

Discover new paths with **FluoroSpot**

Tell the story of every cell

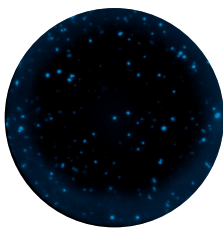
FluoroSpot visualizes the secretory profile as a spot, which is the footprint of one responding cell

Study physiologically relevant secretion

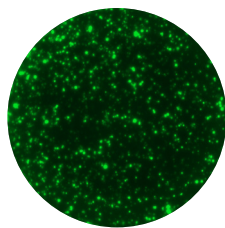
Analytes with different kinetics can be combined without manipulating intracellular processes

World leaders

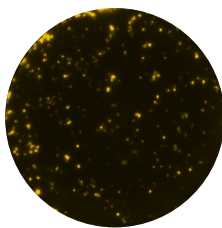
We have focused on spot analysis for over 30 years and know how to best design a FluoroSpot assay



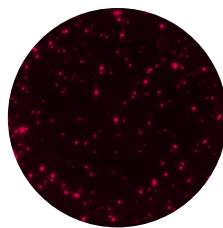
IL-22



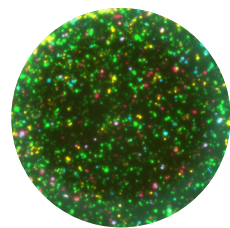
IFN- γ



IL-5



IL-17A



Overlay

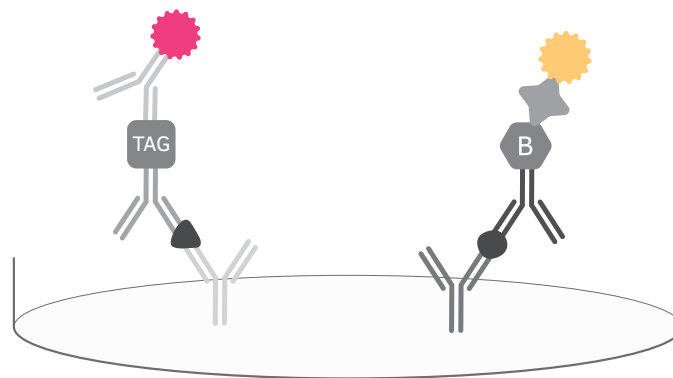
FluoroSpot assay principle

FluoroSpot combines the sensitivity of ELISpot with the capacity to study secretion of several analytes simultaneously, enabling investigations of cell populations with different functional profiles.

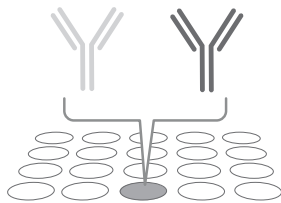
This highly sensitive cellular assay is robust, easy to perform, and suitable for both single tests and large-scale screening.

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 analytes from 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.

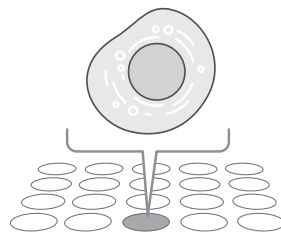


Step-by-step guide



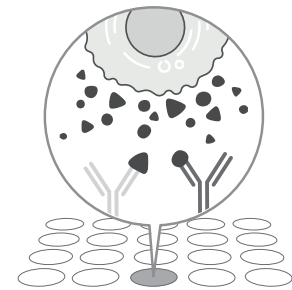
1. Coating

A mixture of capture antibodies is added to a 96-well plate.



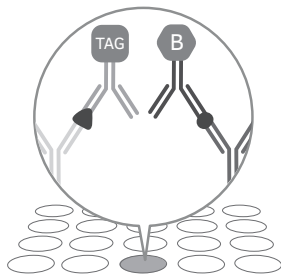
2. Cell incubation

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



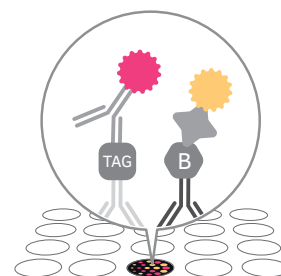
3. Analyte capture

Secreted analytes bind to the capture antibodies surrounding the activated cells.



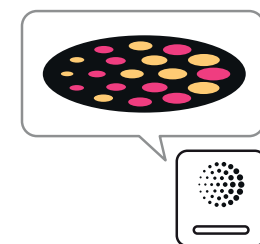
4. Detection antibodies

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



5. Fluorophore-labeled conjugates

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



6. Analysis

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

FluoroSpot kit formats

Flexible or validated, it's your choice. With FluoroSpot^{FLEX} you get to freely choose among our analytes (more than 56 000 unique combinations possible).

