

Product datasheet

Mouse TNF alpha ELISA Kit ab208348

Recombinant SimpleStep ELISA

★★★★☆ [4 Abreviews](#) [177 References](#) [13 Images](#)

Overview

Product name Mouse TNF alpha ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

| Sample | n | Mean | SD | CV% |
|---------|---|------|----|------|
| Overall | 8 | | | 6.7% |

Inter-assay

| Sample | n | Mean | SD | CV% |
|---------|---|------|----|------|
| Overall | 3 | | | 9.8% |

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

Assay type Sandwich (quantitative)

Sensitivity 9.1 pg/ml

Range 46.88 pg/ml - 3000 pg/ml

Recovery

Sample specific recovery

| Sample type | Average % | Range |
|--------------------|-----------|-------------|
| Serum | 97 | 85% - 115% |
| Cell culture media | 111 | 109% - 115% |
| Hep Plasma | 92 | 85% - 103% |
| EDTA Plasma | 99 | 98% - 101% |
| Cit plasma | 87 | 85% - 88% |

Assay time 1h 30m

Assay duration One step assay

Species reactivity

Product overview

Reacts with: Mouse

Mouse TNF alpha ELISA Kit (ab208348) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of TNF alpha protein in cell culture supernatant, serum, cit plasma, edta plasma, and hep plasma. It uses our proprietary SimpleStep ELISA® technology. Quantitate Mouse TNF alpha with 9.1 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 SimpleStep ELISA® kits.

ASSAY SPECIFICITY

This kit recognizes both native and recombinant mouse TNF alpha protein in serum, plasma, and cell culture supernatant samples only.

CROSS REACTIVITY

Recombinant mouse TNF RI, TNF RII, OPG, CD40 receptor, IL1 beta, IL-16, and IL-5 were prepared at 100 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

Recombinant human TNF alpha was prepared at 100 ng/mL, 25 ng/mL, and 3 ng/mL and assayed for cross reactivity. On average, 3.0% cross-reactivity was observed with a standard deviation of 0.7%.

INTERFERENCE

Recombinant mouse TNF RI, TNF RII, OPG, CD40 receptor, IL1 beta, IL-16, and IL-5 and recombinant human TNF alpha were prepared at 100 ng/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

This kit recognizes mouse TNF alpha protein.

CALIBRATION

This immunoassay is calibrated against a highly purified mouse TNF alpha. The NIBSC/WHO unclassified purified mouse TNF alpha preparation 88/532 was evaluated in this kit.

The dose response curve of the unclassified standard 88/532 parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep mouse TNF alpha kit to approximate NIBSC 88/532 units, use the equation below.

NIBSC (88/532) approximate value (units/mL) = 2.0 x SimpleStep mouse TNF alpha value (pg/mL)

Notes

Tumor necrosis factor, or TNF alpha, is a cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFR2. Furthermore, TNF alpha is a ligand of the TNF superfamily which plays a central role in inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis, and morphogenesis. It is mainly secreted by macrophages, and can induce cell death of certain tumor cell lines. Rat and Human TNF alpha are 95% and 79% identical to Mouse TNF alpha, respectively.

TNF alpha is expressed on macrophages and endothelial, epithelial, and tumor cells as a 26 kDa transmembrane protein. TNF-alpha is cleaved by proteolytic processing into six chains: (1) TNF membrane form, (2) Intracellular domain 1 (ICD1), (3) Intracellular domain 2 (ICD2), (4) C-domain 1, (5) C-domain 2 and (6) TNF soluble form. ICD1 and ICD2 are released into the cytosol, while C-domain 1 and C-domain 2 are released into the extracellular space. Furthermore, 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.

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. Likewise, the TNF intracellular domain (ICD) form induces IL-12 production in dendritic cells.

