ELISPOT Assay

Shilpa Deshpande Kaistha
Department of Biotechnology
School of Life Sciences & Biotechnology
CSJM University Kanpur

ELISPOT

- The Enzyme Linked Immunospot technique was developed by Cecil Czerkinskdy in 1983.
- ELISpot is a technique that focuses on measuring the quantity of cytokine-secreting or antigen-specific cells.
- It is an immunostaining technique that uses antibodies to detect the protein analyte.
- Enzyme-linked immunosorbent spot (ELISpot) is a quantitative method for measuring T cell activation.
- It's a commonly used immunoassay for evaluating human clinical trials of vaccines and other forms of immunotherapy

ELISPOT versus ELISA

- Engvall and Perlmann in 1971. ELISA is a biochemical assay that is used to detect the presence of a ligand (mostly protein) by directing an antibody against it.
- ELISpot is a technique that is used to measure cytokine-secreting cells. by Cecil Czerkinsky in 1983
- An ELISPOT/FluoroSpot assay can be 100 to 400 times more sensitive than a conventional ELISA, because the secreted protein is captured directly onto the well of an ELISPOT/FluoroSpot plate before it will be diluted in the culture supernatant, degraded by proteases, or captured by receptors on adjacent cells.
- Disadvantage: ELISPOT only detects the specific cytokines being tested and does not provide direct information on the ability of antigen-specific lymphocytes to mediate other effector functions.

Protocol

- Coat the ELISPOT plate with capture antibody: ELISPOT is performed using a PVDF or nitrocellulose membrane 96-well plate pre-coated with an antibody specific to the secreted protein. .
- Add cells and stimuli to the plate: Cells are then stimulated overnight at 37°C in CO^2 incubator and the secreted protein binds to the antibody. .
- Let the antibodies capture the analyte.
- Add detection antibody:The resulting antibody complex can be detected either through enzymatic action to produce a colored substrate or with fluorescent tags. An advantage to using fluorescence is the ability to identify more than one secreted protein at a time.
- Add streptavidin-enzyme conjugate.
- Add substrate:BCIP/NBT-plus substrate, HRP conjugated Ab
- Analyze the developed plate: Dry the plates and allow the membranes to dry at room temperature
- In the analysis software, set the following parameters for measurement: Automated ELISPOT readers
  - Size/spot diameter
  - Intensity/saturation
  - Circularity/shape
  - Spot development/slope

