Store at 4°C

#7289

SignalKine™ Human TNF-α Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #7124
Swiss-Prot Acc. #P01375

Species Cross-Reactivity: H

Description: SignalKine™ Human TNF-α Sandwich ELISA Kit from Cell Signaling Technology (CST) is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects human TNF-α (hTNF-α) in multiple matrices. Unknown samples being tested for hTNF-α and hTNF-α standards are added to low volume microwells, where the hTNF-α is captured by the coated hTNF-α Rabbit mAb. Following a washing step, a biotinylated hTNF-α Detection Rabbit mAb is added to detect the captured hTNF-α. HRP-linked Streptavidin is then used for detection of the biotinylated hTNF-α Detection Rabbit mAb. HRP substrate, TMB, is added for color development. The magnitude of absorbance for this developed color is proportional to the quantity of hTNF-α in the sample.

SignalKine™ Human TNF-α Sandwich ELISA Kit detects hTNF-α in multiple matrices that can be quantified by generating a standard curve with the recombinant hTNF-α protein standard provided. The hTNF-α standard range is from 15.6 to 1000 pg/ml. Samples containing higher levels of hTNF-α can be diluted to fit into the standards range.

Matrices Tested: This ELISA has been validated in cell culture supernatants, plasma (citrate), plasma (EDTA), plasma (heparin), and serum.

Background: TNF-α, the prototypical member of the TNF protein superfamily, is a homotrimeric type-II membrane protein (1,2). Membrane-bound TNF-α is cleaved by the metalloprotease TACE/ADAM17 to generate a soluble homotrimer (2). Both membrane and soluble forms of TNF-α are biologically active. TNF-α is produced by a variety of immune cells including T cells, B cells, NK cells, and macrophages (1). Cellular response to TNF-α is mediated through interaction with receptors TNF-R1 and TNF-R2 and results in activation of pathways that favor both cell survival and apoptosis depending on the cell type and biological context. Activation of kinase pathways (including JNK, Erk (p44/42), p38 MAPK, and NF-κB) promotes the survival of cells, while TNF-α-mediated activation of caspase-8 leads to programmed cell death (1,2). TNF-α plays a key regulatory role in inflammation and host defense against bacterial infection, notably *Mycobacterium tuberculosis* (3). The role of TNF-α in autoimmunity is underscored by blocking TNF-α action to treat rheumatoid arthritis and Crohn's disease (1,2,4).

Storage: Following reconstitution, reagents are stable at 4°C for 1 month.

Products Included	Volume	Solution Color
hTNF-α Rabbit mAb Coated Microwells*	96 tests	
Human TNF-α Standard (Lyophilized)	1 vial	
SignalKine™ Assay Diluent A01	1.5 ml	colorless
SignalKine™ Sample Diluent S01	25 ml	brown
hTNF-α Detection Rabbit mAb (Biotinylated) (Lyophilized) 100X	1 vial	
Detection Antibody Diluent	6 ml	green
HRP-linked Streptavidin (Lyophilized) 100X	1 vial	
HRP Diluent	6 ml	red
ELISA Wash Buffer (20X)	25 ml	colorless
TMB Substrate	6 ml	colorless
STOP Solution	6 ml	colorless
Sealing Tape	2 sheets	

* 12 8-well modules -Each module is designed to break apart for 8 tests.

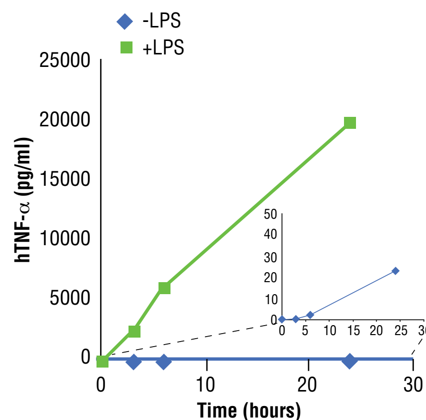

Figure 1. Time course of TNF-α secretion from THP-1 cells after LPS treatment. THP-1 cells were treated with TPA #4174 (16 nM, 18 hr) to induce differentiation. Addition of LPS (1 µg/ml) results in an increase of hTNF-α levels in the supernatant collected after 3, 6, and 24 hours compared with untreated cells using the SignalKine™ Human TNFα Sandwich ELISA Kit. Inset: Low picogram levels of hTNF-α in the untreated sample are detected by the SignalKine™ Human TNFα Sandwich ELISA Kit at 24 hours.