Platform

Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions

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

| Components | 1 x 96 tests | 1 x 96 tests |
|---|--------------|--------------|
| 10X Mouse TNF alpha Capture Antibody | 1 x 600µl | 1 x 600µl |
| 10X Mouse TNF alpha Detector Antibody | 1 x 600µl | 1 x 600µl |
| 10X Wash Buffer PT (ab206977) | 1 x 20ml | 1 x 20ml |
| Antibody Diluent CPI2 | 1 x 6ml | 1 x 6ml |
| Mouse TNF alpha Lyophilized Recombinant Protein | 2 vials | 2 vials |
| Plate Seals | 1 unit | 1 unit |

| Components | 1 x 96 tests | 1 x 96 tests |
|---|--------------|--------------|
| Sample Diluent NBS | 1 x 20ml | 1 x 20ml |
| Sample Diluent NS (ab193972) | 1 x 50ml | 1 x 50ml |
| SimpleStep Pre-Coated 96-Well Microplate (ab206978) | 1 unit | 1 unit |
| Stop Solution | 1 x 12ml | 1 x 12ml |
| TMB Development Solution | 1 x 12ml | 1 x 12ml |

Function Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFR2. 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.

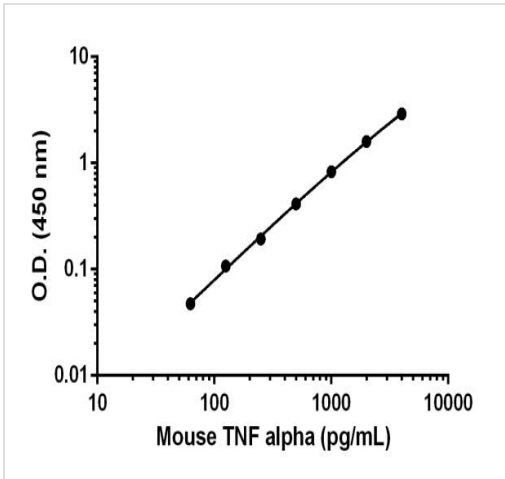
Involvement in disease Genetic variations in TNF are a cause of susceptibility psoriatic arthritis (PSORAS) [MIM:607507]. PSORAS is an inflammatory, seronegative arthritis associated with psoriasis. It is a heterogeneous disorder ranging from a mild, non-destructive disease to a severe, progressive, 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



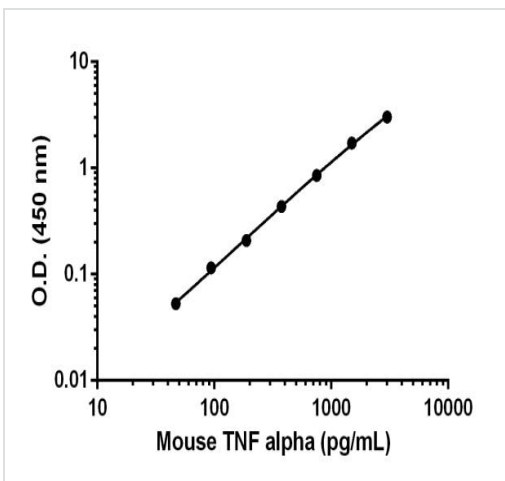
Example of mouse TNF alpha standard curve in Sample Diluent 25BS

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

| Standard Curve Measurements | | | |
|-----------------------------|-------------|-------|-----------|
| Conc. (pg/mL) | O.D. 450 nm | | Mean O.D. |
| | 1 | 2 | |
| 0 | 0.054 | 0.066 | 0.060 |
| 62.5 | 0.107 | 0.107 | 0.107 |
| 125 | 0.172 | 0.163 | 0.167 |
| 250 | 0.257 | 0.250 | 0.253 |
| 500 | 0.481 | 0.468 | 0.474 |
| 1,000 | 0.904 | 0.879 | 0.891 |
| 2,000 | 1.693 | 1.633 | 1.663 |
| 4,000 | 3.001 | 2.924 | 2.962 |

Example of mouse TNF alpha standard curve in Sample Diluent 25BS.