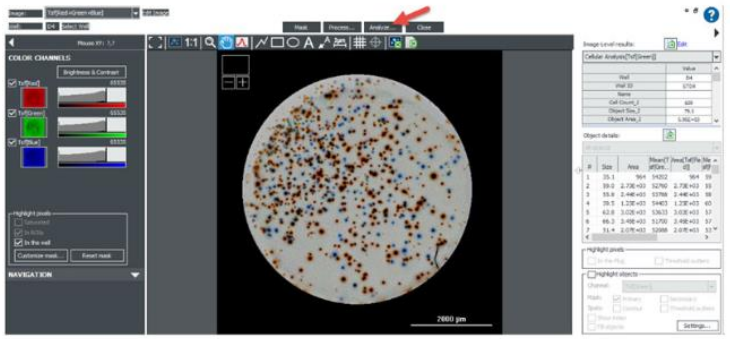
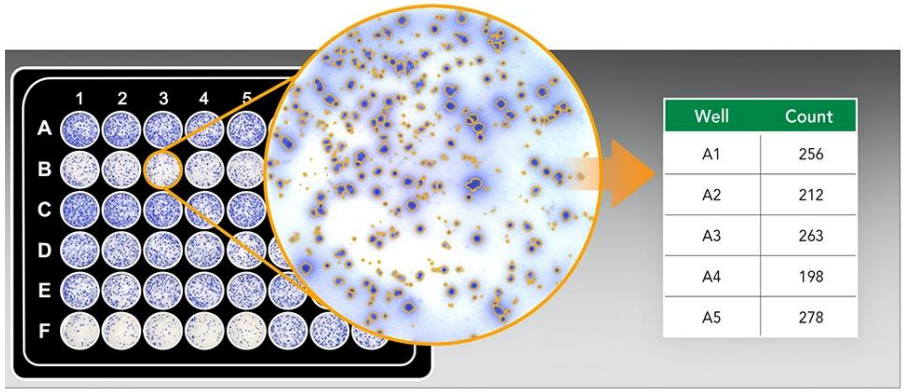
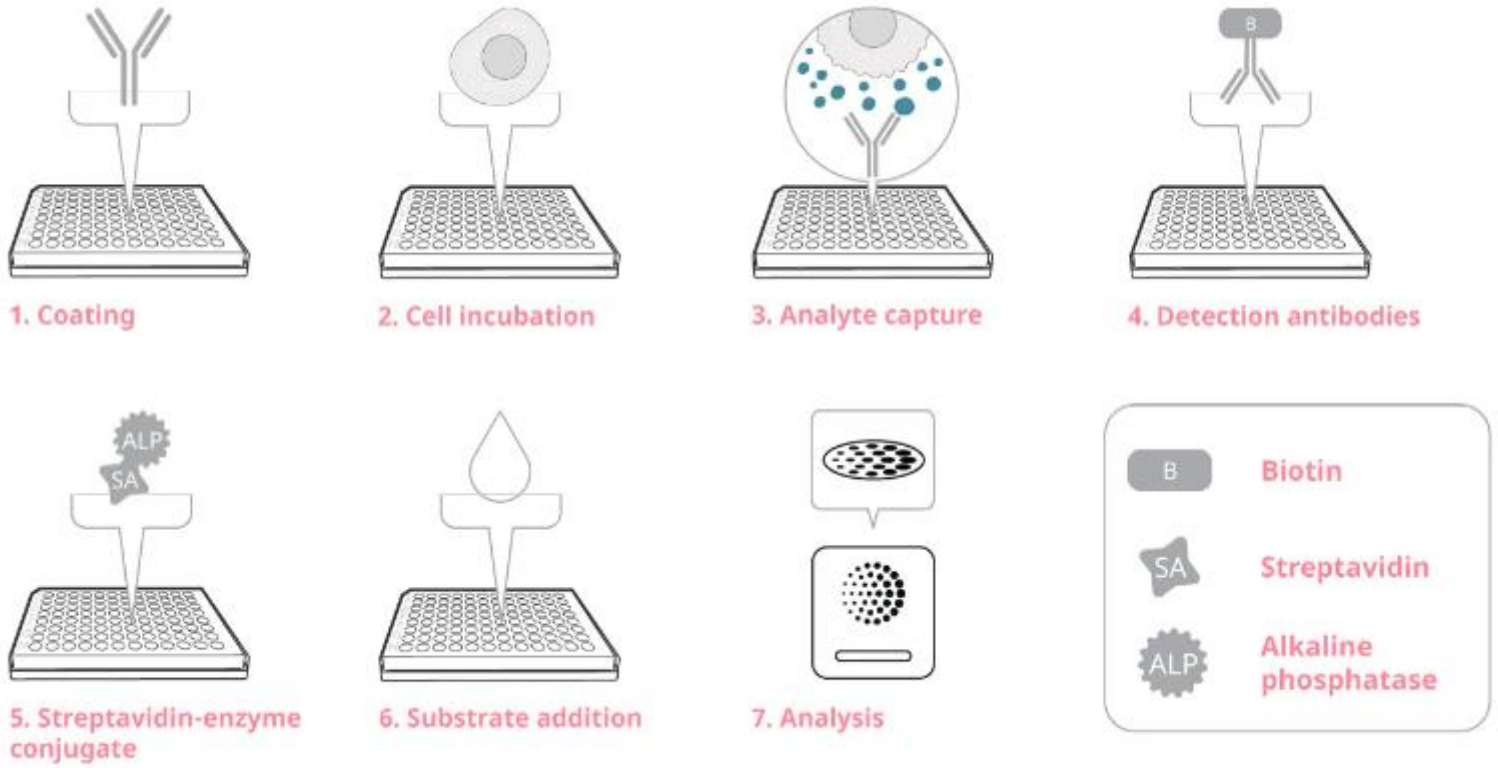


Figure 1. Gen5 image analysis window. When defining subpopulations using single-color control wells, statistics of each population, such as mean and standard deviation (right), can be used to set robust thresholds in order to define red or blue spots.

