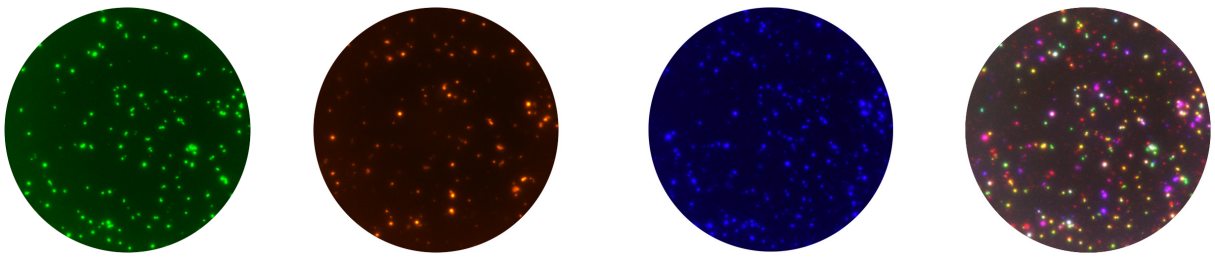
Discover more with **FluoroSpot**

FluoroSpot combines the sensitivity of ELISpot with the capacity to analyze secretion of several analytes simultaneously. This highly sensitive cellular assay is robust, easy to perform, and suitable for both single tests and large-scale screening. Mabtech provides FluoroSpot reagents for detection of a single analyte as well as dual and triple FluoroSpot kits for combined detection of two or three analytes.

- A fluorescence-based system enables **simultaneous detection** of multiple analytes
- FluoroSpot generates **more data**, saves time, and enables the use of fewer cells and less antigen compared to ELISpot



IFN-γ | IL-2 | TNF-α | Overlay

Triple FluoroSpot analysis enables studies of polyfunctional T-cell populations with different cytokine secretion profiles. In total, seven subpopulations can be visualized with triple FluoroSpot: three single secretors, three dual secretors, and one triple-secreting population. The images show a triple FluoroSpot analysis of IFN-γ (FITC), IL-2 (Cy3), and TNF-α (Cy5, color substituted) secretion by human PBMCs stimulated with a pool of viral peptides (CEF). Individual analyte images from the same well and an image overlay, combining images from the three filters, are shown.

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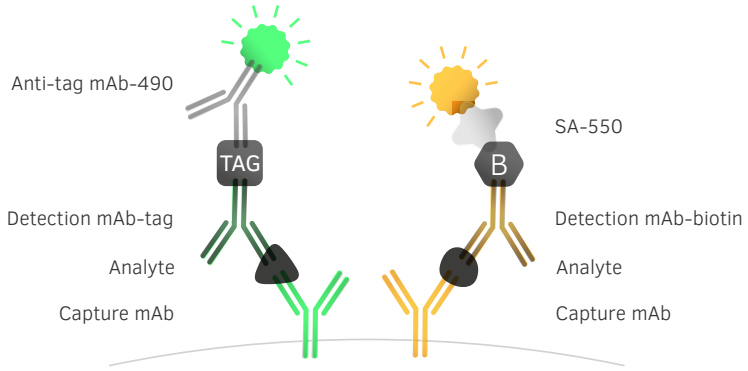
Discover more with FluoroSpot

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- or triple-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.

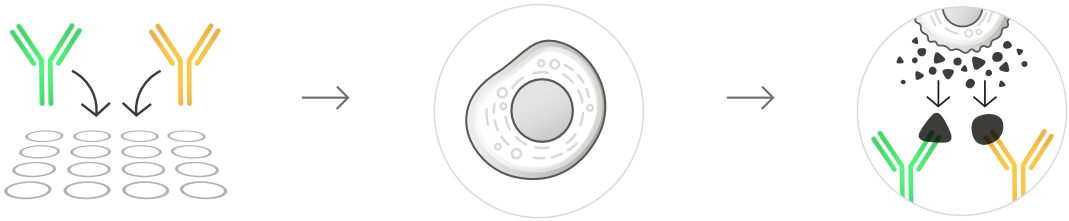


Fluorophore	Filter
490	FITC
550	Cy3
640	Cy5

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

FluoroSpot step-by-step guide

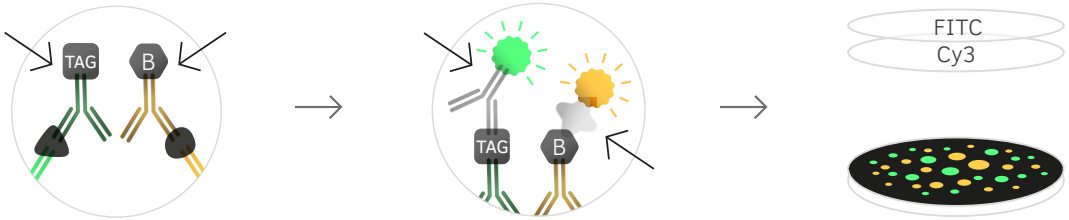
Step-by-step guide to the FluoroSpot assay, demonstrated here by a dual FluoroSpot setup.



