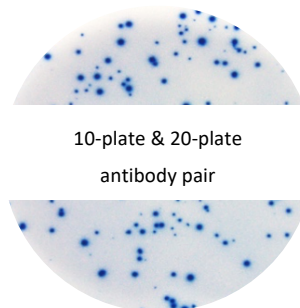


Manual T cell ELISPOT antibody pair



The ELISPOT assay is one of the most sensitive tests to monitor *ex vivo* cellular immune responses at the single cell level. The assay can accurately detect secreted proteins, such as cytokines, released by e.g. T cells in response to antigen. The cell suspensions, used in the test, can originate from blood (PBMC), lymphoid, spleen, bone marrow or CNS tissue.

The accompanying 'Data sheet' (specific for each antibody pair) and 'SDS' can be found at www.ucytech.com/manuals. References of studies using our T cell ELISPOT products, guidelines and recommendations for the performance of the T cell ELISPOT assay can be found at www.ucytech.com/t-cell-elispot-assay-guidelines.

Please note that information provided in this Manual are general guidelines and recommendations for an ELISPOT procedure using enzymatic staining on PVDF membrane-bottomed plates. U-CyTech also offers complete ELISPOT kits.

Contents of the antibody pair

Items	Quantity (10-plate format)	Quantity (20-plate format)	Storage conditions
Coating antibody*	2 vials**	1 vial***	2 to 8 °C
Biotinylated detection antibody*	2 vials**	1 vial***	2 to 8 °C

* Lyophilized

** Each vial contains sufficient material for five 96-well ELISPOT plates

*** Each vial contains sufficient material for twenty 96-well ELISPOT plates

Note: U-CyTech also offers complete ELISPOT kits (see: www.ucytech.com/products/t-cell-elispot).

Warnings

This antibody pair is designed for *research use only* and is not for use in diagnostic or therapeutic procedures.

Hazard information

All components are not classified as hazardous substances/mixtures according to Regulation (EC) no. 1272/2008. Please find the Safety Data Sheet at www.ucytech.com/manuals.

Materials and equipment (required but not provided)

Recommended items when using enzymatic staining on PVDF membrane-bottomed plates:

- Sterile distilled water and demineralized water.
- 70% ethanol.
- PBS: 5.4 mM Na₂HPO₄·2H₂O; 1.3 mM KH₂PO₄; 150 mM NaCl; pH 7.4. For washing only.
- PBS-I: sterile, pyrogen-free liquid PBS pH 7.4 (e.g. Thermo Fisher Scientific cat. no. 10010).
- Cell culture medium: e.g. RPMI-1 supplemented with 100 µg/ml streptomycin, 100 units/ml penicillin, 2 mM L-Glutamine, 10% fetal calf serum (selected on low background staining).
- Cell stimuli: see www.ucytech.com/t-cell-elispot-assay-guidelines.
- Wash buffer: PBS containing 0.05% (w/v) Tween-20.
Note: use PBS only as wash buffer for Human IL-21 T cell ELISPOT assay.
- Blocking buffer: Blocking stock solution (e.g. U-CyTech cat. no. CT363) or cell culture medium.
- Dilution buffer: use Dilution buffer R (e.g. U-CyTech cat. no. CT348).
Note: use Dilution buffer Q (U-CyTech cat. no. CT368) for Human IL-21 T cell ELISPOT assay.
- Conjugate: Streptavidin-HRP conjugate (e.g. U-CyTech cat. no. CT353).
- Substrate: AEC coloring system (e.g. U-CyTech cat. no. CT356. Use fume hood).
- 96-well PVDF membrane-bottomed ELISPOT plates with lid (Millipore cat. no. MSIP S4510).
- Adhesive cover slips (e.g. Greiner Bio-one cat. no. 676001).
- Pipetting devices.
- Tubes and containers/plates to prepare solutions.
- Tissue culture plates for preincubation (optional).
- CO₂ incubator (37 °C, 100% humidity, 5% CO₂).
- Washing device: squirt bottle with a wide spout.
- Laminar Flow Hood (for sterile conditions).
- A reflected light microscope or an automated ELISPOT reader for spot counting.

Storage and stability

Coating and detection antibody

The vials with lyophilized coating and biotinylated detection antibody can be safely stored at 2 to 8 °C until the expiry date (printed on the vials). After reconstitution, the antibodies are stable for at least 12 months at 2 to 8 °C when kept sterile. Divide reconstituted antibody solutions into aliquots for single use. Store aliquots at -32 to -18 °C (stable for at least 2 years).

