

ELISA Pro: Human TNF- α

3512-1HP-1 | 3512-1HP-2 | 3512-1HP-10

Datasheet & Protocol



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Introduction

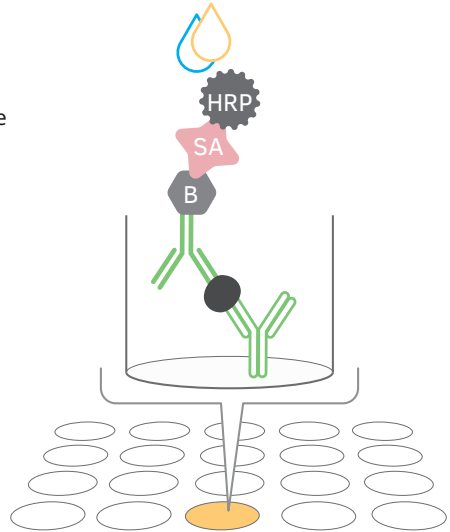
Mabtech's carefully validated ELISA Pro kits provide all the necessary reagents to conveniently quantify analytes in serum, plasma, and cell culture supernatants in a robust, sensitive, and specific manner. For Research Use Only (RUO). Not for use in diagnostic procedures.

ELISA assay principle

ELISA Pro kits are supplied with ELISA strip plates precoated with monoclonal antibody (mAb). Analyte in the sample is captured by the coated mAb and detected by the biotinylated detection mAb followed by Streptavidin-HRP (SA-HRP). Addition of TMB substrate will result in a colored substrate product. The reaction is stopped with sulfuric acid and the optical density can be quantified using an ELISA plate reader. The concentration of analyte is determined by comparison to a serial dilution of the ELISA standard analyzed in parallel.

Analysis of serum and plasma samples

The ELISA Pro kits include ELISA diluent, a buffer that prevents false-positive signals. The buffer blocks heterophilic antibodies from cross-linking the assay antibodies. Heterophilic antibodies are commonly found in human serum/plasma and can also be present in other species. The buffer has been validated using serum/plasma samples from healthy human blood donors.



Shipping and storage

The kit is shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard, which should be stored at -20 °C. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use. Do not combine components from different kit batches or components from other suppliers.

Contents

Component	1-plate kit	2-plate kit	10-plate kit
Pre-coated ELISA strip plate: Anti-TNF- α mAb MT25C5	1 x 96 wells	2 x 96 wells	10 x 96 wells
Recombinant human TNF- α ELISA standard	1 vial	1 vial	1 vial
Detection mAb MT20D9, biotin (0.5 mg/ml)	25 μ l	50 μ l	250 μ l
Streptavidin-HRP	15 μ l	25 μ l	125 μ l
Standard reconstitution buffer A5	1 ml	1 ml	1 ml
Wash buffer concentrate	120 ml	120 ml	5 x 120 ml
ELISA diluent	120 ml	120 ml	3 x 120 ml
Streptavidin-HRP diluent	15 ml	25 ml	120 ml
TMB substrate	15 ml	25 ml	120 ml
Stop solution	15 ml	25 ml	120 ml
Adhesive plate covers	3	6	30

To ensure total recovery of the stated quantity, bottles and vials have been overfilled.

Materials required but not supplied

- Microplate reader capable of reading at 450 nm
- ELISA plate washer; automated or manual (e.g., multipipette or squirt bottle)
- Precision pipettes, tips, and graduated cylinders
- Tubes for standard and sample dilutions
- Distilled or deionized water

Safety information

The Stop solution, 0.18 M H₂SO₄ (< 1%), is irritating to eyes and skin and should be handled with care. The standard should also be handled carefully as the effects of exposure are unknown. Buffers and reagents in solution contain the preservative Kathon CG (0.002%), a potential allergen that may cause sensitization through skin contact. Human and animal samples should be treated as potentially hazardous biologic material. All material should be disposed of in accordance with local regulations. For further information please consult the Safety Data Sheet on our website.

Preparation

- Allow the plates and assay reagents to reach room temperature before starting the assay (except for the TMB substrate which should preferably be used cold).
- Plan the plate layout to include a standard curve, samples, and an assay background control, all in duplicate. The volume per well should not exceed 100 μ l. Include a plate blank (wells with only Substrate and Stop solution) to be used for subtraction before analysis.

Wash buffer

Add 50 ml Wash buffer concentrate to 950 ml distilled or deionized water (sufficient for all washing steps of 1 plate). If crystals have formed in the 20x concentrate, bring to room temperature and mix gently to dissolve.

Samples

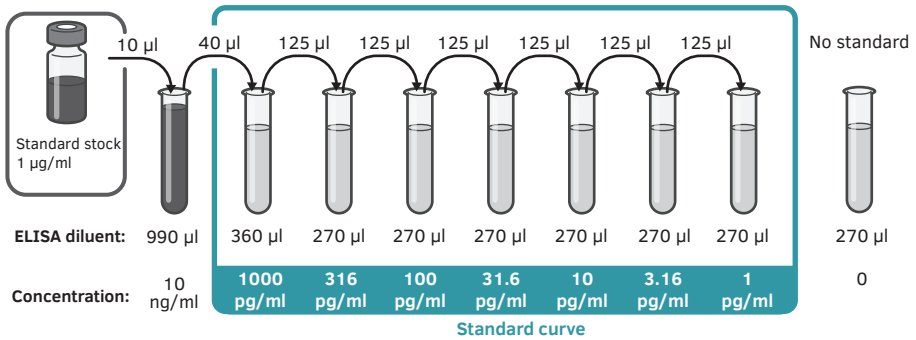
For accurate assay performance, all samples should be diluted at least 2-fold in ELISA diluent. Samples can be diluted in tubes or plates; diluent should be added before the samples and visible precipitates should be removed. The use of strongly hemolyzed and hyperlipemic samples may give inaccurate determination of the concentration. Samples containing high levels of analyte exceeding the standard range of the assay will require further dilution.