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 or absence 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.

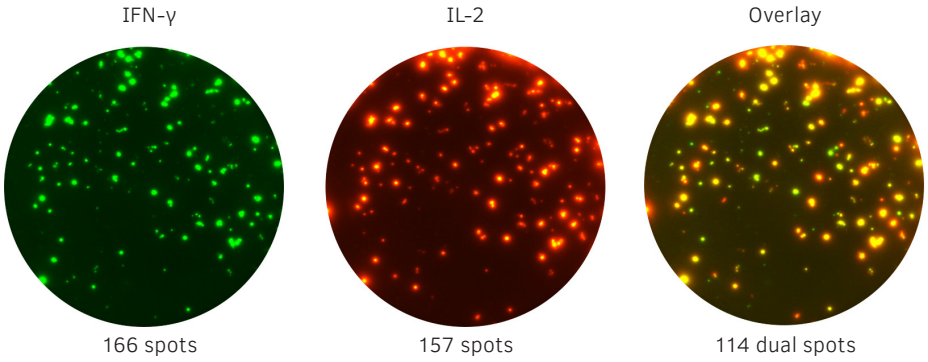
Fluorophore-labeled conjugates
A mixture of fluorophore-labeled anti-tag antibody and streptavidin-fluorophore conjugate is added.

Analysis
The plate is analyzed in a fluorescence 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 single-, dual-, and triple-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. We supply a wide range of FluoroSpot kits for analysis of single-, dual-, and triple-producing T cells where **IFN-γ** can be analyzed alone or in combination with several different analytes.

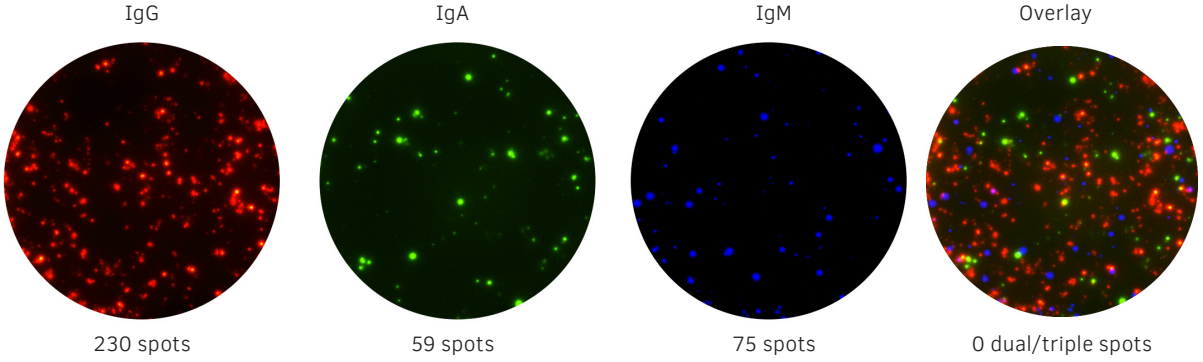


Human IFN-γ/IL-2 dual FluoroSpot
The images show a dual FluoroSpot analysis of cells secreting IFN-γ and/or IL-2 in response to PPD (purified protein derivative). Of the 166 IFN-γ-secreting T cells, 114 also secreted IL-2. Dual-secreting cells were determined as spots with the same position (center point) in an image overlay of the FITC (IFN-γ) and Cy3 (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. Mabtech offers dual FluoroSpot kits for detection of human **IgG/IgA** and **IgG/IgM** and a triple FluoroSpot kit for simultaneous detection of **IgG/IgA/IgM**.

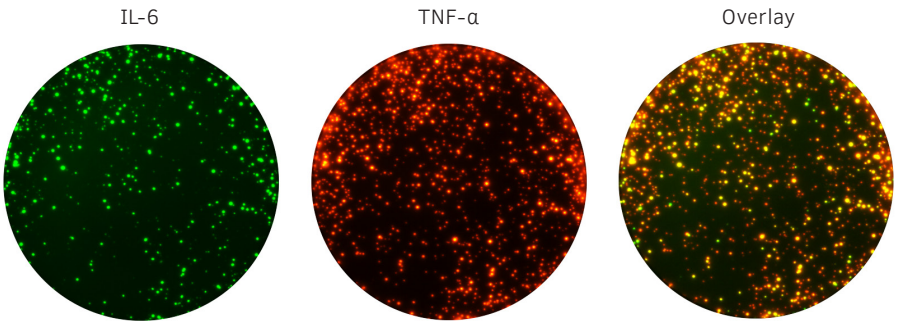


Human IgG/IgA/IgM triple FluoroSpot
In the images shown, PBMCs were pre-incubated in the presence of R848 and recombinant human IL-2, added to a FluoroSpot plate, and incubated overnight. The number of cells secreting IgG (Cy3), IgA (FITC) and IgM (Cy5, color substituted) was analyzed.

