ELISpot Assays

Ultrasensitive Immunoassays for Immune Response Monitoring

ABOUT ELISPOT ASSAYS

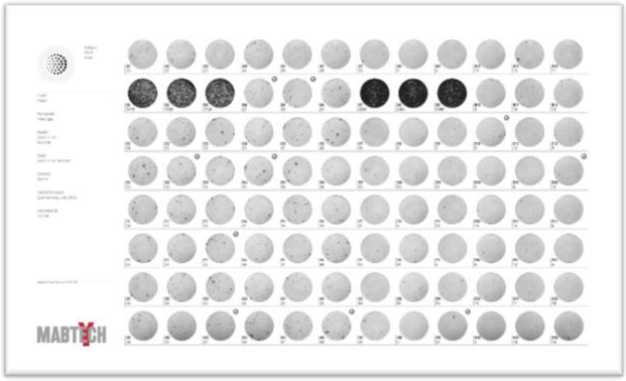
The enzyme-linked immunospot (ELISpot) assay is a refined *ex vivo* adaptation of the ELISA assay, widely utilized across cancer, infectious disease, and immunology research for both basic and translational applications. This assay enables the quantification of antibody-secreting B cells or cells that produce protein antigens, such as cytokine-secreting T cells or growth factor-secreting glial cells and macrophages. One of the ELISpot assay's most notable features is its exceptional sensitivity, which has been shown to surpass that of intracellular cytokine staining (ICS) and traditional ELISA assays by several orders of magnitude.

ELISpot can detect a single cytokine-producing cell among as many as one million bystander cells—an important capability for studies where antigen-specific T cells are typically present at low frequencies *in vivo*.

Key applications of ELISpot include monitoring immune responses in preclinical and clinical trials, stimulating immune cells *ex vivo* to assess the frequency of antigen-specific cells, and evaluating vaccine efficacy.

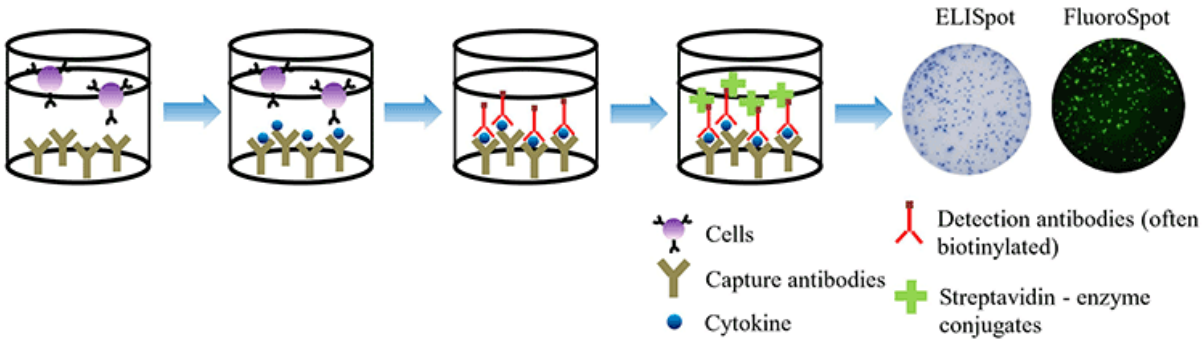
HOW DOES THE ASSAY WORK?

- ❑ Cells are cultured in the bottom of the wells (96-well plate) on a membrane surface coated with a specific capture antibody in the presence or absence of stimuli.



- ❑ Cytokines, immunoglobulins, or other target proteins secreted by the cells are captured immediately upon secretion and throughout the stimulation process. This instant capture makes ELISpot an extremely sensitive sandwich assay, down to the single-cell level.
- ❑ Cells are removed, and the secreted proteins are identified using a detection antibody. Detection reagents are added and visible spots form where each spot corresponds to an individual protein-secreting cell.
- ❑ The secreted proteins are detected either by an enzymatic reaction (precipitating product) or using a fluorescent readout.
- ❑ The analysis can be performed for a single cytokine or a combination of up to 4 cytokines (FluoroSpot).

ELISPOT/FLUOROSPOT ASSAY WORKFLOW



ASSAY BENEFITS

- ❑ More sensitive than other assays: plate-bound antibodies directly capture the secreted proteins released by the cell before they dilute, interact with receptors or are degraded by proteases.
- ❑ Allows the detection of very low frequencies of cytokine secreting cells: a single cytokine-secreting T cell can be reliably identified among a million bystander cells.
- ❑ Feasible to high throughput screening.
- ❑ Provides quantitative information regarding specific cytokines or other secreted immune molecules.

SAMPLE REQUIREMENTS

- ❑ Fresh or thawed PBMCs
- ❑ Other cell suspensions used in our assays: lymphoid, spleen, bone marrow, or CNS tissues.