FluoroSpot^{PLUS} kits, on the other hand, have validated analyte combinations and pre-coated plates to save time and minimize intra-assay variability.

	FluoroSpot^{FLEX} Build your own kit	FluoroSpot^{PLUS} Validated and Pre-coated
FluoroSpot plates	Non-coated	Pre-coated
Capture mAb(s)	✓	In the pre-coated 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)**	✓	✓
Fluorescence enhancer II	✓	✓
Size	1 and 10 plates	2 and 10 plates

*Included for certain cytokine analytes

**Included for certain immunoglobulin analytes

Analysis

The reader should be equipped with filters for excitation (ex) / emission (em) wavelengths:

- ex 490 nm/em 510 nm (FITC)
- ex 550 nm/em 570 nm (Cy3)
- ex 640 nm/em 660 nm (Cy5)
- ex 380 nm/em 430 nm (DAPI)

The Mabtech IRISTM FluoroSpot/ELISpot reader utilizes RAWspotTM technology for accurate identification of spot centers and spot numbers. In addition, it provides information on relative amount of secreted analyte.



Mabtech IRISTM

Mabtech FluoroSpot^{FLEX}

Human	Monkey
GM-CSF	GM-CSF
Granzyme B	IFN- γ
IFN- γ	IgA
IgA	IgG
IgG	IgM
IgG-3	IL-2
IgG-4	IL-4
IgM	IL-5
IL-1 β	IL-6
IL-2	IL-8
IL-3	IL-12/-23 (p40)
IL-4	TNF- α
IL-5	
IL-6	
IL-8 (CXCL8)	
IL-10	
IL-12/-23 (p40)	
IL-13	
IL-17A	
IL-22	
IL-27	
TNF- α	

Mouse	Cow
IFN- γ	IFN- γ
IgG1	IL-2
IgG2a+IgG2c	IL-8 (CXCL8)
IgG2b	
IgG3	
IL-2	
IL-4	
IL-5	
IL-6	
IL-10	
IL-17A	

Mabtech FluoroSpot^{PLUS}

1-color

Human	Monkey	Mouse
IFN- γ	IFN- γ	IFN- γ

2-color

Human	Monkey	Mouse
IFN- γ /Granzyme B	IFN- γ /IL-2	IFN- γ /IL-2
IFN- γ /IL-2		IFN- γ /IL-4
IFN- γ /IL-4		IFN- γ /IL-10
IFN- γ /IL-5		
IFN- γ /IL-10		
IFN- γ /IL-13		
IFN- γ /TNF- α		

3-color

Human	Mouse
IFN- γ /Granzyme B/IL-2	IFN- γ /IL-10/IL-5
IFN- γ /Granzyme B/TNF- α	IFN- γ /IL-17A/IL-5
IFN- γ /IL-2/TNF- α	
IFN- γ /IL-10/Granzyme B	
IFN- γ /IL-10/IL-2	
IFN- γ /IL-10/IL-5	
IFN- γ /IL-10/IL-17A	
IFN- γ /IL-17A/IL-5	
IFN- γ /IL-22/IL-17A	
IL-1 β /IL-6/TNF- α	

4-color

Human
IL-22/IFN- γ /IL-5/IL-17A
IL-22/IFN- γ /IL-10/IL-17A

We continuously expand our product portfolio.

Please visit www.mabtech.com for a complete pricing and product listing.

