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ELISA 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 ELISA antibody pairs

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

Analyte	Species				
	Human	Old World Monkey	New World Monkey	Mouse	Rat
IFN-γ	CT740-10 (10-plate) CT740-20 (20-plate)	CT710-10 (10-plate) CT710-20 (20-plate)	CT770-10 (10-plate) CT770-20 (20-plate)	CT755-10 (10-plate) CT755-20 (20-plate)	CT700-10 (10-plate) CT700-20 (20-plate)
IL-1ß	CT576-10 (10-plate) CT576-20 (20-plate)	CT708-10 (10-plate) CT708-20 (20-plate)			
IL-2	CT741-10 (10-plate) CT741-20 (20-plate)	CT711-10 (10-plate) CT711-20 (20-plate)	CT774-10 (10-plate) CT774-20 (20-plate)	CT762-10 (10-plate) CT762-20 (20-plate)	
IL-4	CT742-10 (10-plate) CT742-20 (20-plate)	CT712-10 (10-plate) CT712-20 (20-plate)		CT757-10 (10-plate) CT757-20 (20-plate)	CT702-10 (10-plate) CT702-20 (20-plate)
IL-5	CT743-10 (10-plate) CT743-20 (20-plate)	CT713-10 (10-plate) CT713-20 (20-plate)		CT764-10 (10-plate) CT764-20 (20-plate)	
IL-6	CT744-10 (10-plate) CT744-20 (20-plate)	CT714-10 (10-plate) CT714-20 (20-plate)	CT776-10 (10-plate) CT776-20 (20-plate	CT763-10 (10-plate) CT763-20 (20-plate)	
IL-7	CT573-10 (10-plate) CT573-20 (20-plate)				
IL-8	CT748-10 (10-plate) CT748-20 (20-plate)	CT718-10 (10-plate) CT718-20 (20-plate)			
IL-10	CT745-10 (10-plate) CT745-20 (20-plate)	CT715-10 (10-plate) CT715-20 (20-plate)		CT765-10 (10-plate) CT765-20 (20-plate)	
IL-12/23p40		CT719-10 (10-plate) CT719-20 (20-plate)	CT775-10 (10-plate) CT775-20 (20-plate)		
IL-12p70	CT750-10 (10-plate) CT750-20 (20-plate)				
IL-13	CT746-10 (10-plate) CT746-20 (20-plate)	CT716-10 (10-plate) CT716-20 (20-plate)	CT771-10 (10-plate) CT771-20 (20-plate)		
IL-17A	CT566-10 (10-plate) CT566-20 (20-plate)	CT551-10 (10-plate) CT551-20 (20-plate)	CT773-10 (10-plate) CT773-20 (20-plate)		
IL-17F	CT568-10 (10-plate) CT568-20 (20-plate)	CT553-10 (10-plate) CT553-20 (20-plate)			
IL-17AF	CT565-10 (10-plate) CT565-20 (20-plate)				
IL-21	CT580-10 (10-plate) CT580-20 (20-plate)				
IL-23	CT567-10 (10-plate) CT567-20 (20-plate)	CT552-10 (10-plate) CT552-20 (20-plate)			
IL-27	CT574-10 (10-plate) CT574-20 (20-plate)				
IL-29	CT575-10 (10-plate) CT575-20 (20-plate)				
IL-31	CT570-10 (10-plate) CT570-20 (20-plate)				

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Analyte	Species				
	Human	Old World Monkey	New World Monkey	Mouse	Rat
IL-33	CT569-10 (10-plate) CT569-20 (20-plate)				
IP-10	CT572-10 (10-plate) CT572-20 (20-plate)	CT555-10 (10-plate) CT555-20 (20-plate)			
Angiopoietin-2	CT577-10 (10-plate) CT577-20 (20-plate)	CT556-10 (10-plate) CT55-20 (20-plate)			
G-CSF	CT769-10 (10-plate) CT769-20 (20-plate)	CT705-10 (10-plate) CT705-20 (20-plate)			
GM-CSF	CT739-10 (10-plate) CT739-20 (20-plate)	CT709-10 (10-plate) CT709-20 (20-plate)			
Granzyme B	CT752-10 (10-plate) CT752-20 (20-plate)				
MCP-1	CT571-10 (10-plate) CT571-20 (20-plate)	CT554-10 (10-plate) CT554-20 (20-plate)			
MICB	CT578-10 (10-plate) CT578-20 (20-plate)				
Perforin	CT753-10 (10-plate) CT753-20 (20-plate)	CT720-10 (10-plate) CT720-20 (20-plate)			
VEGF-A	CT579-10 (10-plate) CT579-20 (20-plate)				
TNF-α	CT747-10 (10-plate) CT747-20 (20-plate)	CT717-10 (10-plate) CT717-20 (20-plate)	CT772-10 (10-plate) CT772-20 (20-plate)	CT761-10 (10-plate) CT761-20 (20-plate)	CT704-10 (10-plate) CT704-20 (20-plate)