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.



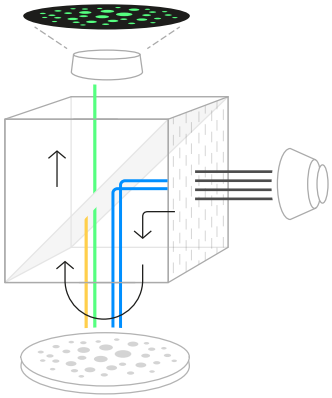
Human IL-6/TNF-α dual FluoroSpot
The images show a dual FluoroSpot analysis of cells secreting IL-6 and/or TNF-α in response to the TLR7/8 ligand R848. Dual-secreting cells were confirmed as spots with the same position in an overlay of the FITC (IL-6) and Cy3 (TNF-α) images. Almost all IL-6-secreting cells also secreted TNF-α.

Analysis

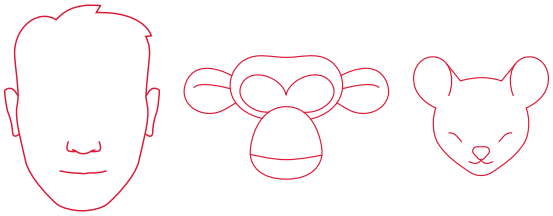
FluoroSpot analysis is performed with an automated reader equipped with specific filters for different fluorophores.

For a triple-analyte analysis, filters for FITC (490/510 nm), Cy3 (550/570 nm), and Cy5 (640/660 nm) are required. Separate images are created from each filter and the spots counted by the automated reader.

Each spot is assigned a coordinate. Based on these coordinates, spots from dual- and triple-secreting cells can be analyzed and visualized in an image overlay.



The principle of FluoroSpot analysis.



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

PRODUCT	SIZE	CODE
Human FluoroSpot kits		
Single FluoroSpot (Cy3 filter required)		
Human GM-CSF FluoroSpot^{BASIC} (550)	for 4 plates	FS1-15-550
Human Granzyme B FluoroSpot^{BASIC} (550)	for 4 plates	FS1-10-550
Human IFN-γ FluoroSpot^{BASIC} (550)	for 4 plates	FS1-01-550
Human IL-1β FluoroSpot^{BASIC} (550)	for 4 plates	FS1-12-550
Human IL-2 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-02-550
Human IL-5 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-08-550
Human IL-6 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-13-550
Human IL-10 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-07-550
Human IL-12/-23 (p40) FluoroSpot^{BASIC} (550)	for 4 plates	FS1-14-550
Human IL-13 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-04-550
Human IL-17A FluoroSpot^{BASIC} (550)	for 4 plates	FS1-03-550
Human IL-22 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-18-550
Human TNF-α FluoroSpot^{BASIC} (550)	for 4 plates	FS1-09-550
Dual FluoroSpot (FITC and Cy3 filters required)		
Human IL-1β/GM-CSF FluoroSpot kit	2 or 10 plates	FS-1215-**2/10**
Human IL-1β/IL-6 FluoroSpot kit	2 or 10 plates	FS-1213-**2/10**
Human IL-1β/ IL-12/-23 (p40) FluoroSpot kit	2 or 10 plates	FS-1214-**2/10**
Human IL-1β/TNF-α FluoroSpot kit	2 or 10 plates	FS-1209-**2/10**
Human IL-6/GM-CSF FluoroSpot kit	2 or 10 plates	FS-1315-**2/10**
Human IL-6/ IL-12/-23 (p40) FluoroSpot kit	2 or 10 plates	FS-1314-**2/10**
Human IL-6/TNF-α FluoroSpot kit	2 or 10 plates	FS-1309-**2/10**
Human IL-17A/IL-22 FluoroSpot kit	2 or 10 plates	FS-0318-**2/10**
Human IgG/IgA FluoroSpot kit	2 or 10 plates	FS-05R06G-**2/10**
Human IgG/IgM FluoroSpot kit	2 or 10 plates	FS-05R17G-**2/10**
Human IFN-γ/Granzyme B FluoroSpot kit	2 or 10 plates	FS-0110-**2/10**
Human IFN-γ/Granzyme B FluoroSpot kit, pre-coated	2 or 10 plates	FSP-0110-**2/10**
Human IFN-γ/IL-2 FluoroSpot kit	2 or 10 plates	FS-0102-**2/10**
Human IFN-γ/IL-2 FluoroSpot kit, pre-coated	2 or 10 plates	FSP-0102-**2/10**
Human IFN-γ/IL-5 FluoroSpot kit	2 or 10 plates	FS-0108-**2/10**
Human IFN-γ/IL-5 FluoroSpot kit, pre-coated	2 or 10 plates	FSP-0108-**2/10**
Human IFN-γ/IL-10 FluoroSpot kit	2 or 10 plates	FS-0107-**2/10**
Human IFN-γ/IL-10 FluoroSpot kit, pre-coated	2 or 10 plates	FSP-0107-**2/10**
Human IFN-γ/IL-13 FluoroSpot kit	2 or 10 plates	FS-0104-**2/10**
Human IFN-γ/IL-17A FluoroSpot kit	2 or 10 plates	FS-0103-**2/10**
Human IFN-γ/IL-22 FluoroSpot kit	2 or 10 plates	FS-0118-**2/10**
Human IFN-γ/TNF-α FluoroSpot kit, pre-coated	2 or 10 plates	FSP-0109-**2/10**
Human TNF-α/GM-CSF FluoroSpot kit	2 or 10 plates	FS-0915-**2/10**
Human TNF-α/ IL-12/-23 (p40) FluoroSpot kit	2 or 10 plates	FS-0914-**2/10**
Triple FluoroSpot (FITC, Cy3 and Cy5 filters required)		
Human IFN-γ/IL-10/IL-17A FluoroSpot kit, pre-coated	2 or 10 plates	FSP-010703-**2/10**
Human IFN-γ/IL-22/IL-17A FluoroSpot kit, pre-coated	2 or 10 plates	FSP-011803-**2/10**
Human IFN-γ/IL-2/TNF-α FluoroSpot kit, pre-coated	2 or 10 plates	FSP-010209-**2/10**
Human IFN-γ/IL-17A/IL-5 FluoroSpot kit, pre-coated	2 or 10 plates	FSP-010308-**2/10**
Human IFN-γ/IL-10/IL-5 FluoroSpot kit, pre-coated	2 or 10 plates	FSP-010708-**2/10**
Human IgG/IgA/IgM FluoroSpot kit	2 or 10 plates	FS-050617-**2/10**

