# ELISPOT assays

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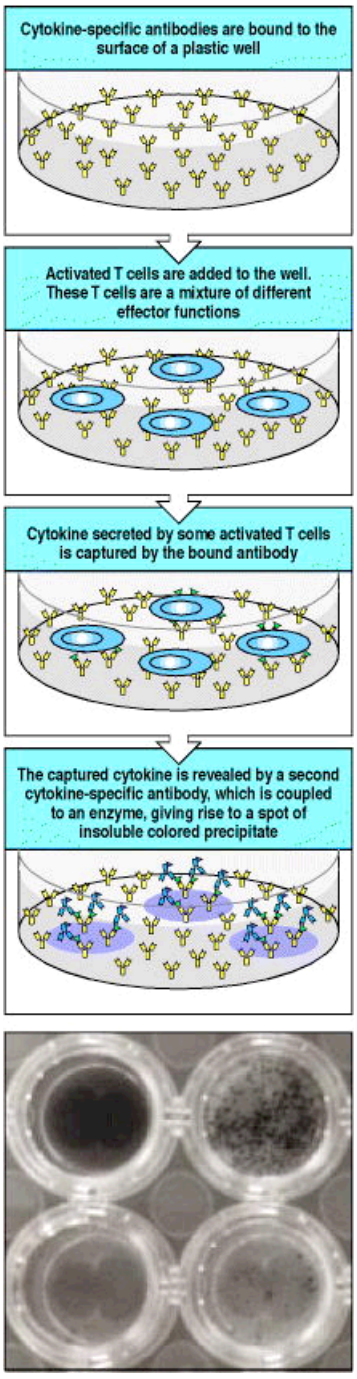
**Principle:**

- ELISPOT assays T cells on the basis of cytokine production. Cytokine secreted by individual activated T cells is immobilized as discrete spots on a plastic plate, which are counted to give the number of activated T cells.




**Objective:**

- Measure the frequency of T-cell responses
- Determination of the amount of activated antigen-specific $CD8^+$ T-cells in a given sample

**Procedure:**

- A well plate is coated with anti-cytokine capture antibodies
- Incubate T-cells in the well after stimulation with the antibody which is investigated
- Cells will secrete cytokines
- Cells are washed off
- Biotynilated antibody is added
- Avidin HRP is added
- Substrate is added and the formation of coloured spots (bottom panel in Figure A-29) is observed (entire procedure is described in Figure A-29)

¬ **Figure A-29 Immunobiology, 6/e (Garland Science 2005)**

ELISPOT is a modification of the ELISA antigen-capture assay.

ELISPOT assays are exceptionally sensitive since the product is captured directly around the secreting cell, before it is either diluted in the supernatant, captured by receptors of adjacent cells, or degraded.

On the other hand, the ELISPOT assay does not give information about the nature of the activated cells, and it can be difficult to determine whether individual cells are capable of secreting mixtures of cytokines. It was therefore important to develop assays that could make these measurements on single cells. One of the methods used for single cells measurements instead of ELSIPOT is flow cytometry.

**Example:**

From Barouch et at, Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes, Nature 415.

The figure shows the response of the T-cells samples taken from three monkeys (893, 833 and 798) at 52 weeks after infection with SHIV. The measured quantity along y-axis is the number of spots (SFC) per $10^6$ Peripheral Blood Mononuclear Cells (PBMCs). Cells were stimulated with p11C epitope (wild-type) and with p11C* (mutated epitope). The p11C epitope is the immunodominant epitope for the $CD8^+$ T-cells response upon infection with the type of SHIV used in the experiment. The response to 4 different concentrations (8μg $ml^{-1}$, 0.8μg $ml^{-1}$, 0.08μg $ml^{-1}$ and 0.008μg $ml^{-1}$) of antigens was measured for each monkey and type of antigen. Monkey 798 had almost no response to the antigens, no matter how high was the concentration used. This monkey died during the $52^{nd}$ week after infection. The other two monkeys were alive at two years upon infection. This shows that the p11C was in fact the epitope needed for the effective immune response.

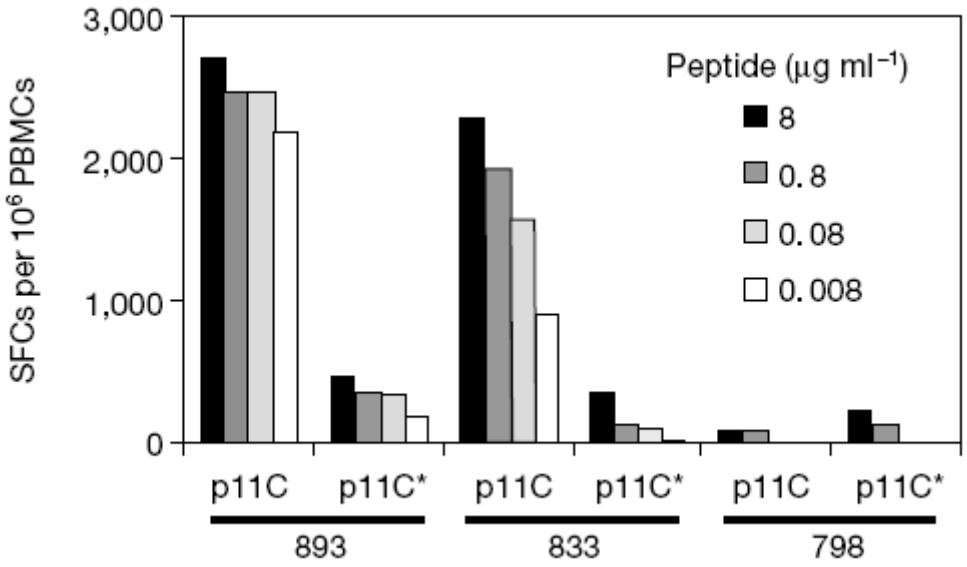



**Figure 4 B from Barouch et al**

**References:**


- Janeway, CA., Travers, P., Walport, M., Shlomchik, M., (2001). Immunobiology 5th Edition: The Immune System in Health and Disease: Appendix
- Barouch et at, Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes, Nature 415
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- http://www.elispot-analyzers.de/english/elispot-animation.html
- http://www.immunospot.com/index.htm