abcam

Product datasheet

Human TNF alpha ELISA Kit ab181421

SimpleStep ELISA®

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Overview

Product name Human TNF alpha ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
PBMC media	8			2.3%

Inter-assay

Sample	n	Mean	SD	CV%
PBMC media	3			5%

Sample type Serum, Plasma, Cell culture media

Assay type Sandwich (quantitative)

Sensitivity 14 pg/ml

Range 31.25 pg/ml - 2000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Serum	99.5	80% - 119%
Cell culture media	100.97	95% - 109%
Heparin Plasma	98	91% - 106%
EDTA Plasma	102	95.7% - 110%
Citrate Plasma	93.09	83.5% - 99%

Assay time 1h 30m

Assay duration	One step assay
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse
Product overview	Abcam's TNF-alpha *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of TNF-alpha protein in Human serum, plasma and culture media. The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.
Notes	TNF-alpha, also known as cachectin or TNFSF1A, is the prototypic ligand of the TNF superfamily which plays a central role in inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis and morphogenesis. It is expressed on macrophages, endothelial, epithelial and tumor cells as a 26kDa transmembrane protein. TNF-alpha is cleaved by proteolytic processing into six chains: (1) TNF membrane form, (2) Intracellular domain 1, (3) Intracellular domain 2, (4) C-domain 1, (5) C-domain 2 and (6) TNF soluble form. Signaling from TNF-alpha differs depending on the type of ligand initiating the signaling event (intracellular, membrane or soluble). As an example, the membrane form of TNF-alpha appears to mediate anti-tumorigenic therapeutic responses whereas the soluble ligand is linked to inflammation and proliferation.
Tested applications	**Suitable for:** Sandwich ELISA
Platform	Microplate

Properties

Storage instructions	Store at +4°C. Please refer to protocols.

Components	**1 x 96 tests**
10X Wash Buffer PT (ab206977)	1 x 20ml
Antibody Diluent 5BI	1 x 6ml
Plate Seals	1 unit
Sample Diluent 50BP	1 x 20ml
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml

Components	1 x 96 tests
TMB Substrate	1 x 12ml
TNFa Capture Antibody (Lyophilized)	1 x 2 vials
TNFa Detector Antibody (Lyophilized)	1 x 2 vials
TNFa Human Lyophilized Recombinant Protein	1 x 2 vials

Function	Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation.
Involvement in disease	Genetic variations in TNF are a cause of susceptibility psoriatic arthritis (PSORAS) [MIM:607507]. PSORAS is an inflammatory, seronegative arthritis associated with psoriasis. It is a heterogeneous disorder ranging from a mild, non-destructive disease to a severe, progressive, erosive arthropathy. Five types of psoriatic arthritis have been defined: asymmetrical oligoarthritis characterized by primary involvement of the small joints of the fingers or toes; asymmetrical arthritis which involves the joints of the extremities; symmetrical polyarthritis characterized by a rheumatoidlike pattern that can involve hands, wrists, ankles, and feet; arthritis mutilans, which is a rare but deforming and destructive condition; arthritis of the sacroiliac joints and spine (psoriatic spondylitis).
Sequence similarities	Belongs to the tumor necrosis factor family.
Post-translational modifications	The soluble form derives from the membrane form by proteolytic processing. The membrane form, but not the soluble form, is phosphorylated on serine residues. Dephosphorylation of the membrane form occurs by binding to soluble TNFRSF1A/TNFR1. O-glycosylated; glycans contain galactose, N-acetylgalactosamine and N-acetylneuraminic acid.
Cellular localization	Secreted and Cell membrane.

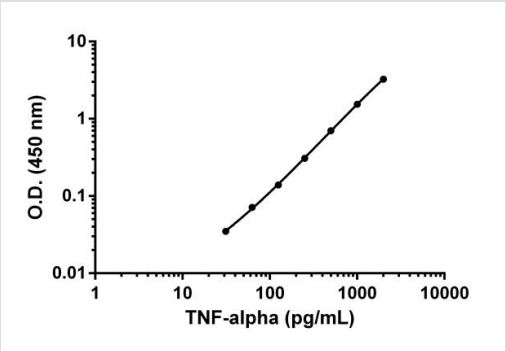
Applications

Our Abpromise guarantee covers the use of **ab181421** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

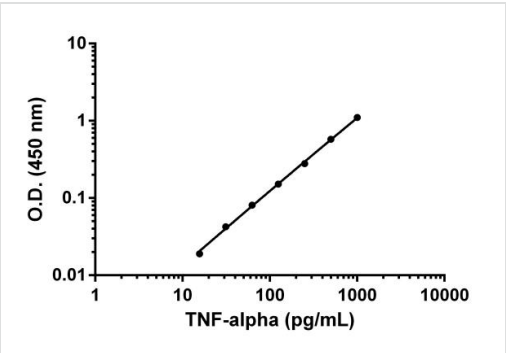
Application	Abreviews	Notes
Sandwich ELISA		Use at an assay dependent concentration.

Images



Example of TNF-alpha standard curve

Background-subtracted data values (mean +/- SD) are graphed.



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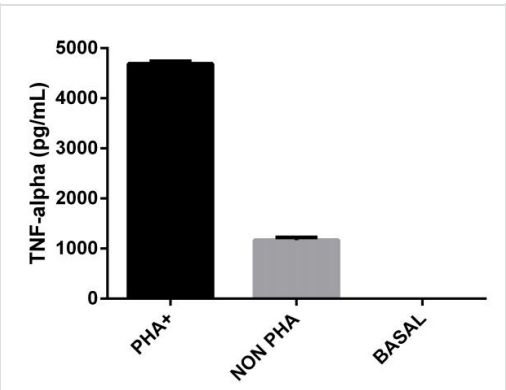

Specificity of TNF-alpha signal on stimulated and non stimulated media supernatants.

Human PBMCs were cultured in RPMI supplemented with 10% fetal calf serum, 2mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were cultured for 2 days at 37°C in the presence or absence of PHA. The concentrations of TNF-alpha were interpolated from the calibration curve and corrected for sample dilution. The mean TNF-alpha concentration was determined to be undetectable in basal media, 1.2 ng/mL in unstimulated PMBC supernatants and 4.7 ng/mL in stimulated PBMC supernatants.

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