abcam

Product datasheet

Human TNF alpha ELISA Kit ab181421

KO VALIDATED Recombinant SimpleStep ELISA

4 Abreviews 195 References 11 Images

Overview

Product name Human TNF alpha ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
PBMC media	8			2.5%

Inter-assay

Sample	n	Mean	SD	CV%
PBMC media	3			3.1%

Sample type Cell culture supernatant, Serum, Hep Plasma, EDTA Plasma, Cit plasma, Cerebral Spinal Fluid

Assay type Sandwich (quantitative)

Sensitivity 4.32 pg/ml

Range 15.63 pg/ml - 1000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture supernatant	110	105% - 113%
Serum	88	83% - 93%
Hep Plasma	98	89% - 110%
EDTA Plasma	100	95% - 104%
Cit plasma	91	86% - 97%
Cerebral Spinal Fluid	83	80% - 88%

Assay time	1h 30m
Assay duration	One step assay
Species reactivity	**Reacts with:** Human
Product overview	Human TNF alpha ELISA kit (ab181421) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of TNF alpha protein in human serum, plasma and culture media. It uses our proprietary SimpleStep ELISA® technology. Quantitate human TNFa with 4.32 pg/mL sensitivity. SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits: -Single-wash protocol reduces assay time to 90 minutes or less -High sensitivity, specificity and reproducibility from superior antibodies -Fully validated in biological samples -96-wells plate breakable into 12 x 8 wells strips A 384-well SimpleStep ELISA® microplate (**ab203359**) is available to use as an alternative to the 96-well microplate provided with SimpeStep ELISA® kits. **ASSAY SPECIFICITY** This kit recognizes both native and recombinant human TNF alpha protein in serum, plasma, and cell culture supernatant samples only. **CROSS REACTIVITY** Recombinant proteins were prepared at 1000 pg/mL and assayed for cross reactivity. No cross reactivity was found for the following targets: - Human IL-2 - Human IL-4 - Human IL-1 alpha - Human IFN gamma - Human TNF beta - Human TNF R1. **INTERFERENCE** Recombinant Human TNF R1 was prepared at 1000 pg/mL and tested for interference. No interference with was observed. **SPECIES REACTIVITY** This kit recognizes human TNF alpha protein. Other species reactivity was determined by measuring 1000 pg/mL recombinant proteins of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration assayed at the same dilution. Reactive species: Primate Reactivity < 3% was determined for the following species: Mouse/Rat **CALIBRATION** This immunoassay is calibrated against a highly purified human TNF alpha. The NIBSC/WHO unclassified purified human TNF alpha preparation 12/154 was evaluated in this kit. The dose response curve of the unclassified standard TNF alpha parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep Human TNF alpha kit to approximate NIBSC 12/154 International units, use the equation below. NIBSC (12/154) approximate value (IU/mL) = 0.094 x SimpleStep Human TNF alpha value (pg/mL).
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.

Platform Microplate

Properties

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

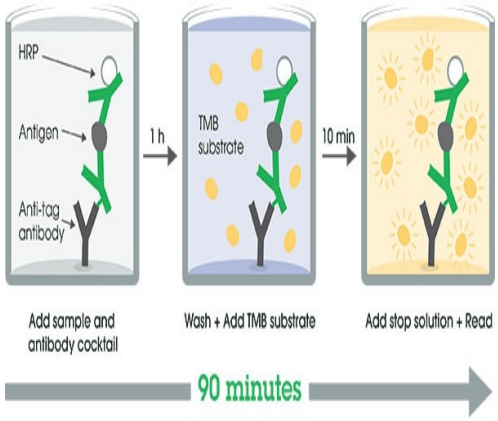
Components	1 x 96 tests	10 x 96 tests	1 x 384 tests
10X Wash Buffer PT (ab206977)	1 x 20ml	1 x 200ml	1 x 20ml
384 well CaptSure™ microplates	0 x 0 unit	0 x 0 unit	1 unit
Antibody Diluent 4BR	1 x 6ml	10 x 6ml	1 x 6ml
Human TNFa Capture Antibody	1 x 600µl	10 x 600µl	1 x 600µl
Human TNFa Detector Antibody	1 x 600µl	10 x 600µl	1 x 600µl
Human TNFa Lyophilized Recombinant Protein	2 vials	2 x 10 vials	2 vials
Plate Seals	1 unit	10 units	1 unit
Sample Diluent NS (ab193972)	1 x 50ml	2 x 250ml	2 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	10 units	0 x 0 unit
Stop Solution	1 x 12ml	1 x 120ml	2 x 12ml
TMB Development Solution	1 x 12ml	1 x 120ml	2 x 12ml