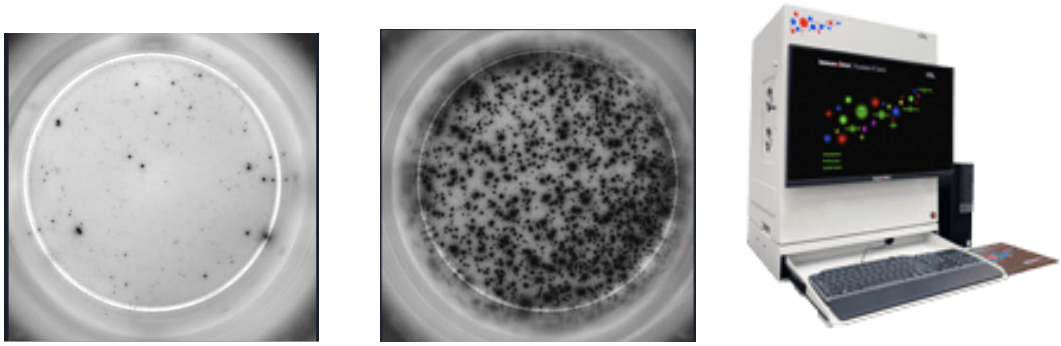


Figure 1. IL-2 production from unstimulated (left) or anti-CD3-stimulated (middle) PBMCs using a CTL ImmunoSpot® Analyzers (right)

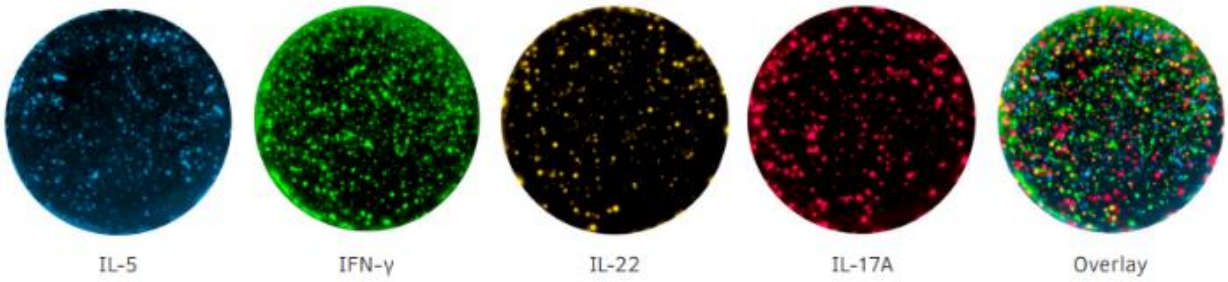


Figure 2. FluoroSpot analysis of IL-5, IFN-γ, IL-22 and IL-17A secretion by normal human PBMCs stimulated with anti-CD8 and anti-CD28 mAbs for 48h. Individual analyte images from the same well and an overlay of the four channels are shown.

SERVICE FEATURES

- ❑ Access to a large collection of PBMCs from humans and animals.
- ❑ Tailored projects with specific donor demographics are available upon request.
- ❑ We use the next generation RAWspot™ technology with Mabtech IRIS™ which has the advantage of consistent and accurate detection of every spot and robust data collection with minimal variability, even if wells are very crowded and spots appear confluent.
- ❑ RAWspot™ uses the wide dynamic range of the image RAW signal generating a 3-dimensional spot volume (RSV) that correlates with the amount of analyte secreted, in addition to accurate detection of spot centers which ensures that multiplexing is reliable.
- ❑ ELISpot and FluoroSpot assays are available for a wide range of species and analytes. Pathway specific stimuli are available in some assays.
- ❑ Extensive data analysis.
- ❑ Timely data delivery: 1-4 weeks.
- ❑ 30+ years of accumulated experience: expert data analysis and interpretation, and high quality scientific and technical support.
- ❑ Additional testing available on state-of-the-art platforms such as FlexMAP 3D, nCounter, Olink, Cytoflex S.
- ❑ qPCR for validation of any gene of interest.
- ❑ We have a library of >4000 validated antibodies to examine samples at protein levels using quantitative automatic western blot.
- ❑ We offer ~100 human cancer cell lines and many types of primary cells for testing drugs and biologicals for specific projects.

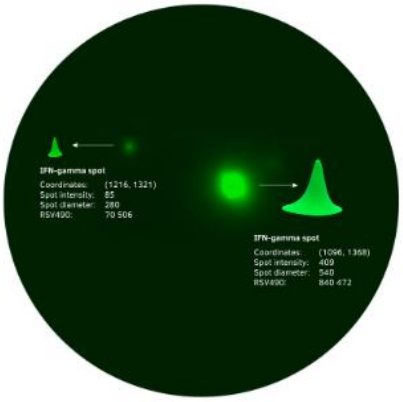


Figure 3. New data dimension with 3-dimentional spot volume (RSV) that correlates with the amount of analyte secreted.

REACTIVITY OF ELISPOT AND FLUOROSPOT TESTING

Human	Dog	Cat (Lion/Cheetah/Leopard)
Non-human primates	Horse (Rhinoceros)	Chicken
Mouse	Rat	Cotton rat
Cow	Ferret,	Hamster
Pig	Goat	Woodchuck
Sheep	Rabbit	Salmon

ANALYTE EXAMPLES

EBI3	IgG3	IL-13
GM-CSF	IgG4	IL-17A
Granzyme B	IgM	IL-17F
IFN-α	IL-1α,	IL-21
IFN-γ	IL-1β	IL-22
IgA	IL-2	IL-23
IgE	IL-3	IL-27
IgEa	IL-4	IL-29 (IFN-λ1)
IgG	IL-5	IL-31
IgG1	IL-6	IP-10 (CXCL10)
IgG2	IL-8 (CXCL8)	Perforin
IgG2a	IL-10	TNF-α
IgG2b	IL-12 (p70)	
IgG2c	IL-12/23 (p40)	