Preparation solutions and reagents

Coating and detection antibody

Reconstitute the lyophilized product by injecting the appropriate volume (printed on the vial) of sterile distilled water into the vial. Mix the solution gently for approximately 15 sec and allow it to stand for 5 min at room temperature (RT: 20-26 °C). Avoid vigorous shaking.

Dilute 100-fold in PBS-I (coating antibody) or dilution buffer (detection antibody).

Sample preparation

See www.ucytech.com/t-cell-elispot-assay-guidelines for information on cell collection and handling, cell sample preparation (including information on preincubation) and guidelines for stimuli, cell concentrations and cell incubation times.

Use 2×10^2 – 1×10^5 cells/well for a polyclonal stimulus (positive control) and 1 – 3×10^5 cells/well for antigen-specific responses and negative controls (no stimulus added). Add 100 µl/well.

T cell ELISPOT assay procedure

Notes:

- All solutions should be at room temperature (RT: 20-26 °C) prior to use.
 - Steps 1 till 11 should be performed under sterile conditions.
 - Estimate the time needed to prepare all cell suspensions which have to be ready for step 10 and plan accordingly.
1. Prewet the PVDF membrane of each well of the ELISPOT plate with 25 µl of 70% ethanol. Incubate for 1 min at RT.
 2. Wash the wells thoroughly twice with 200 µl PBS-I/well. The plate is subsequently emptied with a firm shake-out action.
 3. Add 50 µl of diluted coating antibody solution to each well of the ELISPOT plate. Cover the plate with a lid.
 4. Incubate overnight at 2 to 8 °C.
 5. Remove coating antibody solution and wash the wells 3 times with PBS-I. The plate is subsequently emptied with a firm shake-out action.
 6. Add 200 µl of blocking buffer to each well.
 7. Cover the plate with a lid and incubate for at least 1 hour at RT.
 8. Prepare cell sample suspensions*.
 9. Remove the blocking buffer with a firm shake-out action (do not wash the wells).
 10. Add 100 µl of the cell sample suspensions into the wells.
 11. Cover ELISPOT plate with lid and incubate at 37 °C, 5% CO₂ and 100% humidity. The incubation time can vary from 24 to 72 h. Specific activation conditions will vary, depending on cell type, protein of interest, kinetics of protein release and whether a preincubation step was included in the procedure.*
 12. Remove the bulk of cells with a firm shake-out action and rinse each well 2x with 200 µl PBS-I. The plate is subsequently emptied. Wash the plate 5x with 250 µl wash buffer/well.*
 13. Add 100 µl of diluted detection antibody solution to each well.
 14. Seal the plate with an adhesive cover slip and incubate 2 h at RT (or overnight at 4 °C).
 15. Empty plate. Remove and discard the underdrain from the bottom of the plate and wash both sides of the PVDF membrane 5x with wash buffer.
 16. Add a biotin-specific conjugate into each well. Seal the plate. The volume and incubation time depend on the type and brand of conjugate used.
 17. Empty plate and wash both sides of the PVDF membrane 5x with wash buffer.
 18. Add an appropriate substrate into each well. Cover the plate. The volume and incubation time depend on the type and brand of substrate used.
 19. Stop the reaction by emptying the plate and thoroughly rinse both sides of the PVDF membrane with demineralized water. Air-dry the plate at RT (protected from light).
 20. Count spots by using a reflected light microscope or an ELISPOT reader.
Note: Store the plate at a dry place protected from light to prevent bleaching of spots.

* More information on how to prepare the cells, cell incubation times, stimuli, washing and troubleshooting can be found at www.ucytech.com/t-cell-elispot-assay-guidelines.

This manual is applicable to following U-CyTech's ELISPOT antibody pairs

Note:

Antibody pairs are available in a 10-plate format (CTxxx-10) and 20-plate format (CTxxx-20).

Analyte	Human	Old World Monkey	New World Monkey	Mouse	Rat
IFN- γ	CT640	CT605	CT963	CT655	CT599
IL-1 β	CT651	CT607			
IL-2	CT471	CT621		CT485	
IL-4	CT642	CT612		CT657	CT601
IL-5	CT643	CT613		CT660	
IL-6	CT644	CT614			
IL-10	CT645	CT615		CT658	
IL-12/IL-23 p40	CT649	CT619	CT966		
IL-12p70	CT650				
IL-13	CT646	CT616	CT967		
IL-17A	CT466	CT451	CT968		
IL-17F	CT468	CT453			
IL-21	CT469				
G-CSF	CT652	CT606			
GM-CSF	CT638	CT608			
Granzyme B	CT639				
Perforin	CT653	CT620			
TNF- α	CT647	CT617	CT962	CT661	

If you require assistance, information or have any questions, please contact our Customer Service by e-mail: cs@ucytech.com.