Capture | Detect | Discover

MABTECH

Discover new paths with **FluoroSpot**

Tell the story of every cell

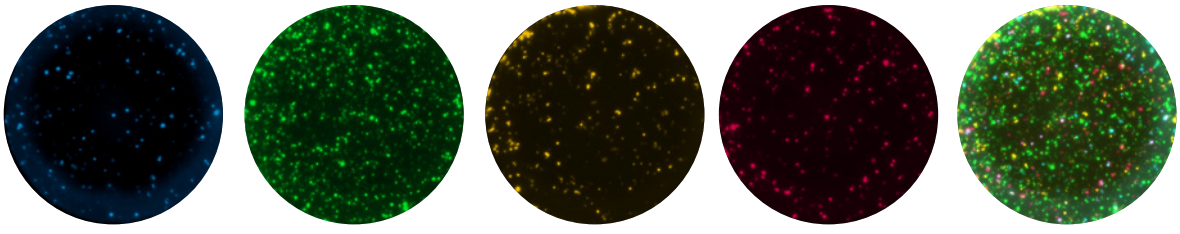
FluoroSpot visualizes the secretory profile as a spot, which is the footprint of one responding cell

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

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FluoroSpot assay principle

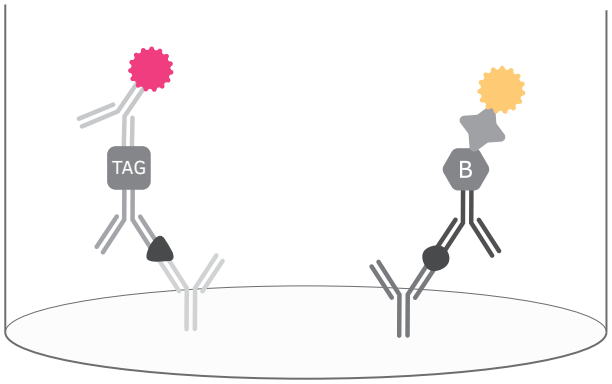
FluoroSpot combines the sensitivity of ELISpot with the capacity to study secretion of several analytes simultaneously, enabling investigation of cell populations with different functional profiles.

Proteins, for example cytokines, secreted by cells are captured immediately after secretion and throughout the stimulation process by specific antibodies.

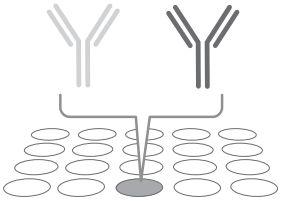
This highly sensitive cellular assay is robust, easy to perform, and suitable for both single tests and large-scale screening.

A sandwich assay principle is applied in FluoroSpot according to the step-by-step guide below.

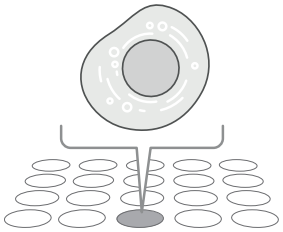
The end result is visible as a spot, where each spot corresponds to a single secreting cell.



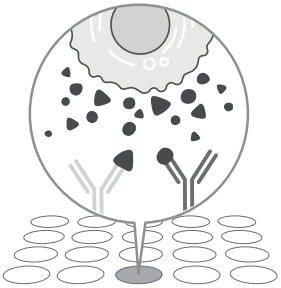
FluoroSpot step-by-step



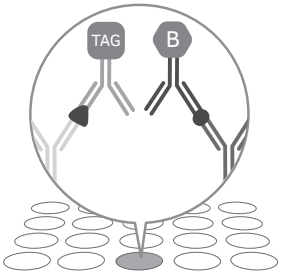
1. Coating
A mixture of monoclonal capture antibodies with different specificities is coated onto the PVDF membrane in a 96-well plate.



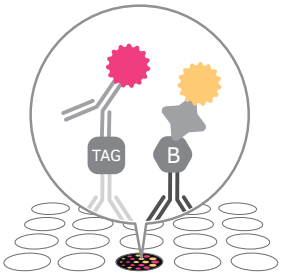
2. Cell incubation
Cells are added in the presence of stimuli and the plate is incubated to enable analyte secretion.



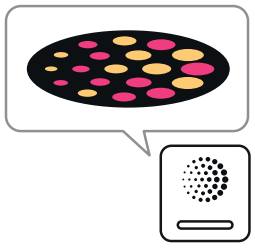
3. Analyte capture
Secreted analytes bind to the capture antibodies surrounding the activated cells.



