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ELISPOT antibody pair

Technical Data Sheet

10-plate and 20-plate format

For research use only.
Not for use in diagnostic or therapeutic procedures.

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This Technical Data Sheet applies for the following U-CyTech ELISPOT antibody pairs

(please find below the catalogue number of the ELISPOT antibody pair)

	Species			
Analyte	Human	Old World Monkey	Mouse	Rat
IFN-γ	CT640-10 (10-plate) CT640-20 (20-plate)	CT605-10 (10-plate) CT605-20 (20-plate) CT610-10 (10-plate) CT610-20 (20-plate)	CT655-10 (10-plate) CT655-20 (20-plate)	CT600-10 (10-plate) CT600-20 (20-plate)
IL-1ß	CT651-10 (10-plate) CT651-20 (20-plate)	CT607-10 (10-plate) CT607-20 (20-plate)		
IL-2	CT641-10 (10-plate) CT641-20 (20-plate)	CT611-10 (10-plate) CT611-20 (20-plate)	CT485-10 (10-plate) CT485-20 (20-plate)	
IL-4	CT642-10 (10-plate) CT642-20 (20-plate)	CT612-10 (10-plate) CT612-20 (20-plate)	CT657-10 (10-plate) CT657-20 (20-plate)	CT601-10 (10-plate) CT601-20 (20-plate)
IL-5	CT643-10 (10-plate) CT643-20 (20-plate)	CT613-10 (10-plate) CT613-20 (20-plate)	CT660-10 (10-plate) CT660-20 (20-plate)	
IL-6	CT644-10 (10-plate) CT644-20 (20-plate)	CT614-10 (10-plate) CT614-20 (20-plate)	CT486-10 (10-plate) CT486-20 (20-plate)	
IL-10	CT645-10 (10-plate) CT645-20 (20-plate)	CT615-10 (10-plate) CT615-20 (20-plate)	CT658-10 (10-plate) CT658-20 (20-plate)	
IL-12/23p40		CT619-10 (10-plate) CT619-20 (20-plate)		
IL-12p70	CT650-10 (10-plate) CT650-20 (20-plate)			
IL-13	CT645-10 (10-plate) CT645-20 (20-plate)	CT616-10 (10-plate) CT616-20 (20-plate)		
IL-17	CT466-10 (10-plate) CT466-20 (20-plate)	CT451-10 (10-plate) CT451-20 (20-plate)		
GM-CSF	CT638-10 (10-plate) CT638-20 (20-plate)	CT608-10 (10-plate) CT608-20 (20-plate)		
Granzyme B	CT639-10 (10-plate) CT639-20 (20-plate)			
TNF-α	CT647-10 (10-plate) CT647-20 (20-plate)	CT617-10 (10-plate) CT617-20 (20-plate)	CT661-10 (10-plate) CT661-20 (20-plate)	

Intended use

The cytokine ELISPOT (Enzyme-Linked ImmunoSPOT) assay is designed to enumerate cytokine-secreting cells in single cell suspensions of lymphoid tissue, central nerve system (CNS) tissue, bone marrow or preparations of peripheral blood mononuclear cells (PBMCs). The assay has the advantage of detecting only activated/memory T cells and has the ability to detect cytokine release in response to antigen by a single cell thereby permitting direct calculation of responder T cell frequencies. The high sensitivity and easy performance, allowing the determination of peptide-reactive T cells without prior in vitro expansion, makes the ELISPOT assay eminently well suited to monitor T cell responses. The higher sensitivity of

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ELISPOT in comparison to that of ELISA (1) or intracellular staining (2) is due to the plate-bound antibodies directly capturing the cytokine released by the cell before it is diluted in the supernatant, trapped by high-affinity receptors or degraded by proteases. The sensitivity of the assay lends itself to measurement of very low frequencies of cytokine-secreting cells (1/300,000).

1. Tanguay, S. and Killion, J.J. 1994. Lymphokine Cytokine Res. 13: 259.
2. Carter, L.L. and Swain, S.L. 1997. Curr. Opin. Immunol. 9: 177.

Brief description ELISPOT assay

Cells are incubated 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 washed away. Areas in which the cytokines have been bound are detected with a combination of biotinylated anti-cytokine detection antibodies and a conjugate. The last step in the assay is the addition of a substrate revealing the site of cytokine secretion (i.e spot formation).