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



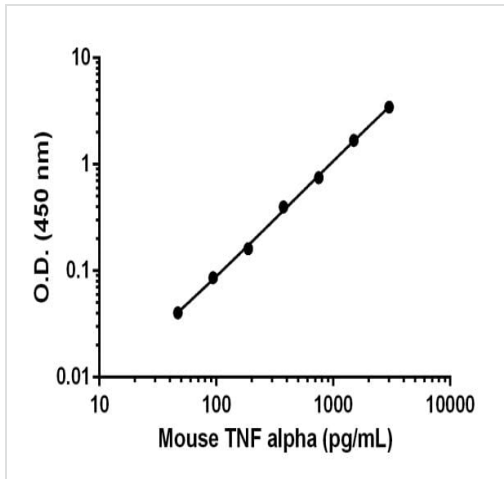
Example of mouse TNF alpha standard curve in Sample Diluent 10BS

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

| Standard Curve Measurements | | | |
|-----------------------------|-------------|-------|--------------|
| Conc. (pg/mL) | O.D. 450 nm | | Mean O.D. |
| | 1 | 2 | |
| 0 | 0.088 | 0.070 | 0.079 |
| 46.88 | 0.132 | 0.132 | 0.132 |
| 93.75 | 0.187 | 0.202 | 0.194 |
| 187.5 | 0.280 | 0.293 | 0.287 |
| 375 | 0.519 | 0.511 | 0.515 |
| 750 | 0.934 | 0.935 | 0.935 |
| 1,500 | 1.797 | 1.806 | 1.801 |
| 3,000 | 3.098 | 3.116 | 3.107 |

Example of mouse TNF alpha standard curve in Sample Diluent 10BS.

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



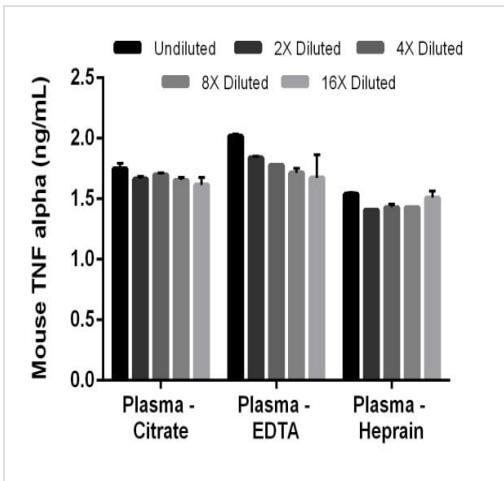
Example of mouse TNF alpha standard curve in Sample Diluent NS

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

| Standard Curve Measurements | | | |
|-----------------------------|-------------|-------|--------------|
| Conc. (pg/mL) | O.D. 450 nm | | Mean O.D. |
| | 1 | 2 | |
| 0 | 0.070 | 0.076 | 0.073 |
| 46.88 | 0.131 | 0.132 | 0.132 |
| 93.75 | 0.198 | 0.183 | 0.191 |
| 187.5 | 0.306 | 0.309 | 0.308 |
| 375 | 0.520 | 0.537 | 0.529 |
| 750 | 0.965 | 0.963 | 0.964 |
| 1,500 | 1.840 | 1.821 | 1.830 |
| 3,000 | 3.046 | 3.134 | 3.090 |

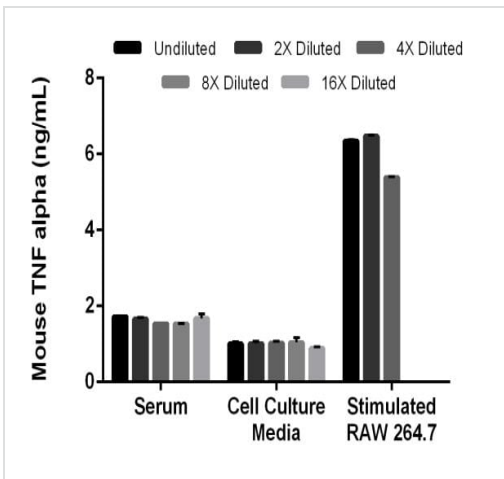
Example of mouse TNF alpha standard curve in Sample Diluent NS.