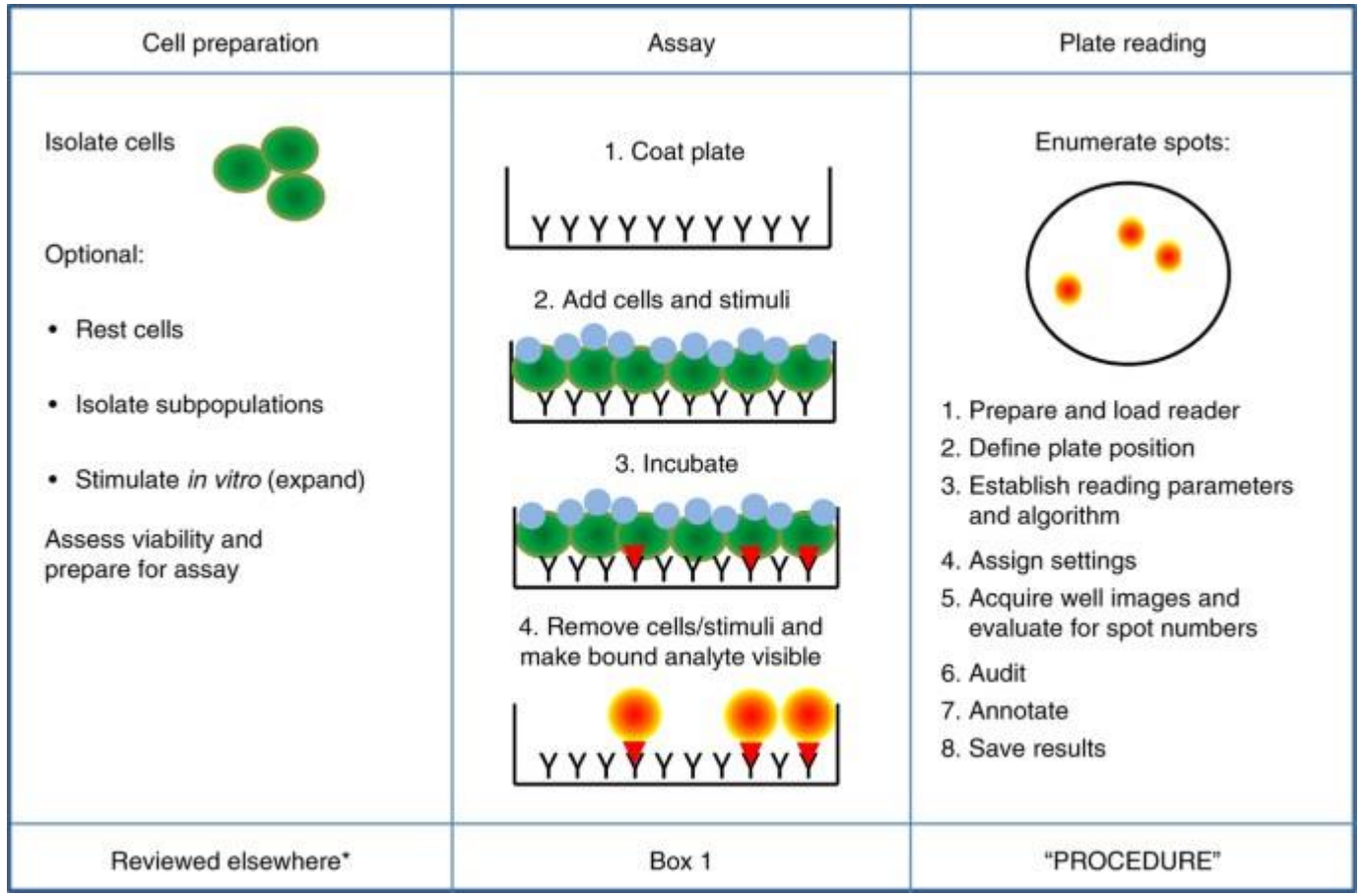


https://www.agilent.com/about/tektalk/en/newsletter-ELISpot.html

ELISpot is an immunoassay used to quantify analyte-secreting cells. Cytokines, immunoglobulins, or other target proteins secreted by cells are captured by specific antibodies immediately after secretion and throughout the stimulation process.