See the back for a list of monkey and mouse FluoroSpot kits →

PRODUCT	SIZE	CODE
Monkey FluoroSpot kits		
Single FluoroSpot (Cy3 filter required)		
Human GM-CSF* FluoroSpot^{BASIC} (550)	for 4 plates	FS1-15-550
Human IL-5* FluoroSpot^{BASIC} (550)	for 4 plates	FS1-08-550
Human IL-6* FluoroSpot^{BASIC} (550)	for 4 plates	FS1-13-550
Human IL-12/-23 (p40)* FluoroSpot^{BASIC} (550)	for 4 plates	FS1-14-550
Human IL-13* FluoroSpot^{BASIC} (550)	for 4 plates	FS1-04-550
Human TNF-α* FluoroSpot^{BASIC} (550)	for 4 plates	FS1-09-550
Monkey IFN-γ FluoroSpot^{BASIC} (550)	for 4 plates	FS1-21-550
Monkey IL-2 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-22-550
Dual FluoroSpot (FITC and Cy3 filters required)		
Monkey IFN-γ/IL-2 FluoroSpot kit	2 or 10 plates	FS-2122-**2/10**
Monkey IgG/IgA FluoroSpot kit	2 or 10 plates	FS-05R24G-**2/10**
Human IgG/IgM* FluoroSpot kit	2 or 10 plates	FS-05R17G-**2/10**
Triple FluoroSpot (FITC, Cy3 and Cy5 filters required)		
Monkey IgG/IgA/IgM FluoroSpot kit	2 or 10 plates	FS-052417-**2/10**

* Reactivity with Human/Non-human primates

Mouse FluoroSpot kits

Single FluoroSpot (Cy3 filter required)		
Mouse IFN-γ FluoroSpot^{BASIC} (550)	for 4 plates	FS1-41-550
Mouse IL-2 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-42-550
Mouse IL-5 FluoroSpot^{BASIC} (550)	for 4 plates	FS1-43-550
Mouse IL-17A FluoroSpot^{BASIC} (550)	for 4 plates	FS1-44-550
Dual FluoroSpot (FITC and Cy3 filters required)		
Mouse IFN-γ/IL-2 FluoroSpot kit	2 or 10 plates	FS-4142-**2/10**
Mouse IFN-γ/IL-5 FluoroSpot kit	2 or 10 plates	FS-4143-**2/10**
Mouse IFN-γ/IL-17A FluoroSpot kit	2 or 10 plates	FS-4144-**2/10**

We continuously expand our product portfolio. Please visit **www.mabtech.com** for a complete pricing and product listing.

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