U-CyTech BV
Yalelaan 48
3584 CM Utrecht
The Netherlands
P +31.85.073 1460
info@ucytech.com
www.ucytech.com

Manual ELISA kit

5-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 'Typical data sheet' (specific for each kit) and 'MSDS' can be found on www.ucytech.com/manuals. References of studies using our ELISA kits, guidelines and recommendations for the performance and data analysis of the ELISA assay can be found on www.ucytech.com/elisa-guidelines.

Contents of the kit

Items	Quantity (5-plate format)	Storage conditions
Coating antibody*	1 vial	4 °C
Standard*	5 vials	4 °C
Biotinylated detection antibody*	1 vial	4 °C
SPP conjugate*	1 vial	≤-20 °C***
BSA stock solution (10%)	2 vials (2x 12 ml)	4 °C
Cytokine stabilization buffer (CSB)**	1 vial (5 ml)	4 °C
Tween-20	1 vial (5 ml)	RT***
TMB substrate solution	2 vials (2x 30 ml)	4 °C***
Stop solution (0.175 M H_2SO_4)	2 vials (2x 30 ml)	4 °C
96-well ELISA plate	8 plates	RT
Adhesive cover slip	10 slips	RT

RT Room temperature (temperature between 20 °C and 26 °C)
* Lyophilized
** For use in serum and plasma samples only, see section "Sample preparation"
*** Store protected from light

Warnings

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

Hazard information

All kit components are not classified as dangerous according to Regulation (EC) no. 1272/2008 and Directive 67/548/EEC or 1999/45/EC and their amendments.
Please find the Material Safety Data Sheet on www.ucytech.com/manuals.

Materials and equipment (required but not provided)

- PBS (pH 7.4; ingredients: $Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4, NaCl and distilled water). Alternatively, use commercially available liquid PBS (pH 7.4) from Thermo Fisher Scientific (cat. no. 10010) or other suppliers.
- Sterile distilled water.
- Pipetting devices.
- Tubes and containers/plates to prepare solutions.
- Ice.
- 37 °C incubator.
- Plate washer: automated or manual (squirt bottle, manifold dispenser).
- Reading device for microtiter-plate (wavelength set to 450 or 650 nm).

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. 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).

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

The vial with lyophilized SPP conjugate is 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. However, it is strongly recommended to divide the solution into small aliquots for single use. These aliquots should be stored protected from light at ≤-20 °C (stable for at least one year).

TMB

The ready-to-use TMB substrate solution should be stored at 4 °C and is stable until the expiry date (indicated on the vial). Avoid exposure to light, heat and contamination with metal ions or peroxidase.

BSA and Cytokine stabilization buffer

The vials with BSA stock solution and Cytokine stabilization buffer can be safely stored at 4 °C until the expiry date (indicated on the vial). After opening, these solutions are stable for at least 6 months when kept sterile.

Tween-20

Tween-20 can safely be stored at RT (protected from light) and is stable until the expiry date (indicated on the vial).

Stop solution

The ready-to-use stop solution can safely be stored at 4 °C and is stable until the expiry date (indicated on the vial).

Preparation solutions and reagents

PBS

PB stock: dissolve 96.0 g of $Na_2HPO_4 \cdot 2H_2O$ plus 17.5 g of KH_2PO_4 in 1 L distilled water, adjust pH to 7.4 and filtrate solution (0.2 µm). Store solution at RT (stable for at least 6 months when kept sterile).

PBS: add 10 ml of the PB stock and 8.8 g of NaCl to 1 L distilled water. It is strongly recommended to prepare PBS freshly each day. Alternatively, when PBS is prepared in advance, the solution should be filter sterilized (0.2 µm) or autoclaved.

Wash buffer

PBS containing 0.05% Tween-20 (add 0.5 ml of Tween-20 to 1 L PBS and mix gently but thoroughly). The volume needed is depending on the washing procedure (manual or automatic washing).

Blocking buffer

PBS containing 1% BSA.