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Matched antibody pair for an ELISA test

U-CyTech offers matched ELISA antibody pairs for researchers who wish more flexibility and want to use their own assay design. These antibodies form the most crucial starting point to perform an ELISA (enzyme-linked immunosorbent assay) for the measurements of cytokine, chemokine and granzyme levels in biological fluids such as cell culture supernatant, plasma or serum.

Please note that U-CyTech also offer complete ELISA kits, which include all necessities and reagents to run 480 ELISA tests.

The usefulness of sandwich ELISAs in cytokine biology is evident from the many reports published on this subject. The assay requires two highly specific antibodies (either mono- or polyclonal antibodies) that bind with high affinity to different epitopes of the analyte of interest. One of the antibodies is immobilized within the wells of a 96-well microtiter plate. This coating antibody functions to selectively immobilize the analyte from crude protein preparations. The second antibody (detection antibody) is labeled with biotin and binds to another epitope of the analyte. Biotin allows the antibody to interact with streptavidin molecules. By using HRP (horseradish peroxidase)-labeled streptavidin, the analyte can be quantified using the enzymatic conversion of an HRP-specific substrate (e.g., TMB).

All antibody pairs, which have been selected by U-CyTech, result in a sensitive ELISA test with a detection limit falling within the low picogram range (≤ 5 pg/ml).

Contents

10-plate format

- 2 vials with coating antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 2 vials with biotinylated detection antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 5 vials with standards supplied in lyophilized form.
- 2 vials with SPP conjugate (Streptavidin-HRP) supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.

20-plate format

- 4 vials with coating antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 4 vials with biotinylated detection antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 10 vials with standards supplied in lyophilized form.
- 4 vials with SPP conjugate (Streptavidin-HRP) supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.

Hazard information

Components of the antibody pair are not classified as dangerous according to Regulation (EC) no. 1272/2008.

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

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Storage and Stability

The vials with lyophilized coating and biotinylated detection antibody can be safely stored in a refrigerator for a defined length of time (expiry date indicated on the vial). After reconstitution, the antibodies are stable for at least 12 months at 4°C when kept sterile. However, it is recommended to divide the reconstituted antibody solutions into small aliquots for single use. These aliquots should be stored at ≤-20°C (stable for at least two years).

The vials with lyophilized cytokine standard can be safely stored at 4°C until the expiry date (indicated on the vial). These vials are for single use only.

The vials with lyophilized SPP conjugate is stable until the expiry date (indicated on the vial) when stored at ≤-20°C in the dark. After reconstitution, the reagent is stable for at least 2 months at 4°C when kept sterile and protected from light. However, it is strongly recommended to divide the solution into small aliquots for single use. These aliquots should be stored at ≤ -20°C in the dark (stable for at least one years).

Materials and Equipment (required but not provided)

- Liquid PBS (pH 7.4).
- Sterile distilled water.
- Bovine serum albumin (BSA; ELISA grade).
- Tween-20 detergent.
- Coloring system e.g. 3,3′,5,5′-Tetramethylbenzidine (TMB) and Stop solution (H_2SO_4). Ready-to-use TMB substrate solution from U-CyTech (cat.no. CT383) is recommended in combination with 0.18 M H_2SO_4 as Stop solution.
- 96-well ELISA plates. Plates from Greiner Bio-one are recommended (Greiner Bio-one cat.no.655092 or U-CyTech cat.no. CT361).
- Adhesive cover slips (Greiner Bio-one cat.no. 676001).
- Pipetting devices for the accurate delivery of volumes required for assay performance.
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
- Reading device for microtiter plates (which fulfills the requirements of the applied substrate).

