



Manufactured By: U-CyTech Biosciences



## U-CyTech's immunoassays



U-CyTech biosciences is a registered Dutch company engaged in fundamental and applied research in the field of cytokine biology. U-CyTech manufactures and sells a wide range of innovative cytokine ELISA, ELISPOT and Fluorospot systems for human, monkey and rodent use. The reagents for these systems are available in two different formats: complete kits and matched antibody pairs.

The ELISA and ELISPOT kits require high-quality reagents and this has stimulated U-CyTech to develop "in house" a wide range of highly specific monoclonal and polyclonal antibodies directed to a wide range of cytokines from different species. The validated compatibility of these antibodies with certain high-quality ancillary reagents guarantees trouble-free operation and optimal results. Additionally, the FluoroSpot assay has been designed to identify individual cells that release two different cytokines. Double-cytokine secreting cells are visualized by using two fluorescent -instead of enzyme- labeled detector antibodies.

## ELISA

U-CyTech has developed various ELISA kits for the detection of cytokines in cell culture supernatant, plasma or serum. The sandwich type ELISA from U-CyTech is a simple and sensitive assay for the determination of cytokine levels in biological fluids. The assay is conducted in antibody-coated microtiter 96-wells plate where serial dilutions with unknown concentrations of cytokines are brought into the wells. The bound cytokine is then allowed to associate with a biotinylated detector antibody and an enzyme-labeled streptavidin polymer. Subsequently, a chromogenic substrate is introduced producing a colored product that can be determined spectrophotometrically.

U-CyTech offers a wide range of ELISA kits for the detection of human and monkey cytokines, chemokines or granzymes in cell culture supernatant, plasma or serum: e.g. GM-CSF, Granzyme B, IFN- $\gamma$ , interleukin family members (IL-1 $\beta$ , IL-2, IL-4, etc.), Perforin, TNF- $\alpha$ .

The monkey ELISA kits have been validated for various Old World monkey species including rhesus macaques, barbary macaques, lion-tailed macaques, pig-tailed macaques, Sulawesi-crested macaques, Japanese macaques, cynomolgous monkeys, langurs, baboons and mangabeys. U-CyTech has also developed ELISA kits for the detection of cytokines from the Marmoset (New World monkey species), including IFN- $\gamma$ , TNF- $\alpha$  and IL-17.

Furthermore, U-CyTech offers various ELISA kits for the detection of rat (IFN- $\gamma$ , IL-4, TNF $\alpha$ ) and mouse (IFN- $\gamma$ , IL-4, IL-5, IL-10, TNF $\alpha$ ) cytokines in cell culture supernatant, plasma or serum. ELISA kits for other cytokines from rat and mouse are under development.

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## ELISPOT

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.

Cells are incubated in the wells of a 96-well ELISPOT plate precoated with a high-affinity antibody, which captures the secreted cytokine, chemokine or granzyme. Subsequently, cells are lysed and debris is washed away. Subsequently, biotinylated anti-cytokine detector antibodies are added that bind to the captured product. The addition of enzyme-labeled streptavidin or anti-biotin antibodies and a substrate results in a colored zone ('spot'). Colored spots reveals the site of cytokine secretion, representing individual secreting T cells and are identified and counted using an automated ELISPOT reader.

The 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 on polystyrene-bottomed plates (or PVDF membrane-bottomed plates) or "red" spots on PVDF membrane-bottomed plates, respectively).

The ELISPOT kits that are offered by U-CyTech are kits for the detection of human and monkey cytokine, chemokine or granzymes producing cells, including kits for GM-CSF, Granzyme B, IFN- $\gamma$ , interleukin family members (IL-1 $\beta$ , IL-2, IL-4, etc.), and TNF- $\alpha$ . The antibodies in the monkey 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, U-CyTech offers various rat (IFN- $\gamma$ , IL-4) and mouse (IFN- $\gamma$ , IL-4, IL-5, IL-10, TNF- $\alpha$ ) cytokine ELISPOT kits. ELISPOT kits for other cytokines from rat and mouse are under development.

## B cell ELISPOT

The B cell ELISPOT assay has been designed to identify and enumerate individual antibody secreting B cells in single cell suspensions of peripheral blood mononuclear cells (PBMC) of humans or monkeys. The technique is based on the same solid-phase immuno-enzymatic principle as the T cell ELISPOT. The first step in the assay is the expansion of memory B cells by polyclonal or antigenic stimulation. Expanded B cells are subsequently enumerated in the ELISPOT 96-well plate coated with anti-immunoglobulines or an antigen of interest. Next, spot forming cells are detected by labeled antibodies directed to an immunoglobulin class of choice.

Each kit contains a reagent for the stimulation of memory B cells, coating antibodies for the determination of the total number of antibody secreting cells, labelled antibodies for the detection of the immunoglobulin class of choice and coloring agents. Kits are available with different coloring agents, either for silver or enzymatic staining producing "black" and "red" spots, respectively.

## FluoroSpot

The dual-color FluoroSpot assay is a modification of the ELISPOT assay and developed for the detection of two cytokines released by a single T cell. The assay is based on the use of antibodies labelled with different fluorophores enabling the detection of T cells producing two pre-selected cytokines. The Fluorospot assay is particularly useful for the analysis of T cell subpopulations with distinct cytokine profiles. Kits are supplied with PVDF membrane-bottomed (PVDF) plates, which are specially optimized for the FluoroSpot assay.

U-CyTech offers a wide range of human and monkey cytokine FluorSpot kits including kits for the combinatorial detection of IFN- $\gamma$  and a secondary cytokine (e.g. IL-2 & IL-10). Fluorospot kits for detection of multiple cytokines are under development.