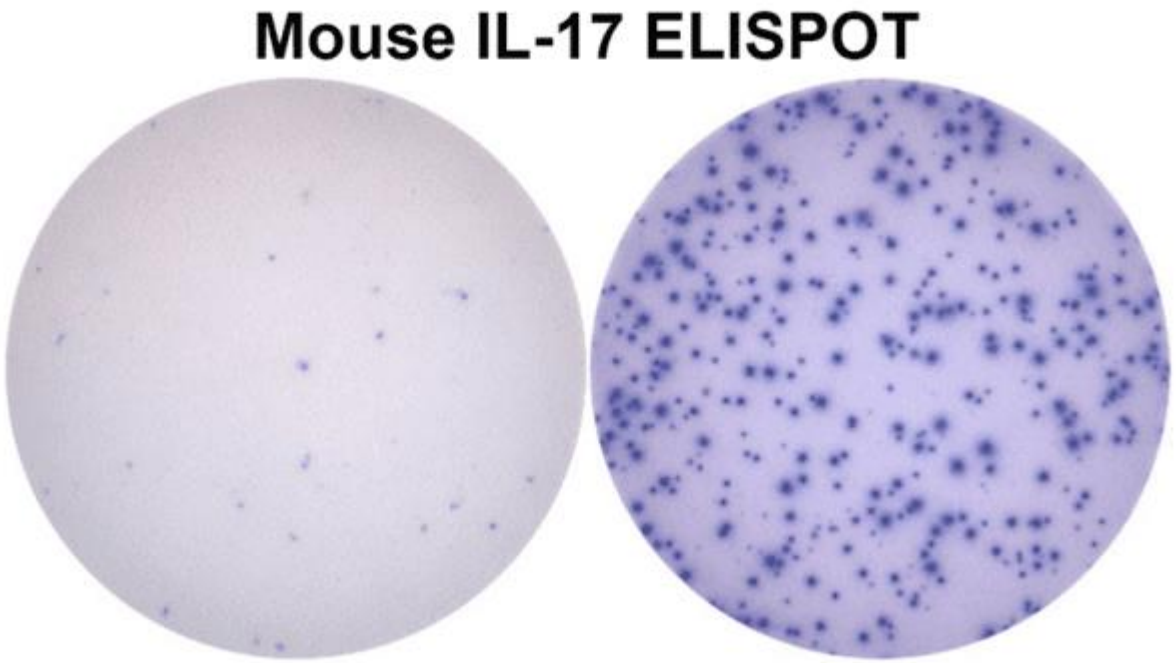


https://www.mabtech.com/knowledge-hub/step-step-guide-elispot

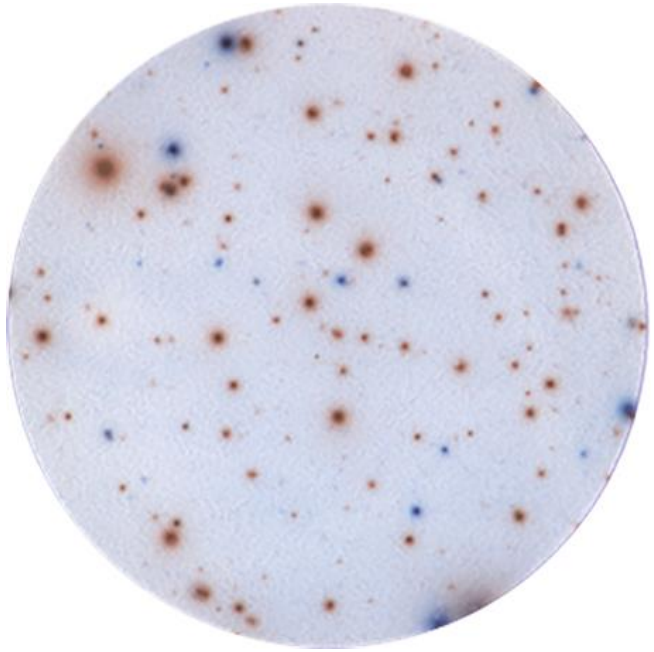


Janetzki, S., Price, L., Schroeder, H. *et al.* Guidelines for the automated evaluation of Elispot assays. *Nat Protoc* **10**, 1098–1115 (2015). https://doi.org/10.1038/nprot.2015.068

ELISPOT Image



https://immunospot.com/mouse-il-17-single-color-elispot.html



https://immunospot.com/human-ifn-gamma-il-17-double-color-elispot.html

Applications of ELISPOT

- Screening of antibody producing cells
- Serial check on immune reactions towards chemicals, and newly developed pharmaceuticals
- Optimization of anti-tumour activities of dendritic cells and T-lymphocytes
- Control of guided vaccinations, e.g. HIV
- Diagnosis and prognostic analysis of autoimmune diseases, e.g. Diabetes, Multiple Sclerosis or Rheumatoid Arthritis
- Monitoring of immune therapy
- Control of de-sensibilization treatment of allergic diseases
- Prediction of rejection crisis in organ transplantation
- Analysis of stem cell function
- Determination of transfection efficacy in gene therapy
- Measuring secretory products of low frequency tissue, tumour and immune cells