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



5. Secondary detection
A mixture of fluorophore-labeled anti-tag antibodies 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 can select your analytes from among more than 56 000 unique possible combinations. FluoroSpot^{PLUS} kits, on the other hand, have validated analyte combinations and pre-coated plates to save time and minimize intra-assay variability. Finally, FluoroSpot Path kits include validated antigens and are designed to study the immune response to specific pathogens.

	FluoroSpot^{FLEX} Build your own kit	**FluoroSpot^{PLUS}** Pre-coated	**FluoroSpot Path** Antigen-specific
FluoroSpot plate(s)	Non-coated	Pre-coated	Pre-coated
Capture mAb(s)	√	Inside plate	Inside 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	1 plate

*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 IRIS™ FluoroSpot/ELISpot reader utilizes RAWspot™ technology for accurate identification of spot centers and spot numbers. In addition, it provides information about relative amount of secreted analyte.

Mabtech IRIS™

Mabtech FluoroSpot[FLEX]

Human

EBI3
GM-CSF
Granzyme B
IFN-α pan
IFN-γ
IgA
IgG
IgG1
IgG2
IgG3
IgG4
IgM
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-8 (CXCL8)
IL-10
IL-12 (p70)
IL-12/-23 (p40)
IL-13
IL-17A
IL-21
IL-22
IL-27
Perforin
TNF-α

Cow

IFN-γ
IL-2
IL-8 (CXCL8)

Goat

IL-4
IL-17A

Rat

IFN-γ
IL22

Monkey

GM-CSF
IFN-γ
IgA
IgG
IgM
IL-2
IL-4
IL-5
IL-6
IL-8
IL-12 (p70)
IL-12/-23 (p40)
IL-13
IL-17A
Perforin
TNF-α

Mouse

IFN-γ
IgG1
IgG2a+IgG2c
IgG2b
IgG3
IL-2
IL-4
IL-5
IL-6
IL-10
IL-17A
IL-22
TNF-α

Sheep

IFN-γ
IL-4
IL-17A

Pig

IFN-γ
IL-2
TNF-α

Chicken

IFN-γ

Mabtech FluoroSpot[PLUS]

Human

1-color

IFN-γ
IL-2

2-color

IFN-γ/Granzyme B*
IFN-γ/IL-2*
IFN-γ/IL-4
IFN-γ/IL-5
IFN-γ/IL-10
IFN-γ/IL-13
IFN-γ/TNF-α

3-color

IFN-γ/Granzyme B/IL-2
IFN-γ/Granzyme B/TNF-α
IFN-γ/IL-2/TNF-α
IFN-γ/IL-4/IL-5
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-1β/IL-6/TNF-α

4-color

IL-6/IL-1β/GM-CSF/TNF-α
IL-22/IFN-γ/IL-5/IL-17A
IL-22/IFN-γ/IL-10/IL-17A

Monkey

1-color

IFN-γ

2-color

IFN-γ/IL-2
IFN-γ/IL-17A
IFN-γ/TNF-α

3-color

IFN-γ/TNF-α/IL-2

Mouse

1-color

IFN-γ
IL-2

2-color

IFN-γ/IL-2
IFN-γ/IL-4
IFN-γ/IL-5
IFN-γ/IL-10
IFN-γ/IL-17A
IFN-γ/TNF-α

3-color

IFN-γ/IL-2/TNF-α
IFN-γ/IL-10/IL-5
IFN-γ/IL-17A/IL-5

*** FluoroSpot Path**

SARS-CoV-2 kits with peptide pools or antigen,
CMV and AdV5 kits with peptide pool,
Mtb kits with ESAT-6, CFP-10, and EspC peptide pools

We are continually expanding our product portfolio.

Please visit **www.mabtech.com** for a current list of products and prices.