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Please note that the information provided below are general guidelines and recommendations for an ELISA procedure.

Preparation Solutions and Reagents

- PBS: 5.4 mM $Na_2HPO_4.2H_2O$; 1.3 mM KH_2PO_4; 150 mM NaCl; pH 7.4
- Wash buffer: 0.5 ml Tween-20 added to 1 L PBS
- Blocking buffer: PBS supplemented with 1% (w/v) BSA
- Dilution buffer: PBS supplemented with 0.5% (w/v) BSA and 0.05% (w/v) Tween-20
- Coating antibody | Biotinylated detection antibody | SPP conjugate:
  Reconstitute the lyophilized antibody by injecting the appropriate volume (indicated on the vial and Data sheet) of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds and allow it to stand for 5 min at room temperature. Avoid vigorous shaking. Dilute 100-fold in the appropriate buffer (indicated on the vial and Data sheet).
- Standard:
  Reconstitute the lyophilized standard by injecting 500 µl of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds and allow it to stand for 5 min at room temperature. Avoid vigorous shaking. Thereafter, the reconstituted standard is places on melting ice and is immediately (preferentially within one hour) diluted in Dilution buffer to the desired concentrations to be used in the standard curve range.
  In general, when TMB substrate solution from U-CyTech (cat.no. CT383) is used; the linear portion of the standard curve falls within the range of 0.5 to 100 pg/ml.

Preparation Samples

- Specimens should be clear, non-hemolyzed and non-lipemic. Excessive hemolysis and the presence of large clots or microbial growth in the sample may interfere with the performance of the test.
- Dilute samples in Dilution buffer (at least 2-fold).
- The diluent for the standard and blank control should preferentially be control serum or plasma originating from the same species. For measuring cytokines in cell culture supernatant, samples should be diluted in Dilution buffer.
- Avoid repeated freeze-thaw cycles of samples.

Washing

- Incomplete washing of the wells will adversely affect the assay.
- Washing can be performed as follows:
  Completely empty the wells. Then fill the wells with at least 250 µl Wash buffer. Let it soak for 10-20 seconds and then empty the wells. Repeat these steps at least six times. After washing, the plate is inverted and tapped dry on absorbent paper.

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Assay procedure

1. Bring 50 µl of diluted coating antibodies in the wells of an ELISA plate and fill up to 100 µl with PBS.
2. Seal the plate with an adhesive cover slip and incubate overnight at 4 °C.
3. Remove coating antibody solution and wash the wells at least 6 times with Wash buffer.
4. Add 200 µl Blocking buffer to each well.
5. Seal the plate with an adhesive cover slip and incubate 1 hour at 37 °C.
6. Dilute samples and standards in Dilution buffer.
7. Remove Blocking buffer by a vigorous 'shake-out' action but do not wash.
8. Add 100 µl of diluted standards and samples to each well.
9. Seal the plate and incubate for 2 hours at 37 °C (or alternatively overnight at 4 °C).
10. Remove standards and samples and wash the wells at least 6 times with Wash buffer.
11. Add 100 µl of diluted detector antibodies to each well.
12. Seal the plate and incubate 1 hour at 37 °C.
13. Remove detection antibody solution and wash the wells at least 6 times with Wash buffer.
14. Add 100 µl of diluted SPP conjugate to each well.
15. Seal the plate and incubate 1 hour at 37 °C.
16. Remove conjugate solution and wash the wells at least 6 times with Wash buffer.
17. Add an HRP- specific substrate solution into each well. The volume and incubation conditions depend on the type and brand of substrate used. When TMB substrate solution from U-CyTech (cat.no. CT383) is used; incubate the plate at room temperature in the dark with an incubation time between 15 and 25 minutes. This chromogenic substrate solution produces a blue-colored product, which can be read at 370 nm or 655 nm. The intensity of the color is related to the amount of analyte in the sample.
18. Stop the reaction when possible. When TMB substrate solution from U-CyTech (cat.no. CT383) is used; add 100 µl 0.18 M H_2SO_4 (resulting in a yellow color that can be read at 450 nm).

Technical assistance

If you require assistance, information or have any questions, please contact our company:

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Please, find frequently asked questions and data sheets of our ELISA antibody pairs on:
www.ucytech.com/FAQs_ELISA and www.ucytech.com/manuals.