Background References:

(1) Aggarwal, B.B. (2003) *Nat Rev Immunol* 3, 745-56.
(2) Hehlgans, T. and Pfeffer, K. (2005) *Immunology* 115, 1-20.
(3) Lin, P.L. et al. (2007) *J Investig Dermatol Symp Proc* 12, 22-5.
(4) Brennan, F.M. and McInnes, I.B. (2008) *J Clin Invest* 118, 3537-45.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Sensitivity/Lower Limit of Detection (LLD): (< 5.2 pg/ml) Multiple assays were evaluated to calculate Sensitivity/LLD. The standard curve was run along with replicates of the zero standard. Sensitivity/LLD was determined by calculating the mean signal of the zero replicates + 2 standard deviations. This value was read off the standard curve and the concentration was determined.

The LLD range for this SignalKine™ Human TNF-α Sandwich ELISA Kit was 4.0 – 6.5 pg/ml. The mean LLD was 5.2 pg/ml.

Calibration: The hTNF-α standard used in this ELISA is a recombinant human TNF-α Val77-Leu233 (Accession # P01375) which was produced in E. coli at Cell Signaling Technology (Human Tumor Necrosis Factor-α #8902).

A natural human TNF-α standard (88/786) obtained from the WHO/NIBSC was evaluated in our assay. A conversion factor relating the CST and the WHO/NIBSC hTNF-α was determined. The following formula can be used to calculate results from the SignalKine™ Human TNF-α Sandwich ELISA Kit to the NIBSC values in International Units/ml (IU/ml):

SignalKine™ Human TNF-α Sandwich ELISA Kit value (pg/ml) x 0.0465 = NIBSC (88/876) value (IU/ml)

1 µg WHO/NIBSC hTNF-α = 1.05 µg CST hTNF-α.

Specificity: A panel of recombinant cytokines and growth factors at 1 ng/ml were tested on this ELISA. No cross-reactivity was detected with the following recombinant cytokines and growth factors:

hEGF, hBAFF, hGM-CSF, hG-CSF, HGF, hIFN- γ, hIL-1α, hIL-1β, hIL-2, hIL-3, hIL-4, hIL-6, hIL-8, hIL-10, hIL-13, hIL-17A, hIL-17F, Leptin, hSCF, hTGF-β1, hTGF-β2, hTGF-β3, mIL-3, mTNF-α, hIGF-I.

Spike/Recovery: Recombinant hTNF-α was spiked into cell culture supernatant, plasma, and serum at three different concentrations. The percent recovery is determined by comparing hTNF-α concentrations, using this ELISA, with expected concentrations.

% Recovery			
Expected Concentrations	50 pg/ml	150 pg/ml	600 pg/ml
Cell Culture Supernatant	104.4	93.5	96.8
Plasma (Citrate)	103.1	93.8	90.3
Plasma (EDTA)	97.7	99.6	90.2
Plasma (Heparin)	97.4	97.2	89.9
Serum	92.1	93.8	90.4

Linearity of Dilution: hTNF-α spiked into various matrices and then serially diluted with SignalKine™ Sample Diluent S01 or cell culture media. Percent recoveries were then determined to demonstrate the ELISA's linearity with dilution.

% Recovery					
hTNF-α (pg/ml)	Cell Culture Supernatant	Plasma (Citrate)	Plasma (EDTA)	Plasma (Heparin)	Serum
800	99.3	95.3	91.5	99.5	93.5
400	104.4	91.8	97.0	99.6	98.5
200	103.3	94.0	92.9	96.3	93.5
100	122.0	101.0	84.2	98.0	83.7
50	109.8	91.2	82.3	87.6	72.5

Precision/Reproducibility:

Intra-assay Precision – Multiple replicates at three concentrations looking at the cell culture supernatant assay, and plasma (citrate), plasma (EDTA), plasma (heparin), serum assay were run on a single plate and the values were assessed for precision/reproducibility.

Inter-assay Precision – Multiple replicates at three concentrations looking at the cell culture supernatant assay, and plasma (citrate), plasma (EDTA), plasma (heparin), serum assay were run on multiple plates over multiple days and assessed for precision/reproducibility.