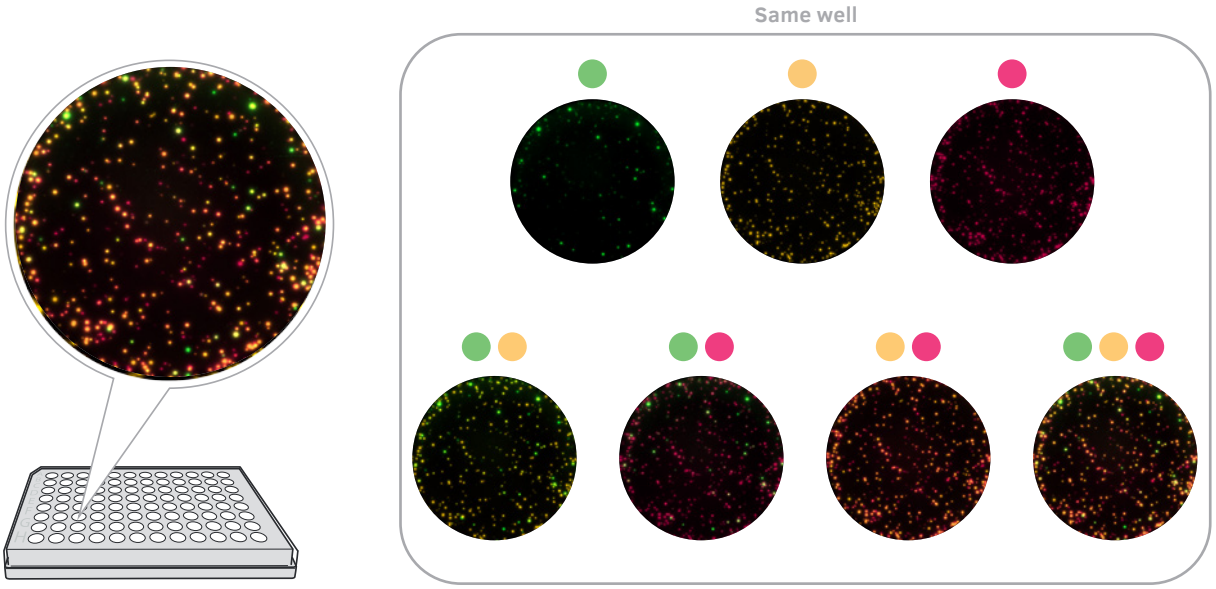
Functionality and sensitivity in one assay

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

The polyfunctional profile of every cell can be assessed for example by a three-color FluoroSpot assay in which seven different cell populations are explored in the same well (see image below).

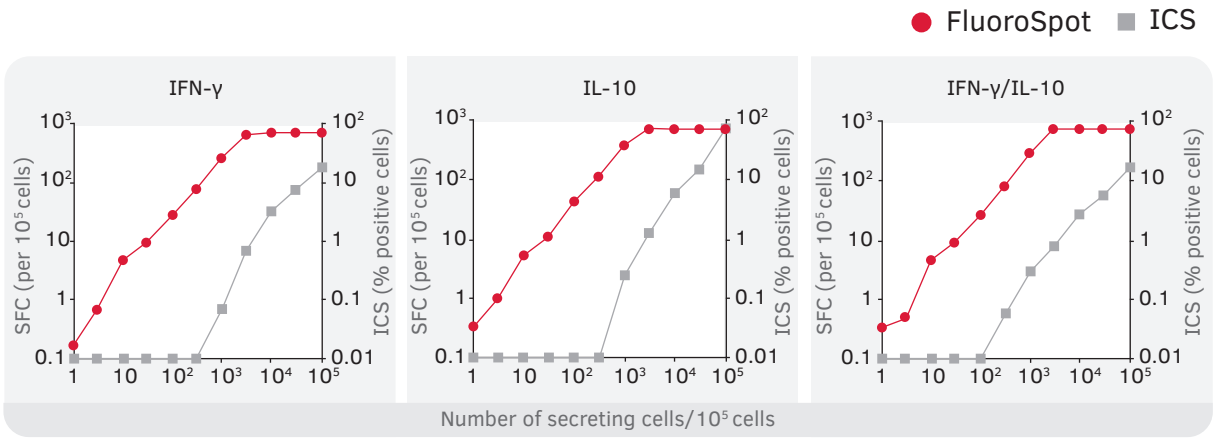
With a four-color FluoroSpot assay, 15 different cell populations can be identified.

FluoroSpot is one of the most sensitive cellular assays available; it is 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

Looking at the same well using different filters, a three-color FluoroSpot assay can be used to identify: Three cell populations secreting one analyte, three populations secreting two analytes, and one 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 10^5 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.

As seen in the graphs above, FluoroSpot could detect cytokine secretion when as few as 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)

About Mabtech

Mabtech AB is a Swedish biotech company that was 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.

Nacka 11/2021

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