Capture | Detect | Discover

MABTECH

B cell profiling with ELISpot & FluoroSpot

Extraordinarily sensitive

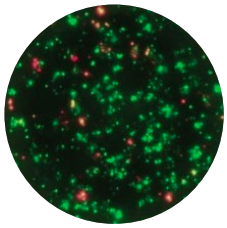
Detection levels of rare antigen-specific cells as low as one in 100,000 cells

Unravel B cell profiles

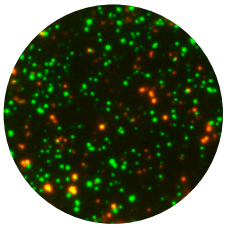
Investigate B cell properties such as antigen-specificity, isotype, and subclasses

Both ASC and memory B cell applications

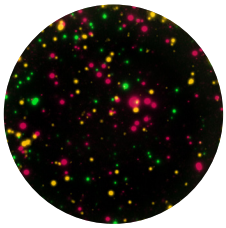
Directly assess frequency of ASCs and memory B cells in circulation



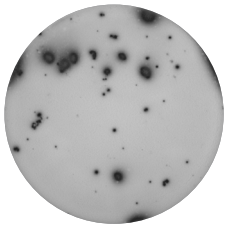
SARS-CoV-2 Spike & RBD specific IgG memory B cells



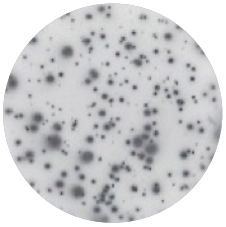
Monkey IgG/IgM ASCs



Human IgG/IgA/IgM ASCs



SARS-CoV-2 RBD specific IgG memory B cells



Mouse IgA memory B cells

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Why B cell ELISpot and FluoroSpot?

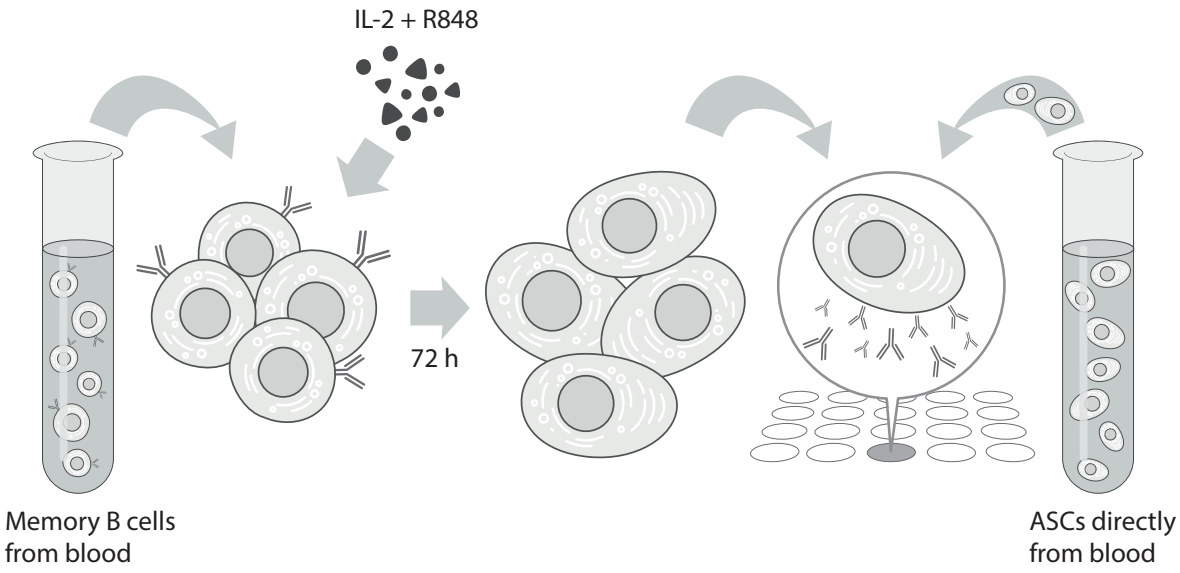
B cell ELISpot and FluoroSpot assays are powerful tools in measuring immunoglobulins directly upon secretion. Commonly, these assays are used to directly enumerate antibody-secreting cells (ASCs) and long-term memory B cells. Because of their extreme sensitivity, the methods enable identification of rare antigen-specific B cells in as few as one in 100,000 cells – a difficult challenge for other methods such as flow cytometry. The B cell FluoroSpot assay utilizes the sensitivity of ELISpot, but in a multiplex format using fluorescence for detection. This allows for analysis of multiple antigens, immunoglobulin isotypes, or subclasses in a single well.

The main applications of B cell ELISpot and FluoroSpot include the detection of B cell responses to infections and responses elicited by vaccination, but it's up to you which direction to explore!

For all your B cell needs

Whether you're investigating total B cells in circulation or rare antigen-specific cells, we've got the ELISpot/FluoroSpot kit for you. With our kits you can accurately trace antibody secretion down to the single cell level. You'll be able to determine each cell's isotype, subclass, and even antigen-specificity.