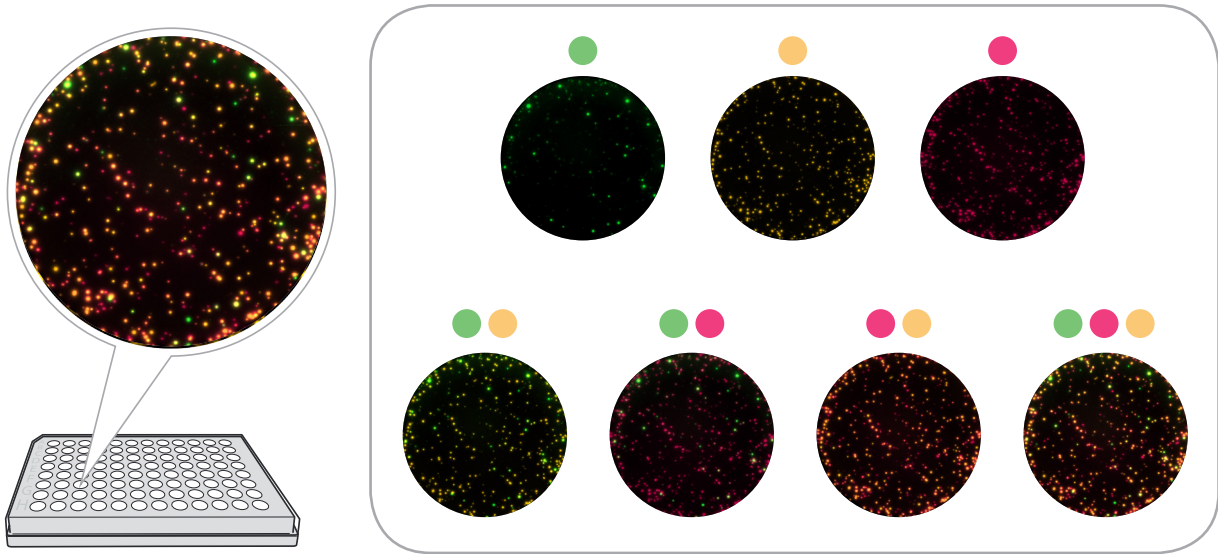
Functionality and sensitivity in one assay

FluoroSpot is ideal for delineating the functional pattern of cytokines and/or immunoglobulins as number of responding cells.

The polyfunctional profile of every cell can be assessed by e.g. a triple-color FluoroSpot assay in which seven different cell populations are explored (see image below). With a four-color

FluoroSpot assay, 15 different cell populations can be identified.

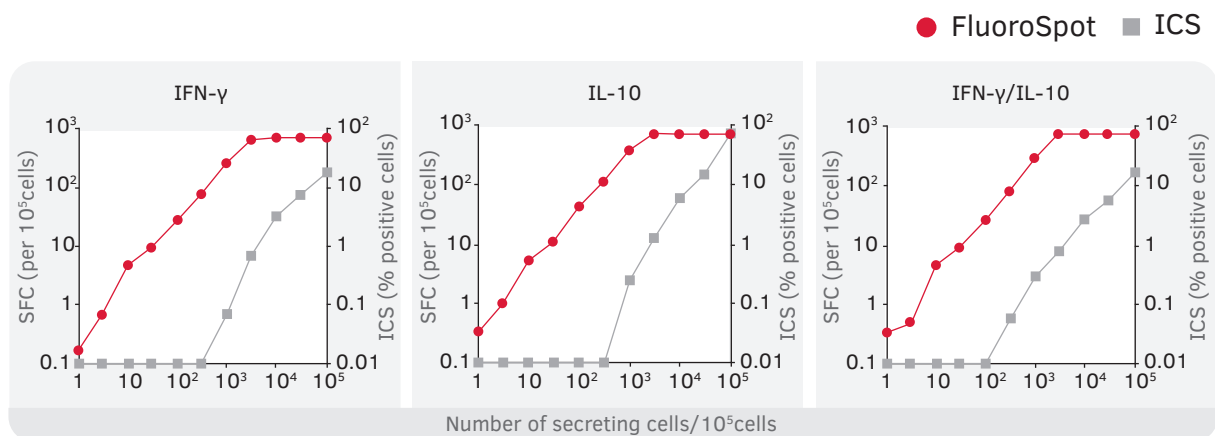
FluoroSpot is one of the most sensitive cellular assays available, up to 500 times more sensitive than intracellular cytokine staining (ICS) (see comparison graphs below). If one cell secretes the analyte, it is detected and visualized as one spot.



Seven different cell populations

A triple-color FluoroSpot assay can be used to identify: Three cell populations secreting only one analyte, three that

secrete two analytes, and one cell population secreting all three analytes.



FluoroSpot is 500 times more sensitive than ICS

To compare the sensitivity, increasing numbers of transfected CHO cells constitutively secreting IFN-γ and IL-10 were mixed with 10⁵ non-transfected cells. As seen, FluoroSpot detected cytokine secretion when as few as

10 transfected cells were added. In 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.



About Mabtech

Mabtech AB is a Swedish biotech company founded in 1986. Our mission is to aid researchers to reach new frontiers and develop novel drugs, by supplying optimal immunoassays based on high quality monoclonal antibodies and instruments.