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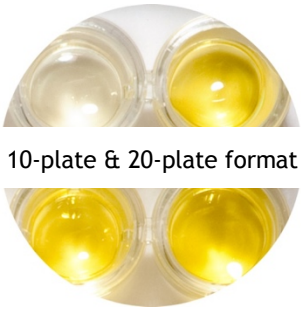
Technical Data Sheet
ELISA antibody pair



10-plate & 20-plate format

The ELISA is one of the primary and most popular methods to detect and measure signaling proteins like cytokines and granzymes. The ELISA test is rapid, simple to perform and is one of the most sensitive and reliable technologies available.

The accompanying 'Data sheet' (specific for each antibody pair, including sensitivity of the antibody pair) and 'SDS' can be found at www.ucytech.com/manuals. References of studies using our ELISA products, guidelines and recommendations for the performance and data analysis of the ELISA assay can be found at www.ucytech.com/elisa-guidelines.

Please note that information provided in this Technical Data Sheet are general guidelines and recommendations for an ELISA procedure. U-CyTech also offers complete ELISA kits.

Contents of the antibody pair

Items	Quantity (10-plate format)	Quantity (20-plate format)	Storage conditions
Coating antibody*	2 vials**	4 vials**	4 °C
Standard*	5 vials	10 vials	4 °C
Biotinylated detection antibody*	2 vials**	4 vials**	4 °C
SPP conjugate*	2 vials**	4 vials**	-20 °C***

* Lyophilized
** Each vial contains sufficient material for five 96-well ELISA plates
*** Store protected from light
Note: U-CyTech also offers complete ELISA kits (see: www.ucytech.com/products/elisa).

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 (SDS) at www.ucytech.com/manuals.

Materials and equipment (required but not provided)

- PBS: 5.4 mM $Na_2HPO_4.2H_2O$; 1.3 mM KH_2PO_4; 150 mM NaCl; pH 7.4 (sterile).
- Sterile distilled water.
- Wash buffer: PBS containing 0.05% (w/v) Tween-20.
- Blocking buffer: PBS containing 1% (w/v) Bovine serum albumin (BSA; ELISA grade).
- Dilution buffer: PBS containing 0.5% (w/v) BSA and 0.05% (w/v) Tween-20.
- Coloring system e.g. 3,3′,5,5′-Tetramethylbenzidine (TMB) and, if applicable, stop solution. 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 (e.g. Greiner Bio-one cat. no.655092 or U-CyTech cat. no. CT361).
- Adhesive cover slips (e.g. Greiner Bio-one cat. no. 676001).
- Pipetting devices.
- Tubes and containers/plates to prepare solutions.
- Ice.
- Plate washer: automated or manual (squirt bottle, manifold dispenser).
- Reading device for microtiter-plate (which fulfills requirements of applied substrate).

Storage and stability

Coating and detection antibody

The vials with lyophilized coating and biotinylated detection antibody can be safely stored at 4 °C until the expiry date (indicated on the vials). After reconstitution, the antibodies are stable for at least 12 months at 4 °C when kept sterile. Divide reconstituted antibody solutions into aliquots for single use. Store aliquots at ≤-20 °C (stable for at least 2 years).

Standard

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

Conjugate (Streptavidin-HRP)

The vials with lyophilized SPP conjugate are stable until the expiry date (indicated on the vial) when stored protected from light at -20 °C. After reconstitution, the reagent is stable for at least 2 months at 4 °C when kept sterile and protected from light. Divide solution into aliquots for single use. Store aliquots protected from light at -20 °C (stable for at least 1 year).

Sample preparation

Read www.ucytech.com/specimen-collection-and-handling for information on specimen collection and handling.

Dilute samples in dilution buffer (at least 1:1).

Preparation solutions and reagents

Standard

Reconstitute the lyophilized standard by injecting 500 µl 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. Thereafter, the reconstituted standard is placed on melting ice and is immediately (preferentially within 1 hour) diluted in dilution buffer to the desired concentrations to be used in the standard curve range.

Coating antibody | Detection antibody | Conjugate

Reconstitute the lyophilized product by injecting the appropriate volume (indicated 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 RT. Avoid vigorous shaking.
Dilute 100-fold in PBS (coating antibody) or dilution buffer (detection antibody/conjugate).

Note:
Do not use commercially available PBS tablets for the preparation of the coating solution (the filler in the tablets interferes with the coating process).

ELISA procedure

Note: All solutions should be at RT (temperature between 20 °C and 26 °C) prior to use.
Read www.ucytech.com/directions-washing-elisa-plates for more information on washing.

1. Add 50 µl of diluted coating antibody solution to each well of the ELISA plate and fill up to 100 µl with PBS. Seal the plate to prevent evaporation.
2. Incubate overnight at 4 °C.
3. Remove coating antibody solution and wash the wells at least six times with wash buffer.
4. Add 200 µl of blocking buffer to each well.
5. Seal the plate and incubate for 1 hour at RT.
6. Prepare blank (dilution buffer only), samples and standard dilutions.
7. Remove the blocking buffer (do not wash the wells).
8. Add 100 µl of diluted standard/blank/samples to the wells.
9. Seal the plate and incubate for 2 hours at RT (or alternatively overnight at 4 °C).
10. Remove standards/blank/samples and wash the wells at least six times with wash buffer.
11. Add 100 µl of diluted detection antibody solution to each well.
12. Seal the plate and incubate for 1 hour at RT.
13. Remove detection antibody solution and wash the wells at least six times with wash buffer.
14. Add 100 µl of diluted conjugate to each well.
15. Seal the plate and incubate for 1 hour at RT (protected from light).
16. Remove conjugate and wash the wells at least six times with wash buffer.
17. Add an HRP-specific substrate into each well. The volume and incubation time depend on the type and brand of substrate used.
    When TMB substrate solution from U-CyTech (cat. no. CT383) is used, add 100 µl/well and incubate between 15 and 25 minutes at RT in the dark. This substrate yields a soluble blue product that absorbs at 650 nm.
18. Stop the reaction when applicable.
    When TMB substrate solution from U-CyTech (cat. no. CT383) is used; add 100 µl/well of 0.18 M H_2SO_4 (resulting in a yellow color) and read the plate at 450 nm within 30 minutes.

More information on data analysis and troubleshooting can be found at www.ucytech.com/elisa-guidelines.

This Technical Data Sheet is applicable to following U-CyTech's ELISA 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	CT740	CT710	CT770	CT755	CT700
IL-1β	CT576	CT708			
IL-2	CT741	CT711	CT774	CT762	
IL-4	CT742	CT712		CT767	CT702
IL-5	CT743	CT713		CT764	
IL-6	CT744	CT714	CT777	CT763	
IL-8 (CXCL8)	CT748	CT718			
IL-10	CT745	CT715		CT765	
IL-12/23p40		CT719	CT775		
IL-12p70	CT750				
IL-13	CT746	CT716	CT771		
IL-17A	CT564	CT557	CT773		
IL-17F	CT568	CT553			
IL-21	CT580				
IL-23	CT567	CT552			
IL-27	CT574				
IL-29	CT575				
IL-31	CT570				
IL-33	CT569				
IP-10 (CXCL10)	CT572	CT555			
Angiopoietin-2	CT577	CT556			
G-CSF	CT769	CT669			
GM-CSF	CT739	CT709			
Granzyme B	CT752				
Perforin	CT753	CT720			
TNF-α	CT747	CT717	CT772	CT761	CT704

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