Precision/Reproducibility						
Cell Culture Supernatent Assay	**Intra-Assay Precision**			**Inter-Assay Precision**		
Expected Conc. (pg/ml)	50	150	600	50	150	600
Average (pg/ml)	48.7	162.9	656.9	47.0	152.9	633.8
Std Deviation	2.8	10.8	14.3	2.3	6.5	21.0
CV (%)	5.8	6.6	2.2	4.9	4.2	3.3
Plasma (Citrate), Plasma (EDTA), Plasma (Heparin), Serum	**Intra-Assay Precision**			**Inter-Assay Precision**		
Expected Conc. (pg/ml)	50	150	600	50	150	600
Average (pg/ml)	47.7	162.7	634.6	46.0	152.9	626.1
Std Deviation	3.9	13.6	56.9	2.0	10.3	30.5
CV (%)	8.1	8.4	9.0	4.4	6.8	4.9

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SignalKine™ Sandwich ELISA Protocol

This ELISA was developed for the detection and quantization of cytokines, chemokines and growth factors in cell culture media, serum and plasma. Recombinant protein standards are run alongside samples. Concentration of target in samples can be determined from the standard curve. We recommend running replicates of standards and samples.

Reagent Preparation

All reagents should be brought to room temperature before use.

Wash Buffer: Prepare 1X wash buffer by diluting ELISA Wash Buffer (20X) in dH_2O.

SignalKine™ Assay Diluent A01: Buffered solution supplied as a 5X solution. 10 µl of Assay Diluent 1 is added to each well before addition of 40 µl of standard and samples.

SignalKine™ Sample Diluent S01: Diluent for diluting standards and samples when working with plasma (citrate), plasma (EDTA), plasma (heparin), or serum samples.

hTNF-α Detection Rabbit mAb (Biotinylated): Supplied lyophilized. Reconstitution with 60 µl of dH_2O yields a 100X Concentrated Stock solution. Incubate at room temperature for 15 minutes with occasional gentle mixing to fully reconstitute. Dilute 100X solution 1:100 in Detection Antibody Diluent (green solution). A 96 well plate will need 50 µl/well of 1X solution, so approximately 5 ml of reagent is needed. Dilute 50 µl of 100X stock into 5 ml of Detection Antibody Diluent or dilute as much as needed if not using full 96 well plate.

HRP-linked Streptavidin: Supplied lyophilized. Reconstitution with 60 µl of dH_2O yields a 100X concentrated stock solution. Incubate at room temperature for 15 minutes with occasional gentle mixing to fully reconstitute. Dilute 100X solution 1:100 in HRP Diluent (red solution). A 96 well plate requires 50 µl/well of 1X solution, so approximately 5 ml of reagent is needed. Dilute 50 µl of 100X stock into 5 ml of HRP Diluent or dilute as much as needed if not using full 96 well plate.

TMB Substrate: Supplied as a single component colorimetric HRP substrate. A blue color reaction is indicative of the presence of HRP and a positive result.

STOP Solution: STOP solution is added to TMB substrate to stop the HRP reaction. STOP solution converts blue solution to yellow, which is then read on a microplate reader at 450 nm.

hTNF-α Standard Preparation

Add 0.1 ml dH_2O to reconstitute Human TNF-α Standard (Lyophilized) (10,000 pg) to yield a 100,000 pg/ml solution. Let sit at room temperature for 15 minutes with occasional gentle mixing to fully reconstitute standard protein.

For detection in plasma (citrate), plasma (EDTA), plasma (heparin), and serum, samples and protein standards are diluted with **SignalKine™ Sample Diluent S01**, (provided in kit). For cell culture media, samples and protein standards are diluted in **fresh cell culture media** corresponding to sample media (not supplied).

A standard range of 1000, 500, 250, 125, 62.5, 31.25, 15.6 and 0 pg/ml is set up. This can be achieved by diluting the protein standard, (100,000 pg/ml) 1:100, which yields 1000 pg/ml. From the 1000 pg/ml solution, serial two-fold dilutions are performed to achieve the other standard concentrations.

We recommend diluting 10 µl of the 100,000 pg/ml protein standard into 990 µl of either **SignalKine™ Sample Diluent S01**, or **cell culture medium** to yield the 1000 pg/ml standard. Serial two-fold dilution can be achieved by setting up six tubes with 200 µl of the appropriate diluent and then serially transferring 200 µl from the preceding tube. Mix well before each transfer. Use the appropriate diluent as the 0 pg/ml standard. (See graphic representation to right).