For 1 ELISA plate: mix 2 ml BSA stock solution (10%) gently but thoroughly with 18 ml PBS.

Dilution buffer

PBS containing 0.5% BSA and 0.05% Tween-20. This buffer can be prepared at once for 5 ELISA plates by making at least 250 ml under sterile conditions. Add 12.5 ml of BSA stock solution (10%) and 125 µl of Tween-20 to 250 ml PBS, mix gently and store at 4 °C. This solution will be stable for at least one month when kept sterile.

For 1 ELISA plate, 20 ml of dilution buffer is needed for detection and conjugate solutions, and at least 20 ml for standards and samples (this volume will depend on the number of sample dilutions).

Coating antibody

Reconstitute the lyophilized antibody by injecting 250 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 RT. Avoid vigorous shaking.

For 1 ELISA plate: 50 µl is gently but thoroughly mixed with 5 ml PBS.

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).

Detection antibody

Reconstitute the lyophilized antibody 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 RT. Avoid vigorous shaking.

For 1 ELISA plate: 100 µl is gently but thoroughly mixed with 10 ml dilution buffer.

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 RT. Avoid vigorous shaking. Thereafter, the reconstituted standard is placed on melting ice and is immediately (preferentially within 1 hour) diluted as described in section "Preparing the standard curve".

Note: the quantity (expressed in ng/vial) of the standard is indicated on the vial and is variable for each kit and batch. After reconstitution, the concentration can be calculated as follows: divide the quantity (indicated on the vial) by the volume used for reconstitution. For example, the concentration of a standard vial containing 5 ng/vial will be 10 ng/ml (= 10,000 pg/ml) after reconstitution in 0.5 ml distilled water.

SPP conjugate

Reconstitute the contents of the vial by injecting 500 µl of sterile distilled water into the vial. Mix the solution gently for approximately 15 sec and allow it to stand protected from light for 5 min at 4 °C. Avoid vigorous shaking.

For 1 ELISA plate: 100 µl is gently but thoroughly mixed with 10 ml dilution buffer.

TMB substrate solution (ready-to-use)

Bring TMB substrate solution to RT prior to use.

Stop solution (ready-to-use)

Bring stop solution to RT prior to use.

Sample preparation

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

Samples should be aliquoted and stored frozen at ≤-20 °C to prevent cytokine degradation. Prior to assay, frozen samples should be completely thawed and mixed well. Avoid repeated freeze-thaw cycles of samples.

Dilute samples in dilution buffer (at least 1:1). It is recommended to analyze a series of dilutions of the sample to ensure that sample measurements fall within the assay range (see also www.ucytech.com/data-analysis).

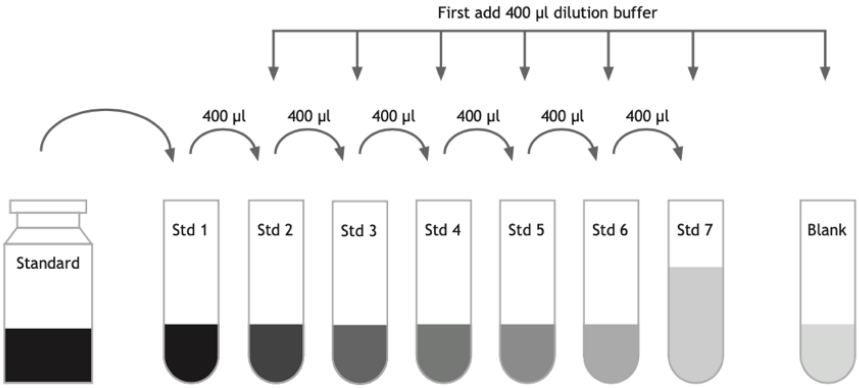
When measuring cytokines in serum or plasma, add 1/20 volume of CSB (ready-to-use) to the pure serum or plasma sample before further dilution in dilution buffer. CSB inhibits the degradation of cytokines in serum or plasma samples and is therefore not required for other samples such as cell culture supernatant.

