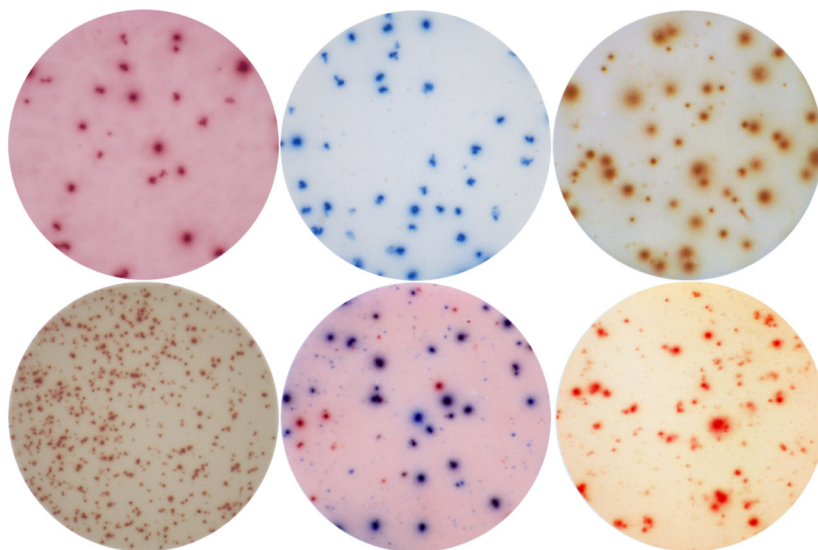


**A** major quest in the field of immune monitoring is the reliable enumeration of antigen-specific T and B cells to assess the magnitude of immunity, and define the class of the immune response by measuring the lymphocytes' secretory products. Recently, cancer immunotherapy proficiency panels compared to what extent ELISPOT, tetramer staining, and intracellular cytokine staining (ICS) would be suited to meet this goal. The authors concluded, "Only the ELISPOT assay... achieved the threshold wherein it was felt to be a practical, reliable, reproducible, and robust assay for clinical use." (Weber, et al., *Clin Cancer Res.*, 2011 17:1664-1673).

## The Unique Strengths of ELISPOT

ELISPOT is a technique that permits the visualization of individual antigen-specific T or B cells by detecting the molecules they secrete upon antigen stimulation. Unique to the technique is:

- **Exquisite sensitivity.** Optimized ELISPOT assays enable reliable detection of as few as one in a million (0.0001%) antigen-specific T or B cells within all lymphocytes – flow cytometry in comparison has a detection limit of 1 in 1,000 (0.1%).
- **Efficient cell utilization.** As few as a single cell can be tested. Typical assays using PBMC are done with 100,000 cells per test condition. Miniaturized assays can be run with 25,000 cells per test condition, requiring about one tenth of the PBMC as flow cytometry-based assays. Moreover, the cells survive the ELISPOT assay unaffected and can be reutilized for other tests.
- **Fully automated, objective analysis.** Intelligent modern



In ELISPOT assays, analytes are captured around secreting cells and visualized. For T cell assays, antigen-induced production of cytokines, such as IFN- $\gamma$ , IL-2, IL-4, IL17, or granzyme B, perforin and TRAIL can be measured. The assay permits the detection of the rare antigen-specific T cells within the PBMC plated. Spot numbers delineate the frequency of the analyte-producing cells. Spot morphologies provide insight into productivity and secretion kinetics of each analyte. ImmunoSpot® analyzers automatically recognize spots of different morphologies and apply statistics-based gating, thereby providing scientifically validated and user-independent results.

software solutions permit automated spot recognition and gating that yield instant results and eliminate

subjectivity. In contrast, analysis of flow-based assays remains manual, slow, and subjective.



- **High-throughput Suitability.**

Due to the efficient cell utilization, 10 million cells are sufficient for an assay with 400 test conditions. Automated analysis and documentation of such an assay requires less than 10 minutes.

- **GLP Suitability.** The ELISPOT assay is the most robust among cellular immune diagnostic tests and can be readily qualified and validated for regulated work.

## Why ImmunoSpot®?

CTL is the only company that has been dedicated to all aspects of the ELISPOT technology. We built the first ELISPOT reader (US patent #6,410,252) and remain committed to the continuous improvement of the instrumentation and software to meet the highest standards of basic scientists and of regulatory requirements. We were the first to qualify and validate ELISPOT assays and develop the capability of testing hundreds of samples a day in a GLP-compliant fashion. Over 100 of our publications are dedicated to establishing the scientific foundations of ELISPOT analysis. The ImmunoSpot® product line evolved as the crystallization of CTL's nearly 20 year focus on becoming **THE ELISPOT Source**.

## ImmunoSpot® Products and Services

CTL offers everything needed for maximized, standardized ELISPOT work:

- **Reference PBMC** with established high-resolution HLA-type and defined CD4 and CD8 cell reactivity to various antigens. A library of over 150 human PBMC donors is available for selection of collectives and controls with desired features.
- **Serum-free Media** permitting loss-free cryopreservation and optimized testing of PBMC, eliminating one of the largest assay variables: serum.
- **Scanning and Analysis Services** with transparent and objective counting and gating algorithms, ImmunoSpot® QC included.
- **Hands-on ELISPOT Training and Workshops** for the jumpstart of the ELISPOT technique in your facility.
- **Consultation** for sample logistics, ELISPOT assays, data analysis, standardization, high-throughput testing, GLP-compliant ELISPOT testing, and more.
- **T Cell and B Cell Test Kits** that are optimized for streamlined work flow and robust performance.

- **Visual Light and Fluorescent Analyzers** with sophisticated software for up to eight-color analysis.
- **GLP-compliant Contract Research** offering turnkey solutions from PBMC shipping and cryo-preservation to assay development, qualification, validation, and high-throughput testing.



**Even first-time users have obtained close to identical results using the ImmunoSpot® platform (Zhang, et al., *J. Immunotoxicology*, 2009, 6:227-34). Request a free ImmunoSpot T cell or B cell Test Kit sample to see for yourself how easy we've made it to get started with ELISPOT and obtain accurate results.**