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

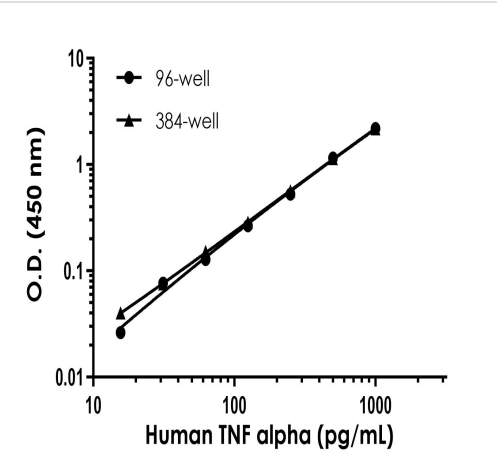
	spondylitis).
Sequence similarities	Belongs to the tumor necrosis factor family.
Post-translational modifications	The soluble form derives from the membrane form by proteolytic processing. The membrane form, but not the soluble form, is phosphorylated on serine residues. Dephosphorylation of the membrane 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.

Images



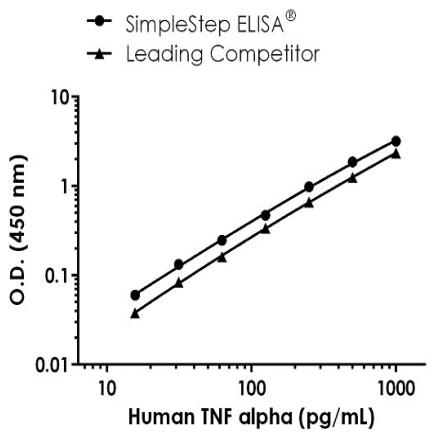
SimpleStep ELISA Protocol Diagram

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



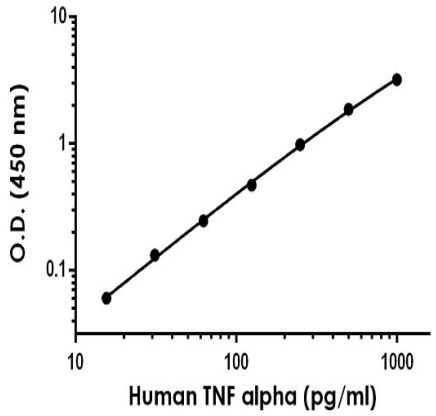
Example of human TNF alpha standard curve in Sample Diluent NS in 96-well vs. 384-well plate.

Example of human TNF alpha standard curve in 96-well vs. 384-well plate. Background-subtracted data values (mean +/- SD) are graphed.



Human TNF alpha standard curve comparison

Standard Curve comparison between human TNF alpha SimpleStep ELISA kit and traditional ELISA kit from leading competitor. SimpleStep ELISA kit shows increased sensitivity.



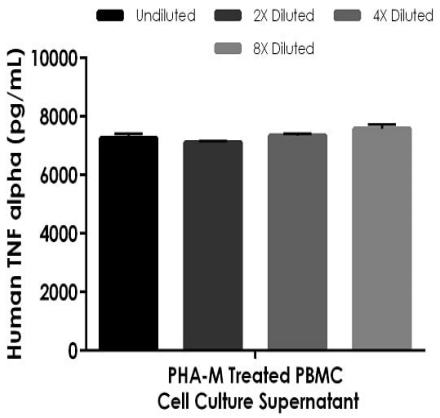
Example of human TNF alpha standard curve in Sample Diluent NS.

The TNF alpha standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.061	0.068	0.065
15.63	0.127	0.123	0.125
31.25	0.189	0.206	0.197
62.5	0.312	0.312	0.312
125	0.555	0.521	0.538
250	1.065	1.030	1.048
500	1.925	1.941	1.933
1000	3.362	3.161	3.262

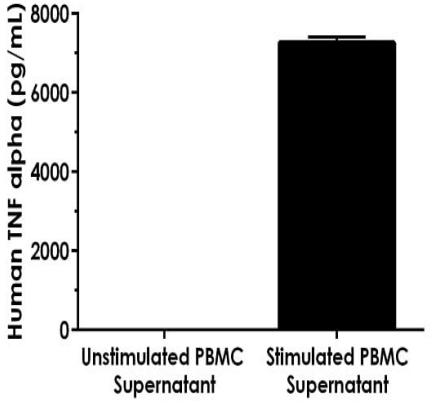
Raw data value for example of TNF standard curve in Sample Diluent NS

Raw data values are shown in the table



Interpolated concentrations of native TNF alpha in human PBMC cell culture supernatant samples.