It is recommended to test samples in triplicate.

Preparing the standard curve

The analyte concentration in the unknown samples can be determined by a standard curve. The standard curve is generated from the results of 7 two-fold serial dilutions (Std 1-7) of the reconstituted standard. The recommended assay range for each specific ELISA kit can be found in the Typical data sheet on www.ucytech.com/manuals. It is recommended to test the standard dilutions (Std 1-7) in triplicate.

- Take 8 tubes and add 400 µl dilution buffer to 7 of these tubes (Std 2 till Std 7 and Blank).
- Prepare in the remaining tube (Std 1) the highest concentration to be used in the standard curve (see Typical data sheet) by mixing an appropriate volume of reconstituted standard with dilution buffer. The final volume of Std 1 should be 800 µl. Allow the mixture to stand for at least 15 sec before using in further dilutions.
- Perform two-fold serial dilutions: transfer 400 µl diluted standard from Std 1 to the next tube (Std 2), mix well and repeat this step until Std 7.



Notes:

- If less than 10 µl of the reconstituted standard is needed to prepare Std 1, it is recommended to dilute the reconstituted standard 10 times in dilution buffer (mix 10 µl reconstituted standard with 90 µl dilution buffer) and use this to prepare Std 1.
- A standard curve, including blank, should be run on each ELISA plate.
- Use vials with standard only once.
- It is recommended to test standard dilutions in triplicate.
- Standard dilutions should be used as soon as possible (preferentially within 1 hour).
- Depending on the biological origin of the unknown samples, also other appropriate dilution buffers may be used for the preparation of the standard curve, depleted with the endogenous protein to be quantified (e.g. cell culture medium, serum).

ELISA procedure

Note: All solutions should be at RT 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 (or alternatively 2 hours at 37 °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 37 °C.
6. Prepare blank, 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 37 °C (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 37 °C.
13. Remove detection antibody solution and wash the wells at least six times with wash buffer.
14. Add 100 µl of diluted SPP conjugate to each well.
15. Seal the plate and incubate for 1 hour at 37 °C (protected from light).
16. Remove SPP conjugate and wash the wells at least six times with wash buffer.
17. Add 100 µl of TMB substrate solution into each well.
18. Leave the plate protected from light for 20 min at RT.
    Note: The substrate yields a soluble blue product that absorbs at 650 nm.
19. After incubation of the substrate (do not empty the wells), stop the reaction by adding 100 µl of stop solution into each well (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 on www.ucytech.com/elisa-guidelines.

This manual is applicable to following U-CyTech's ELISA kits

Analyte	Human	Old World Monkey	New World Monkey	Mouse	Rat
IFN-γ	CT201A	CT141A	CT340A	CT301A	CT071A
IL-1β	CT526A	CT139A			
IL-2	CT202A	CT142A	CT344A	CT309A	
IL-4	CT203A	CT143A		CT306A	CT073A
IL-5	CT204A	CT144A		CT296A	
IL-6	CT205A	CT145A	CT346A	CT299A	
IL-7	CT523A				
IL-8	CT212A	CT151A			
IL-10	CT206A	CT146A		CT307A	
IL-12/23p40		CT149A	CT345A		
IL-12p70	CT210A				
IL-13	CT208A	CT147A	CT341A		
IL-17A	CT516A	CT501A	CT343A		
IL-17F	CT518A	CT503A			
IL-21	CT530A				
IL-23	CT517A	CT502A			
IL-27	CT524A				
IL-29	CT525A				
IL-31	CT520A				
IL-33	CT519A				
IP-10	CT522A	CT157A			
Angiopoietin-2	CT527A	CT158A			
G-CSF	CT389A	CT155A			
GM-CSF	CT200A	CT140A			
Granzyme B	CT211A				
MCP-1	CT521A	CT156A			
Perforin	CT319A	CT154A			
TNF-α	CT209A	CT148A	CT342A	CT303A	CT075A

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