The ELISPOT procedure can be preformed on either PVDF membrane- or polystyrene-bottomed plates (see below).

Contents

10-plate format

- 2 vials with anti-cytokine coating antibody supplied in lyophilized form; each vial contains sufficient antibody to coat five 96-well ELISPOT plates.
- 2 vials with biotinylated detection antibody supplied in lyophilized form; each vial contains sufficient antibody for five 96-well ELISPOT plates.

20-plate format

- 1 vial with anti-cytokine coating antibody supplied in lyophilized form; vial contains sufficient antibody to coat twenty 96-well ELISPOT plates.
- 1 vial with biotinylated detection antibody supplied in lyophilized form; vial contains sufficient antibody for twenty 96-well ELISPOT plates.

Hazard information

Components of the antibody pair are not classified as dangerous according to Regulation (EC) no. 1272/2008 and Directive 67/548/EC or 1999/45/EC and their amendments.

Please find the Material Safety Data Sheet on www.ucytech.com/manuals.

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Storage reagents

The vials with lyophilized coating and detector antibodies can be safely stored in a refrigerator for a defined length of time (expiry date indicated on the vials). After reconstitution, the reagents are stable for minimal 6 months at 4°C when kept sterile. However, it is strongly recommended to divide the reconstituted antibody preparations into small aliquots for single use. These aliquots should be stored at ≤ -20°C. Under these conditions the reagents are stable for minimal two years.

Auxiliary reagents/materials needed

General

- Sterile distilled water.
- PBS (phosphate buffered saline): home-made, filter-sterilize or autoclave.
- For washing purposes only.
- Wash buffer: PBS containing 0.05% Tween-20.
- PBS-I (Sterile and pyrogen free PBS): Invitrogen cat. no. 10010-015 is recommended.
- Culture medium: see Addendum*.
- Cell stimuli: see Addendum*.
- Pipetting devices.
- Plate washer: automated or manual, see Addendum*.
- CO_2-incubator (37°C, 100% humidity, 5% CO_2).
- Tissue culture plates for prestimulation (optional).
- Microscope or an immunospot image analyzer for spot counting.

Recommended reagents/materials for enzymatic staining on PVDF membranes

- PVDF membrane-bottomed 96-well plates (Millipore cat. no. MSIP S4510).
- Blocking: *Blocking stock solution R* (U-CyTech cat. no. CT360) or cell culture medium.
- Dilution buffer for detection antibody and conjugate: *Dilution buffer R* (U-CyTech cat. no. CT348).
- Conjugate: Streptavidin-HRP (U-CyTech cat. no. CT353).
- Substrate: AEC coloring system (U-CyTech cat. no. CT356) is recommended or can be prepared yourself as follows:
    A. AEC stock: dissolve 100 mg AEC (3-amino-9-ethyl-carbazole; Sigma cat. no. A-5754) in 10 ml DMF (N,N-Dimethylformamide; Sigma cat. no. D-4551). Caution: dispense DMF in fume hood and store solution in glassware.
    B. Prepare 0.1 M Acetate buffer: add 148 ml 0.2 M acetic acid/glacial acidic acid to 352 ml sodium acetate. Adjust volume to 1 ltr distilled water; adjust pH to 5.0.
    C. For final AEC substrate solution: add 333 µl of AEC stock to 10 ml 0.1 M Acetate buffer. Filter through 0.45 µm filter. Add 5 µl H_2O_2 (30%) and use immediately.

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Recommended reagents/materials for silver staining on two types of plates

- Transparent polystyrene-bottomed plates (NUNC cat. no. 442404 or U-CyTech cat. no. CT350) or PVDF membrane-bottomed plates (Millipore cat. no. MSIP S4510).
- Blocking: *Blocking stock solution B* (U-CyTech cat. no. CT362) for both polystyrene- and PVDF-bottomed plates or alternatively use cell culture medium.
- Dilution buffer for detection antibody and conjugate: *Dilution buffer T* (U-CyTech cat. no. CT358) for polystyrene-bottomed plates or *Dilution buffer B* (U-CyTech cat. no. CT364) for PVDF-bottomed plates.
- Conjugate: φ-labeled anti-biotin antibodies (GABA) (U-CyTech cat. no. CT353) for both types of plates.
- Substrate: Activator I and II (U-CyTech cat. no. CT355) for both types of plates.