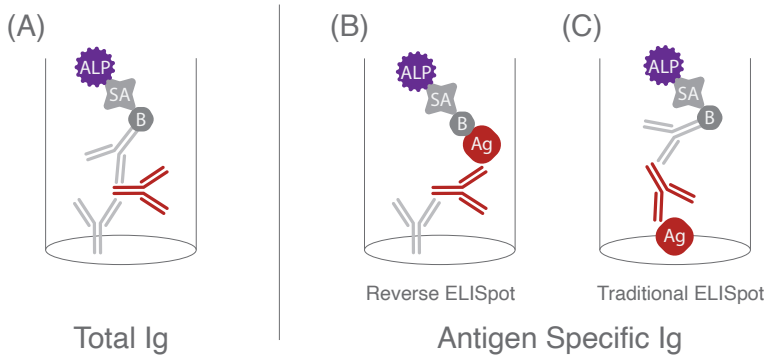
ASCs can be directly assessed with our kits from *in vivo* stimulation following vaccination or infection. Memory B cells require *ex vivo* stimulation to differentiate into ASCs for assessment with ELISpot/FluoroSpot. We've optimized and validated the process with all the required stimulation reagents (R848 + IL-2) included in our kits.



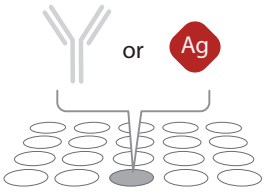
B cell ELISpot

The B cell ELISpot assay counts ASCs using a sandwich assay of capture and detection antibodies or antigen. Total Ig B cell ELISpot (A) captures and detects all ASC antibodies and is often performed in parallel to determine the frequency of antigen-specific cells. The antigen-specific assay may be performed in two different ways: either with Reverse ELISpot (B), using a tagged antigen for detection, or traditional ELISpot (C) with the antigen coated on the plate and using a detection antibody. With both total and antigen-specific ELISpot, every spot represents one single cell.

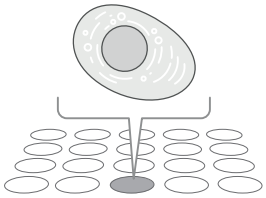
Reverse ELISpot has several advantages for detection of antigen-specific cells. It produces more distinct spots without any risk for antigen denaturation and it also dramatically reduces the amount of antigen required for each assay.



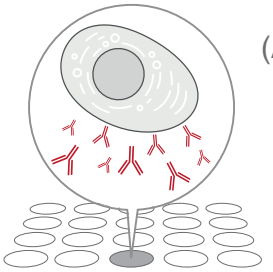
ELISpot step-by-step guide



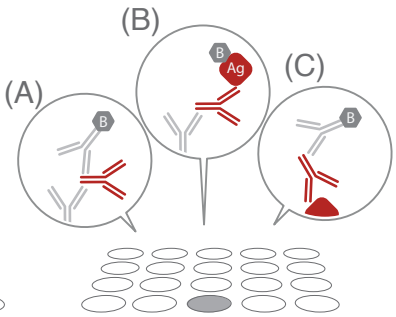
1. Coating
Monoclonal capture antibodies or antigen are added to ethanol-treated PVDF membrane plate.



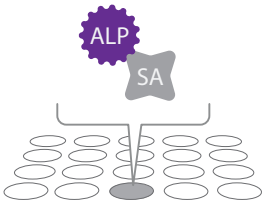
2. Cell incubation
Cells are added to wells and secrete antibodies.



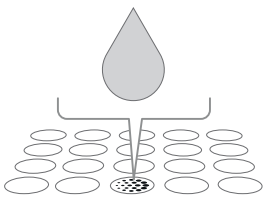
3. Antibody capture
Secreted antibodies bind to capture antibodies or bound antigen in close proximity of the cells.



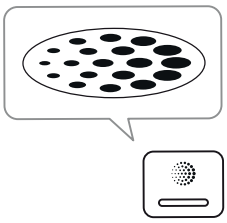
4. Detection
Cells are washed away before biotinylated detection antibodies (A, C) or tagged antigen (B) are added.



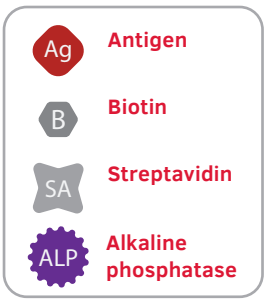
5. Streptavidin-enzyme conjugate
Addition of streptavidin-conjugate enables the formation of spots in the membrane.



6. Addition of substrate
A colorimetric substrate forms an insoluble precipitate when catalyzed by the enzyme.



7. Analysis
The result is analyzed in an automated spot reader. Each spot corresponds to a single antibody secreting cell.

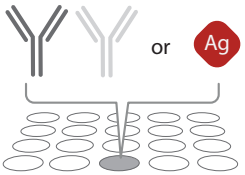


B cell FluoroSpot

FluoroSpot combines the sensitivity of ELISpot with the capacity to multiplex your assay. Several immunoglobulin isotypes, subclasses, and antigen-specificities of either memory B cells or ASCs can be assessed simultaneously utilizing fluorophore conjugated detection. This enables the analysis of unique B cell profiles from a single well.

