

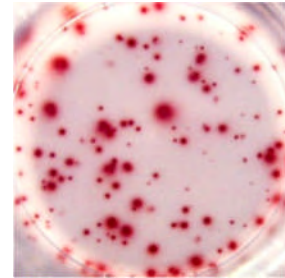


ELISPOT

ELISPOT assay

U-CyTech biosciences offers a wide range of human, monkey, mouse and rat cytokine ELISPOT kits including kits for the detection of cytokine, chemokine or granzyme producing cells such as GM-CSF, Granzyme B, IFN- γ , interleukin family members (IL-2, IL-4, IL-10, etc.) and TNF- α . These ELISPOT kits are available in different formats (2- or 5-plate kits) and can be supplied with different coloring agents (either for silver or enzymatic staining producing "black" spots or "red" spots, respectively).

The antibodies in the monkey kits kits have been validated for detecting cytokine producing cells of various Old World monkeys including barbary macaques, lion-tailed macaques, pig-tailed macaques, Sulawesi macaques, Japanese macaques, cynomolgous monkeys, Langurs, baboons and mangabeys. ELISPOT kits for other monkey cytokines will follow, including kits for Marmoset (New World Monkey species) cytokines.



Additionally, human and monkey "ELISPOT" kits have been developed for the detection of two cytokines released by a single T cell with the use of fluorescent-labeled antibodies (the so-called FluoroSpot). FluoroSpot kits for the detection of multiple cytokines are under development.

Intended use of the ELISPOT assay

The cytokine ELISPOT assay is designed to enumerate cytokine secreting cells in single cell suspensions of lymphoid tissue, CNS tissue, bone marrow or preparations of peripheral blood mononuclear cells (PBMC). The assay has the advantage of detecting only activated/memory T cells and the cytokine release can be detected at the single cell level, allowing direct determination of T cell frequencies. The high sensitivity and easy performance, allowing a direct enumeration of peptide-reactive T cells without prior *in vitro* expansion, makes the ELISPOT assay eminently well suited to monitor T cell responses.

Application

- The ELISPOT assay is an effective tool to enumerate antigen-specific T cells in the circulation of immunized humans and animals at much lower frequencies than possible with other currently available methods (1, 2).
- The ELISPOT assay has proven to be a sensitive and unique system to follow disease progression in human individuals or animals. Several studies have indicated that alterations in the frequency of cytokine producing cells in different compartments of the body adequately reflect changes in immune function (3).

- The ELISPOT assay can be used to determine effects of drugs, chemicals or other compounds on cytokine secretion *in vitro*, thereby providing data on their putative modulatory effects on immune function *in vivo* (4, 5).
- The ELISPOT assay can be used to determine the frequency of antigen-specific cytotoxic T cells (CTL) in fresh unstimulated peripheral blood lymphocytes from vaccinated non-human primates (6, 7).
- The ELISPOT assay is currently being used increasingly for the quantitative assessment of peptide reactive T lymphocytes from PBMC in infectious diseases (8, 9), in the course of vaccination trials aimed at the induction of tumor-specific T cells (10, 11), or in the assessment of immune-mediated pathogenesis of autoimmune diseases (12,13).
- The ELISPOT assay is able to monitor the stimulatory effects of candidate adjuvants on cellular immune responses to co-administered vaccine proteins (14, 15).

Brief description of the ELISPOT assay

Cells are incubated for a defined length of time in the wells of the ELISPOT plate precoated with a high-affinity monoclonal antibody to which the cytokine, produced during incubation, will bind. Subsequently, cells are lysed and debris is washed away. Areas in which the cytokine has been captured by the coating antibody are detected with a combination of biotinylated anti-cytokine detector antibodies and enzyme-labeled streptavidin or anti-biotin antibodies. The last step in the assay is the addition of a substrate yielding a colored zone ('spot'), which reveals the site of cytokine secretion. The different steps of the assay are illustrated in the ELISPOT Flow diagram.

