

## ELISPOT Assay

**Test Name: ELISPOT (Enzyme-Linked Immunospot)**

**Test Code(s):** Test offered for research and clinical studies only

**Background:** The Enzyme-Linked Immunospot (ELISPOT) Assay is a powerful tool for detecting and enumerating individual cells that secrete a particular biomarker of interest *ex vivo*. Originally developed for analysis of antibody secreting cells, the ELISPOT Assay has been adapted to detect cells secreting multiple biomarkers such as cytokines, chemokines, and granzymes [1-4].

**Method:** In the ELISPOT procedure a color reaction is visualized as a spot for each individual cell producing the biomarker of interest (proteins, antibodies, cytokines, etc.). The specificity and the sensitivity of ELISPOT is attained by employing high affinity capture and detection antibodies and detection with enzyme-amplification, which allows a measurement of even very low frequency biomarker-producing cells. Plates are scanned and evaluated for the number of antigen specific cells using powerful imaging system - the latest model of ELISPOT reader.

**Specimen Requirements:**

- Whole blood (8mL minimum; dependent on number of antigens requested) is collected in a Sodium Heparin tube (green top) or a Sodium Heparin Cell Preparation Tube (CPT) and maintained at ambient temperature (22 ± 5°C).
- CPT™ tubes should be centrifuged before the shipment as recommended by the manufacturer [5].
- Fresh whole blood specimens must arrive in the laboratory within 24 hours of collection, using overnight shipping with the earliest morning delivery.
- Assay can be performed using frozen viable lymphocytes. Frozen PBMC should be delivered on dry ice or in liquid nitrogen containers.
- Contact IBT for additional information

**Tests and Services Available:**

- Testing with the following challenge antigens is available: Tetanus Toxoid, CMV, VZV, PPD, *Candida Albicans*, Mumps, Dust Mite Mix, and Ragweed.
- Human IFN $\gamma$ , TNF $\alpha$ , IL-2, IL-4, IL-5, IL-10, and Granzyme assays are available as quantitative biomarkers.
- PHA (Phytohemagglutinin) and CEF (Cytomegalovirus, Epstein-Barr and Flu virus) peptide mix are available as positive controls.
- ELISPOT Recall Antigen Panel (IFN $\gamma$ ) - panel code 402942 (Tetanus toxoid, *Candida Albicans*, CMV, Mumps, VZV, and CEF peptide mix)

- Custom assay development for other antigens and biomarkers and scanning of previously developed plates are available by sponsor request.

**Units Reported:**

The number of biomarker-secreting cells per 200,000 or 300,000 PBMC is reported; customized reports including multiple parameters are available by request.

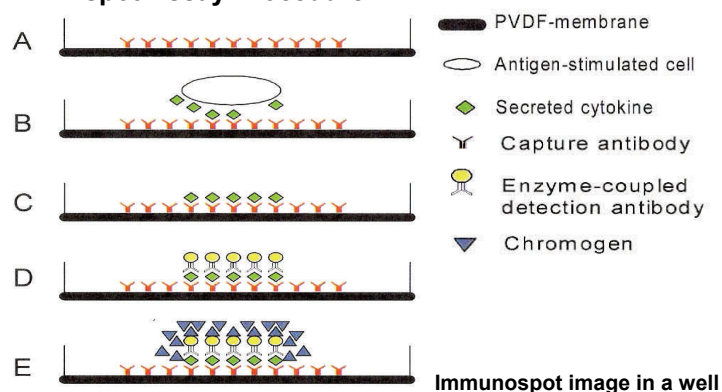
**Interpretative Comments:**

Reference ranges for each antigen / biomarker combination may be established for each project.

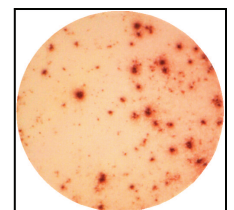
**References:**

1. Yamshchikov G., Franklin J. "Evaluation of cell-mediated immunocompetence using ELISPOT assay." Poster presentation at the iSBTc 21st Annual Meeting, October 2006, Los Angeles, California.
2. Klinman D.M., Nutman T.B., "ELISPOT Assay to Detect Cytokine Secreting Murine and Human Cells", Current Protocols in Immunology, 1994, 6.19.1.-6.19.8, John Wiley and Sons, Inc.
3. "Techniques for Immune Function Analysis", 2003, Application Handbook, BD Biosciences.
4. NCCLS. Performance of Single Cell Immune Response Assays; Approved Guideline. NCCLS document I/LA26-A (ISBN 1-56238-546-1).
5. BD Vacutainer CPT Cell Separation Tubes with Sodium Heparin, BD Biosciences, REF 362753

**Elispot Assay Procedure**



- A-Plate coated with antibodies to a biomarker
- B-Cells are stimulated with the antigen
- C-Cells washed from the plate
- D-Secondary and detection antibody applied
- E-Color reaction development



For more information, contact  
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