Just like with ELISpot, the FluoroSpot assay builds on the sandwich assay, but instead of enzymatic reactions for detection, fluorophore conjugated detection antibodies or antigens are utilized to visualize B cell responses.

FluoroSpot step-by-step guide



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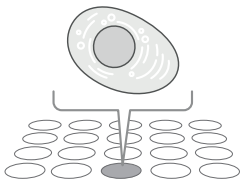

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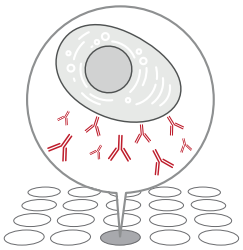

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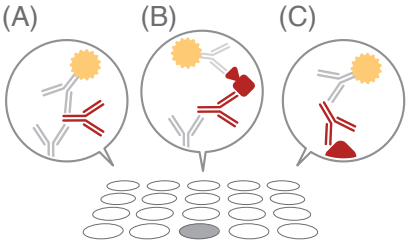
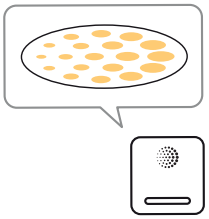


4. Detection
Cells are washed away before biotinylated detection antibodies (A, C) or antigen (B) are added.



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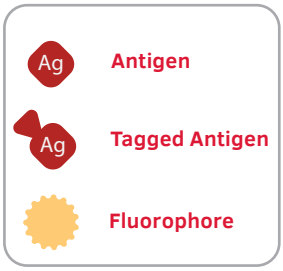
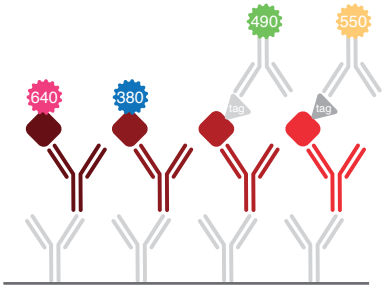
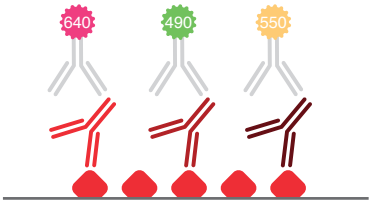
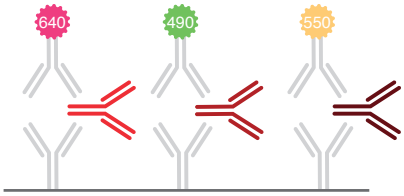


Applications



Multiple antigens
Antigens can be directly conjugated to a fluorophore or recombinantly expressed with a peptide tag (e.g. WASP, GAL) and detected with an anti-tag conjugated antibody. This allows for analysis of multiple antigens in a single well.





Multiple isotypes or subclasses

Coating with antigen or capture antibodies and detecting secreted antibodies with fluorosphore conjugated detection antibodies allows for detection of antigen-specific or total isotypes and subclasses in a single well.

B cell ELISpot[BASIC] Kits

ELISpot[BASIC] kits include capture and detection antibodies, stimuli (R848+IL-2), and streptavidin ALP or HRP conjugates. Our BASIC kits let you customize the detection of antigen-specific cells, using either antigen coating or antigen detection.

Human
IgA
IgE
IgG
IgG1
IgG2
IgG3
IgG4
IgM

Monkey
IgA
IgG
IgM

Pig
IgG

Cow
IgG

Mouse
IgA
IgE
IgE[a]
IgG
IgG1
IgG2a
IgG2b
IgG2c
IgG3
IgM

B cell FluoroSpot[FLEX] Kits

With FluoroSpot[FLEX], you have the flexibility to design your own kit including capture and detection antibodies, plates, stimuli (R848+IL-2), fluorophores, and Fluorescence Enhancer. The kits let you customize the detection of antigen-specific cells, using either antigen coating or detection.

Human
IgA
IgG
IgG3
IgG4
IgM

Monkey
IgA
IgG
IgM

Mouse
IgG1
IgG2a+IgG2c
IgG2b
IgG3

B cell ELISpot/FluoroSpot Path Kits

Path kits include validated, recombinant antigens designed to study immune responses to individual pathogens.

Human
IgG (SARS-CoV-2, RBD)
IgG (SARS-CoV-2, Spike)
IgG (SARS-CoV-2, Spike/RBD)

Analysis

ELISpot/FluoroSpot readers are used to count each individual spot in each well. FluoroSpot readers should be equipped with filters for the following excitation/emission wavelengths:

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

Our B cell ELISpot and FluoroSpot kits can be read with any ELISpot/FluoroSpot reader. But here at Mabtech, we love ELISpot and FluoroSpot so much, we made our very own! The Mabtech ASTOR ELISpot reader and IRIS FluoroSpot/ELISpot reader utilize RAWspot technology for accurate identification of spot centers and spot numbers. In addition, they provide information about the relative amount of secreted antibody - taking your readings to new heights.

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.

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