Sample Preparation

This assay has been validated with cell culture medium, plasma (citrate), plasma (EDTA), plasma (heparin), and serum samples. After sample collection, store in single use aliquots at -20°C. Avoid multiple freeze/thawing.

Particulates in cell culture media should be removed by centrifugation. Note any contaminated, clotted, or hemolyzed samples and interpret these results judiciously.

Absorbance values falling within the hTNF-α standard curve range can be determined directly from the standard curve. Sample absorbance values higher than the 1000 pg/ml hTNF-α value should be diluted so that the absorbance values fall into standard curve range.

Procedure

1. Once microwell strips have reached room temperature, take out as many strips as needed and insert into strip holder. Unused microwell strips should be resealed and stored at 4°C.
2. Add 10 µl of 5X Assay Diluent 1 to each well.
3. Add 40 µl of hTNF-α Standards and sample to the wells. Cover with plate sealer and incubate for 2 hours at room temperature.
4. Wash plates 4 times with 1X Wash Buffer.

Note: Maximum wash volume is 170 µl/well. These are low volume microwells and have a smaller volume capacity than full size microwells.

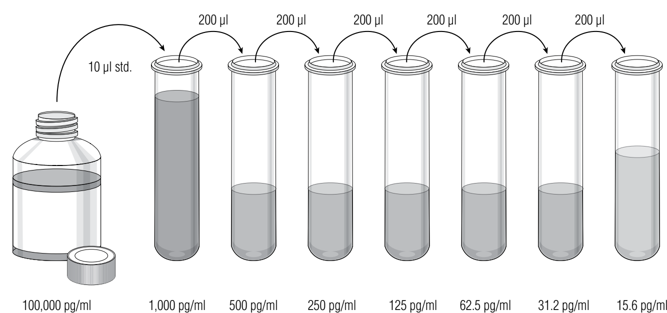
5. Add 50 µl of 1X hTNF-α Detection Rabbit mAb (Biotinylated) per well. Cover with plate sealer and incubate for 1 hour at room temperature.
6. Wash plates 4 times with 1X Wash Buffer.
7. Add 50 µl of 1X HRP-linked Streptavidin per well. Cover with plate sealer and incubate for 30 minutes at room temperature.
8. Wash plates 4 times with 1X Wash Buffer.
9. Add 50 µl of TMB substrate per well. Cover with plate sealer and incubate for 10 minutes at room temperature.
10. After the 10 minutes incubation, add 50 µl of STOP solution per well.
11. Read on a microplate reader, absorbance wavelength set at 450 nm. Plate should be read within 30 minutes.

Calculation of Results

Average replicates for standards and samples.

Using a graphing/curve fitting software, plot a 4-parameter logistic curve fit. A standard curve can also be generated by plotting the standard concentration on the x-axis and the absorbance corresponding to the standard concentration on the y-axis. A best-fit curve is drawn through the points on the graph.

Sample concentrations are determined by interpolating from the standard curves. Account for sample dilution by multiplying determined concentration by dilution factor.



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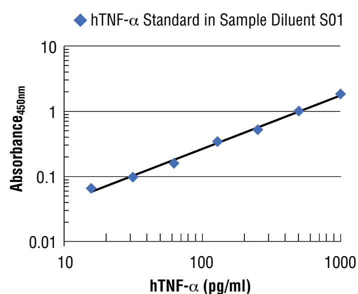
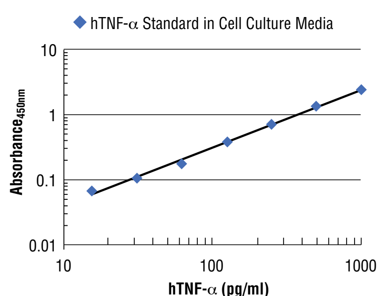
SignalKine™ Sandwich ELISA Protocol

Typical Data

Represented below are hTNF-α Standard Curves, one diluted in SignalKine™ Sample Diluent S01, and a second diluted in cell culture media (RPMI + 10% FBS). Although these are typical of the standard curves that will be generated using this kit, a new set of standards should be run for each new experiment.





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