ELISA standard

Reconstitute the ELISA standard to a stock solution of 1 µg/ml by adding 1 ml Standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

Preparation of standard curve

Dilute the standard stock solution to create a standard curve as shown. The indicated volumes are sufficient for duplicates. The last vial is used as an assay background control, i.e., the standard should be omitted. Prepare the standard curve within 30 minutes of use.



Detection antibody

Dilute the detection mAb in ELISA diluent to 1 µg/ml within 15 minutes of use. For each plate, add 24 µl detection mAb to 12 ml ELISA diluent.

Streptavidin-HRP

Dilute the Streptavidin-HRP 1:1000 in Streptavidin-HRP diluent within 15 minutes of use. For each plate, add 12 µl Streptavidin-HRP to 12 ml Streptavidin-HRP diluent.

Protocol

Prepare the reagents, standard curve, and samples as described in the Preparation section. Assemble the required number of strips in the plate frame and label the top of each strip. Store the remaining strips in the foil bag containing the desiccant at 4-8 °C.

1. Wash the plate 5 times with wash buffer, 300 μ l per well. After the final wash, invert and tap the plate firmly against absorbent paper. Immediately proceed to the next step.
2. Add 100 μ l per well of samples (diluted at least 2-fold), standard, and assay background control. Mix by tapping the plate. Cover the plate with an adhesive plate cover and incubate for 2 hours at room temperature.
3. Wash as in step 1.
4. Add 100 μ l per well of detection mAb. Cover the plate and incubate for 1 hour at room temperature.
5. Wash as in step 1.
6. Add 100 μ l per well of Streptavidin-HRP. Cover the plate and incubate for 1 hour at room temperature.
7. Wash as in step 1.
8. Add 100 μ l of TMB substrate to each well. Incubate at room temperature, protected from direct light for 15 minutes.
9. Add 100 μ l of Stop solution to each well to stop the color development.
10. Measure absorbance at 450 nm within 15 minutes. Preferably use a reader capable of subtracting a reference wavelength between 570 and 650 nm.

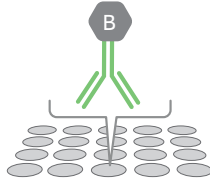
We recommend the use of an ELISA software utilizing a 4- or 5-parameter curve fit. Subtract the mean absorbance value of the blank from the samples, standard and assay background control prior to creating the standard curve and analyzing the results.

Wash



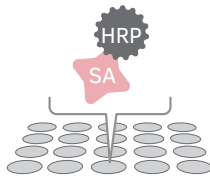
Add samples,
standard, and assay
background control
Incubate 2 hours

Wash



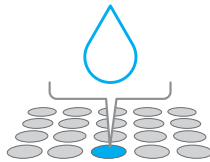
Add detection
antibody
Incubate 1 hour

Wash

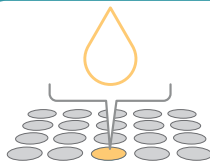


Add Streptavidin-HRP
Incubate 1 hour

Wash



Add TMB substrate
Incubate 15 minutes



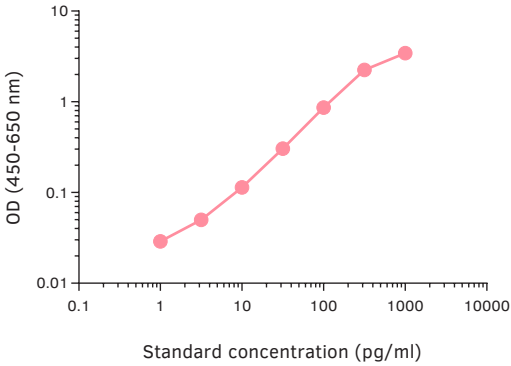
Add Stop solution



Measure absorbance
at 450 nm within
15 minutes

Performance

Human TNF- α



Representative standard curve

Standard range 3.16-1000 pg/ml

Sensitivity 2 pg/ml

The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 3 standard deviations to the mean OD of background wells.

Calibration

The standard of this ELISA has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One ng equals 46 U of NIBSC standard 12/154. Please note that the calibration is batch specific.

Precision

Sample	Intra-assay			Inter-assay		
	1	2	3	1	2	3
n	10	10	10	4	4	4
Mean (pg/ml)	610.9	247.6	121.0	567.8	242.7	122.6
SD	41.0	14.7	6.4	30.3	8.1	3.2
CV%	6.7	5.9	5.3	5.3	3.3	2.6

Intra-assay and inter-assay precision were determined at 3 different concentrations of analyte (10 replicates per concentration in 4 assays).

Recovery

	Spike concentration (pg/ml)	Average recovery % (range)
Plasma	500	88 (83-98)
	250	97 (95-102)
	125	98 (92-104)

Three concentrations of standard were spiked in a human plasma pool. Five replicates per concentration were tested in 4 assays.

Linearity

Recovery of a high concentration of standard in human plasma after serial dilution in ELISA diluent (1:2 to 1:8) ranged from 94% to 106% with a mean recovery of 102%.

Specificity

The kit is based on a matched pair of mAbs specific for native and recombinant human TNF- α .

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



The products are for research use only.

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