PBMC cells were treated with 1.5% PHA-M for 46 hours in 10F RPMI. The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curve and corrected for sample dilution. Undiluted samples are PHA-M treated PBMC cell culture supernatant 10%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TNF alpha concentration was determined to be 7326.31 pg/mL in PHA-M treated PBMC cell culture supernatant.



Interpolated concentrations of native TNF alpha PBMC cell culture supernatant samples.

PBMC cells were stimulated with 1.5% PHA-M or vehicle control in 10F RPMI and incubated for 46 hours. The concentrations of TNF alpha were measured in duplicate and interpolated from the TNF alpha standard curves. Undiluted samples are PHA-M stimulated PBMC cell culture supernatant 10% and unstimulated PBMC cell culture supernatant 10%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TNF alpha concentration was back calculated determined to be 7265.71 pg/mL in PHA-M stimulated PBMC cell culture supernatant and undetectable in the unstimulated PBMC control.

Dilution Factor	Interpolated value	10% PHA-M Treated PBMC Cell Culture Supernatant
Undiluted	pg/mL	726.56
	% Expected value	100
2	pg/mL	355.55
	% Expected value	98
4	pg/mL	183.66
	% Expected value	101
8	pg/mL	94.78
	% Expected value	104

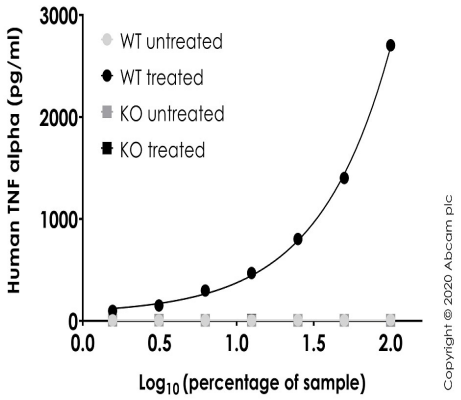
Linearity of dilution - native TNF alpha

Native TNF alpha was measured in biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	2.5% Human Serum	2.5% Human Plasma (Citrate)	2.5% Human Plasma (EDTA)	2.5% Human Plasma (Heparin)
Undiluted	pg/mL	447.20	451.09	483.71	499.11
	% Expected value	100	100	100	100
2	pg/mL	223.35	234.34	244.59	237.17
	% Expected value	100	104	101	95
4	pg/mL	119.50	127.27	123.94	120.73
	% Expected value	107	113	102	97
8	pg/mL	60.23	59.54	62.05	60.11
	% Expected value	108	106	103	96
16	pg/mL	28.78	NL	NL	NL
	% Expected value	103	NL	NL	NL

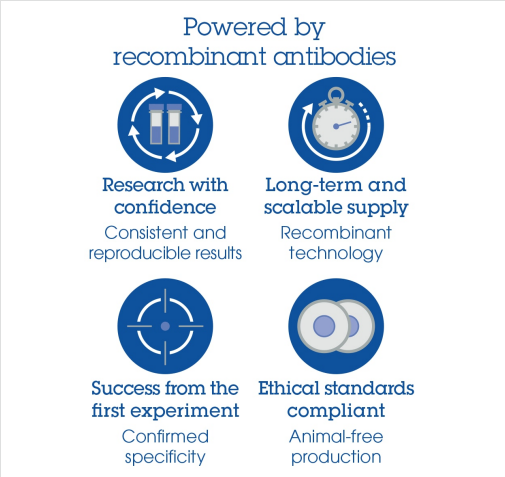
Linearity of dilution - recombinant TNF alpha

Recombinant TNF alpha was spiked biological samples and diluted in a 2-fold dilution series in Sample Diluent NS. Twenty individual healthy human female/male donors were measured in duplicate for the presence of TNF alpha. All values were below the detectable range of the assay
NL – Non-Linear



Sandwich ELISA - Human TNF alpha ELISA kit (ab181421)

Human TNF alpha concentration was interpolated from the standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human TNF alpha ELISA kit (ab181421). Wild-type THP-1 and TNF alpha knockout THP-1 (**ab273761**) cells were assessed in duplicate (n=2). Cells were either treated with 100 ng/ml LPS for 16 h to induce expression of TNF alpha or not treated with LPS. Data are represented as the mean and error bars represent standard deviation."



Sandwich ELISA - Human TNF alpha ELISA Kit (ab181421)

To learn more about the advantages of recombinant antibodies see **here**.

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