References ELISPOT products

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Comparison of cytokine ELISpot assay formats for the detection of islet antigen autoreactive T cells. Report of the third immunology of diabetes society T-cell workshop.

J. Autoimmun. **21**:365-76 (2003). [Abstract](#)

U-CyTech products used in this study:

Human IFN- γ ELISPOT kit

Human IL-4 ELISPOT kit

Human IL-5 ELISPOT kit

Human IL-10 ELISPOT kit

Human IL-13 ELISPOT kit

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Enumeration of lymphokine-secreting cells as a quantitative measure for cellular immune responses in rhesus macaques.

J. Med. Primatol. **24**:271-81 (1995). [Abstract](#)

U-CyTech products used in this study:

Monkey IFN- γ ELISPOT kit

Monkey IL-2 ELISPOT kit

Monkey species: *Macaca mulatta*

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Discontinuation of treatment with IFN-beta leads to exacerbation of experimental autoimmune encephalomyelitis in Lewis rats. Rapid reversal of the antiproliferative activity of IFN-beta and excessive expansion of autoreactive T cells as disease promoting mechanisms.

J. Neuroimmunol. **84**:14-23 (1998). [Abstract](#)

U-CyTech products used in this study:

Rat IFN- γ ELISPOT kit

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T-cell reactivity during tapering of immunosuppression to low-dose monotherapy prednisolone in HLA-identical living-related renal transplant recipients.

Transplantation **87**:907-14 (2009). [Abstract](#)

U-CyTech products used in this study:

Human IFN- γ ELISPOT kit

Human IL-10 ELISPOT kit

Human Granzyme B ELISPOT kit

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Assessment of the inhibitory effect of immunosuppressive agents on rat T cell interferon-gamma production using an ELISPOT assay.

J. Immunol. Methods **144**:203-13 (1991). [Abstract](#)

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Rat IFN- γ ELISPOT kit

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Vaccine **27**:2830-7 (2009). [Abstract](#)

U-CyTech products used in this study:

Monkey IFN- γ ELISPOT kit

Monkey IL-4 ELISPOT kit

Monkey species: *Papio anubis*

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J. Immunol. **164**:4968-78 (2000). [Abstract](#)

U-CyTech products used in this study:

Monkey IFN- γ ELISPOT kit

Monkey species: *Macaca mulatta*

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Analysis of anthrax and plague biowarfare vaccine interactions with human monocyte-derived dendritic cells. *J. Immunol.* **175**:7235-43 (2005). [Abstract](#)

U-CyTech products used in this study:

Human IFN- γ ELISPOT kit

Human IL-2 ELISPOT kit

Human IL-4 ELISPOT kit

Human IL-13 ELISPOT kit

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A single-nucleotide synonymous mutation in the gag gene controlling human immunodeficiency virus type 1 virion production.

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Human IFN- γ ELISPOT kit

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U-CyTech products used in this study:

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Human IFN- γ ELISPOT kit

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N. Engl. J. Med. **345**:1036-40 (2001). [Abstract](#)

U-CyTech products used in this study:

Human IFN- γ ELISPOT kit

Human IL-4 ELISPOT kit

Human IL-5 ELISPOT kit

Human IL-10 ELISPOT kit

Human IL-13 ELISPOT kit

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Human IFN- γ ELISPOT

Human IL-10 ELISPOT

Human IL-13 ELISPOT

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Vaccine **20**:2296-302 (2002). [Abstract](#)

U-CyTech products used in this study:

Monkey IFN- γ ELISPOT kit

Monkey IL-2 ELISPOT kit

Monkey IL-4 ELISPOT kit

Monkey IL-13 ELISPOT kit

Monkey species: *Macaca mulatta*

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The co-administration of CpG-ODN influenced protective activity of influenza M2e vaccine.

Vaccine **27**:4320-4 (2009). [Abstract](#)

U-CyTech products used in this study:

Mouse IFN- γ ELISPOT kit