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



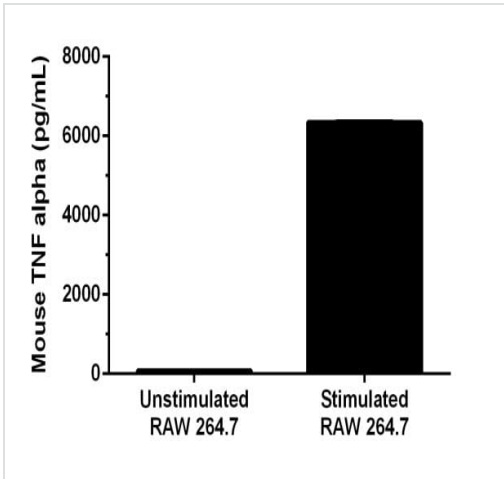
Interpolated concentrations of spiked TNF alpha in mouse plasma samples

The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curves and corrected for sample dilution. Undiluted samples are as follows: plasma (citrate) 100%, plasma (EDTA) 100%, and plasma (heparin) 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TNF alpha concentration was determined to be 1.68 ng/mL in plasma (citrate), 1.81 ng/mL in plasma (EDTA) and 1.47 ng/mL in plasma (heparin).



Interpolated concentrations of spiked mouse TNF alpha in mouse serum and cell culture samples and native mouse TNF alpha in mouse RAW 264.7 LPS stimulated cell culture supernatants

The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 100%, cell culture 100%, and RAW 264.7 LPS stimulated cell culture supernatants 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TNF alpha concentration was determined to be 1.63 ng/mL in serum, 1.00 ng/mL in cell culture and 6.07 ng/mL in RAW 264.7 LPS stimulated cell culture supernatants.



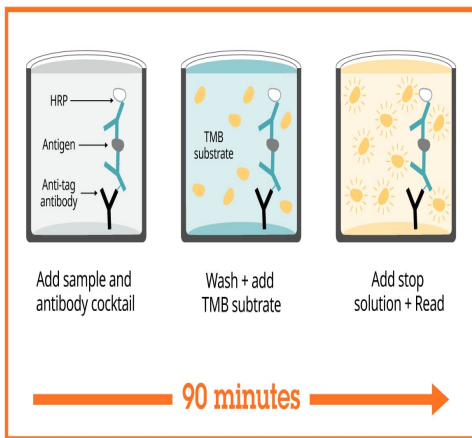
Interpolated concentrations of native TNF alpha in mouse RAW 264.7 LPS stimulated and unstimulated cell culture supernatant samples

The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curves and corrected for sample dilution. Undiluted samples are as follows: RAW 264.7 LPS stimulated supernatant 50% and RAW 264.7 LPS unstimulated supernatant 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TNF alpha concentration was determined to be 6346.4 pg/mL in RAW 264.7 LPS stimulated supernatant and 83.7 pg/mL in RAW 264.7 LPS unstimulated supernatant. RAW 264.7 cells were cultured in HGMEM with 100 µg/mL Kanamycin and 2 mM L-glutamine. Cells were starved for 24 hours and treated in the presence and absence of 5 µg/mL of LPS.

| Sample Diluent Buffer | n= | Minimal Detectable Dose |
|-----------------------|----|-------------------------|
| Sample Diluent NS | 24 | 9.8 pg/mL |
| Sample Diluent 10BS | 24 | 12.1 pg/mL |
| Sample Diluent 25BS | 24 | 9.1 pg/mL |

Assay sensitivity

The calculated minimal detectable dose (MDD) is determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentrations. The MDD is dependent on the Sample Diluent buffer used.



Sandwich ELISA - Mouse TNF alpha ELISA Kit (ab208348)

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.

Powered by
recombinant antibodies



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Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Sandwich ELISA - Mouse TNF alpha ELISA Kit
(ab208348)

To learn more about the advantages of recombinant antibodies see [here](#).

Get more done with
SimpleStep ELISA



Easy to use
Single-wash 90-minute protocol



Flexible
Matched antibody pairs available



Precision antibodies
High sensitivity, specificity and reproducibility



Scalable
Now in 10-pack and 384-well formats

Sandwich ELISA - Mouse TNF alpha ELISA Kit
(ab208348)

To learn more about the advantages of SimpleStep ELISA[®] kits see [here](#).

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