* The accompanying Addendum ELISPOT assay contains guidelines and troubleshooting for ELISPOT analyses. The Addendum ELISPOT assay is also available on our website (www.ucytech.com) or contact U-CyTech biosciences (order@ucytech.com).

ELISPOT protocol

Note: use ELISPOT plates and reagents under aseptic conditions (e.g. use a Laminar Flow Hood or Biosafety cabinet) for the steps up to 'cell activation'.

DAY 1

When using PVDF-membrane-bottomed plates, the membrane should be prewetted with 25 µl 70% ethanol. After an incubation time of 1-2 minutes, the wells are thoroughly washed with PBS-I. Immediately thereafter a proper volume of coating antibodies is added to the wells.

Coating antibody

Reconstitute the lyophilized contents by injecting an appropriate volume (indicated on the vial) of sterile distilled water. Mix gently and allow it to stand for 2 minutes at room temperature.

Take out (aseptically) a required volume and dilute 100-fold with PBS-I. Add 50 µl/well and fill up to 100 µl with PBS-I. Cover the plate with a lid and incubate overnight at 4°C.

DAY 2

Blocking

Discard coating antibody solution. Wash wells 3x with PBS-I and add 200 µl/well Blocking solution. Cover the plate with a lid and incubate 1 h at 37°C.

Cell activation

- Discard Blocking solution. Do not wash.
- Prepare mitogen or antigen in Culture medium. Add 50 µl/well.

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- Prepare cell suspensions at different cell concentrations (e.g. 1x10^5 cells/ml to 2x10^6 cells/ml). Add 50 µl/well of each cell concentration.
- Cover the plate with a lid and incubate at 37ºC, 5-7% CO_2 and 100% humidity.
- The optimal incubation time can vary from 12 to 24 h depending on the nature of the stimulatory agents and kinetics of cytokine secretion (see Addendum ELISPOT assay).

It is advised to include a preincubation step in the procedure before the cells are transferred to the ELISPOT plate. The rationale is that exogenous proteins must be internalized, processed and presented by antigen presenting cells (APC) via MHC class II molecules to $CD4^+$ T cells. Using TT and PPD as model antigens it has been shown that a preincubation step in a tube prior to incubating the cells in the ELISPOT plate is required for optimal antigen presentation (Schmittel, A. et al. 2001. J. Immunol. Meth. 247: 17).

DAY 3

Removal of cells

Remove the bulk of cells with a vigorous 'shake-out' action and immediately fill the wells with PBS of room temperature (200 µl/well). Remove PBS by a firm 'shake-out' action. Repeat once. Thereafter wells are washed 5x with Wash buffer (see Addendum ELISPOT assay).

Detection antibody

Reconstitute the lyophilized contents by injecting an appropriate volume (indicated on the vial) of sterile distilled water. Mix gently and allow it to stand for 2 minutes at room temperature.

Take out (aseptically) a required volume and dilute 100-fold in Dilution buffer. Add 100 µl/well. Cover the plate with a lid and incubate 1 h at 37°C or overnight at 4°C.

From this step on, it is critical to wash / rinse both sides of the PVDF membrane (see Addendum ELISPOT assay).

Conjugate (Streptavidin-HRP or GABA)

Discard detection antibody solution and wash wells 5x with Wash buffer (see Addendum ELISPOT assay). Bring properly diluted conjugate solution into each well. Seal the plate with an adhesive cover slip and incubate 1 h at 37°C.

Substrate

- Discard Conjugate solution and wash wells 5x with Wash buffer (see Addendum ELISPOT assay).
- Bring a proper volume of substrate solution into each well and incubate for 20-30 minutes at room temperature in the dark.
- When clear spots have developed, stop the reaction by rinsing the wells with demineralised water.
- Air-dry the plate overnight at 37°C in the dark. Use either a microscope or an Immunospot image analyzer for spot counting.

The quality of the spots is preserved for several months (enzymatic staining) or indefinitely (silver staining) when the plate is stored at a dry place in the dark.

For further information, please visit our website (www.ucytech.com) or contact U-CyTech biosciences (info@ucytech.com).