Cy3), ex 640 nm/em 660 nm (Cy5) and for four-color analysis also ex 380 nm/em 430 nm (DAPI).

Mabtech IRIS™ FluoroSpot/ELISpot reader utilizes RAWspot™ technology for accurate identification of spot centers and spot numbers. In addition, it provides information on relative spot volume.

Mabtech IRIS™



Mabtech develops and supplies FluoroSpot reagents for **human**, **cow**, **monkey**, and **mouse** research.

Mabtech FluoroSpot kits

Human

ANALYTE(S)	AVAILABLE FLUOROSPOT KIT FORMATS
1-color (for Cy3-eq filter)	
GM-CSF	Basic
Granzyme B	Basic
IFN-γ	Basic
IL-1β	Basic
IL-2	Basic
IL-3	
IL-5	Basic
IL-6	Basic
IL-8 (CXCL8)	Basic
IL-10	Basic
IL-12/-23 (p40)	Basic
IL-13	Basic
IL-17A	Basic
IL-22	Basic
TNF-α	Basic
2-color (for FITC- and Cy3-eq filters)	
IL-1β/GM-CSF	Non-coated
IL-1β/IL-6	Non-coated
IL-1β/IL-12/-23	Non-coated
IL-1β/TNF-α	Non-coated
IL-6/GM-CSF	Non-coated
IL-6/IL-12/-23	Non-coated
IL-6/TNF-α	Non-coated
IL-17A/IL-22	Non-coated
IgG/IgA	Non-coated
IgG/IgM	Non-coated
IFN-γ/Granzyme B	Non-coated, pre-coated
IFN-γ/IL-2	Non-coated, pre-coated
IFN-γ/IL-5	Non-coated, pre-coated
IFN-γ/IL-10	Non-coated, pre-coated
IFN-γ/IL-13	Non-coated, pre-coated
IFN-γ/IL-17A	Non-coated
IFN-γ/IL-22	Non-coated
IFN-γ/TNF-α	Pre-coated
TNF-α/GM-CSF	Non-coated
TNF-α/IL-12/-23	Non-coated
3-color (for FITC-, Cy3- and Cy5-eq filters)	
IL-6/IL-1β/TNF-α	Pre-coated
IFN-γ/Granzyme B/IL-2	Pre-coated
IFN-γ/Granzyme B/TNF-α	Pre-coated
IFN-γ/IL-10/Granzyme B	Pre-coated
IFN-γ/IL-10/IL-2	Pre-coated
IFN-γ/IL-10/IL-17A	Pre-coated
IFN-γ/IL-22/IL-17A	Pre-coated
IFN-γ/IL-2/TNF-α	Pre-coated
IFN-γ/IL-17A/IL-5	Pre-coated
IFN-γ/IL-10/IL-5	Pre-coated
IgG/IgA/IgM	Non-coated
4-color (for FITC-, Cy3-, Cy5 and DAPI-eq filters)	
IL-6/IL-1β/IL-10/TNF-α	Pre-coated
IL-22/IFN-γ/IL-10/IL-17A	Pre-coated
IL-22/IFN-γ/IL-5/IL-17A	Pre-coated

Monkey

ANALYTE	AVAILABLE FLUOROSPOT KIT FORMATS
1-color (For Cy3-eq filter)	
GM-CSF	Basic
IFN-γ	Basic
IL-2	Basic
IL-5	Basic
IL-6	Basic
IL-8 (CXCL8)	Basic
IL-12/-23 (p40)	Basic
TNF-α	Basic
2-color (For FITC- and Cy3-eq filters)	
IFN-γ/IL-2	Non-coated, pre-coated
IgG/IgA	Non-coated
IgG/IgM	Non-coated
3-color (For FITC-, Cy3- and Cy5-eq filters)	
IgG/IgA/IgM	Non-coated

Mouse

1-color (For Cy3-eq filter)	
IFN-γ	Basic
IL-2	Basic
IL-5	Basic
IL-10	Basic
IL-17A	Basic
2-color (For FITC- and Cy3-eq filters)	
IFN-γ/IL-2	Non-coated
IFN-γ/IL-5	Non-coated
IFN-γ/IL-10	Pre-coated
IFN-γ/IL-17A	Non-coated
3-color (For FITC-, Cy3- and Cy5-eq filters)	
IFN-γ/IL-10/IL-5	Pre-coated
IFN-γ/IL-17A/IL-5	Pre-coated
4-color (For FITC-, Cy3-, Cy5 and DAPI-eq filters)	
IgG subclasses	Non-coated

Cow

1-color (For Cy3-eq filter)	
IFN-γ	Basic
IL-2	Basic
IL-8 (CXCL8)	Basic
2-color (For FITC- and Cy3-eq filters)	
IFN-γ/IL-2	Non-coated

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. Because of our strong focus on research and continued efforts to optimize ELISpot and FluoroSpot, Mabtech has been a world leader in this field for many years. Our close international collaboration with both academia and industry is leading the way for future developments to help the research community achieve optimal results.

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