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ab229393
Mouse TNF alpha CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of TNF alpha in mouse serum, plasma, and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

TNF alpha *in vitro* CatchPoint SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of TNF alpha protein in mouseserum, plasma, and cell culture supernatants.

The CatchPoint 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. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

Tumor necrosis factor, or TNF alpha, is a cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 20 minutes

Read fluorescence at Ex/Cutoff/Em 530/570/590 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab208348).

6. Materials Supplied

Item	Quantity	Storage Condition
Mouse TNF alpha Capture Antibody 10X	600 µL	+4°C
Mouse TNF alpha Detector Antibody 10X	600 µL	+4°C
Mouse TNF alpha Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+4°C
Sample Diluent NS*	50 mL	+4°C
Sample Diluent NBS	20 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

*Sample Diluent NS is provided but not necessary for this product.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The incubation times provided in this protocol were optimized for fastest results with good signal. It is possible to increase the signal with longer incubation times, further optimization might be necessary.
- Keep in mind any RFU values shown are relative, NOT absolute. RFU from one plate reader are not comparable to another, especially if different make or model.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Sample Diluent **[BS]** may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 Sample Diluent 25BS:

Prepare Sample Diluent 25BS by diluting Sample Diluent NBS with Sample Diluent NS. To make 10mL Sample Diluent 25BS combine 2.5 mL Sample Diluent NBS with 7.5 mL Sample Diluent NS.

9.2 Sample Diluent 10BS:

Prepare Sample Diluent 10BS by diluting Sample Diluent NBS with Sample Diluent NS. To make 10 mL Sample Diluent 10BS combine 1 mL Sample Diluent NBS with 9 mL Sample Diluent NS.

9.3 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.4 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI. Mix thoroughly and gently.

9.5 CatchPoint HRP Development Solution

Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer.

For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the TNF alpha by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the TNF alpha standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL **Stock Standard** Solution.

For **serum and plasma (heparin) samples** reconstitute the TNF alpha standard sample by adding Sample Diluent 25BS.
For **plasma (citrate), plasma (EDTA) samples** reconstitute the TNF alpha standard sample by adding Sample Diluent 10BS.
For **cell culture supernatant samples** reconstitute the TNF alpha standard sample by adding Sample Diluent NS.

10.2 Label 11 tubes, Standards 1– 11.

10.3 Add 120 µL appropriate diluent (see section 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2-11.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #11 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 11 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #2-8.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	180	120	10,000	6,000
2	Standard#1	150	150	6,000	3000
3	Standard#2	150	150	3000	1500
4	Standard#3	150	150	1500	750
5	Standard#4	150	150	750	375
6	Standard#5	150	150	375	187.5
7	Standard#6	150	150	187.5	93.75
8	Standard#7	150	150	93.75	46.88
9	Standard#8	150	150	46.88	23.44
10	Standard#9	150	150	23.44	11.72
11	Standard#10	0	150	0	0

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Mouse Plasma - Heparin	6.25 – 100
Mouse Plasma - EDTA	6.25 – 100
Mouse Plasma - Citrate	6.25 – 100
Mouse Serum	6.25 – 100
RAW 264.7 LPS Stimulated Supernatant	12.5 – 50
Cell Culture Media	6.25 – 100

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute citrate and EDTA samples into Sample Diluent 10BS and assay. Dilute heparin samples into Sample Diluent 25BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent 25BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well fluorescence or "edge effects" have not been observed with this assay.
- Ensure plate and all materials are equilibrated to room temperature during use.
- Cover the plate with a plate seal during incubation steps.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 20 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.

13.8 Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org

Mode:	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

*For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

13.9 Analyze the data as described below.

14. Calculations

14.1 Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices

14.2 Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.

14.3 Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

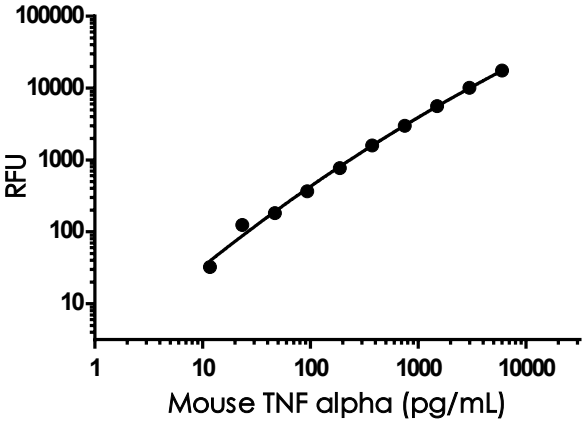
Δ Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.4 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.5 Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (pg/mL)	RFU		Mean RFU
	1	2	
0	87	92	90
11.72	117	129	123
23.44	248	187	218
46.88	309	241	275
93.75	464	448	456
187.5	864	858	861
375	1,740	1,630	1,685
750	3,078	3,072	3,079
1,500	5,742	5,659	5,700
3,000	10,919	9,588	10,254
6,000	17,785	17,412	17,599

Figure 1. Example of mouse TNF alpha standard curve in Sample Diluent NS. The TNF alpha standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. 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 CatchPoint SimpleStep standard curve. To convert sample values obtained with the CatchPoint 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 CatchPoint SimpleStep mouse TNF alpha value (pg/mL)

17. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 8.5 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of TNF alpha recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Mouse Plasma – Heparin	92	85 – 103
Mouse Plasma – EDTA (100%)	99	98 – 101
Mouse Plasma – Citrate	87	85 – 88
Mouse Serum (100%)	97	85 – 115
Cell Culture Media (100%)	111	109 – 115

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native mouse TNF alpha was measured in RAW 264.7 LPS stimulated media in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS. Recombinant mouse TNF alpha was spiked into cell culture media and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% RAW 264.7 LPS Stimulated Media	100% Cell Culture Media
Undiluted	pg/mL	3,173.3	1,010.3
	% Expected value	100	100
2	pg/mL	1619.1	512.1
	% Expected value	102	101
4	pg/mL	673.6	258.3
	% Expected value	85	102
8	pg/mL	NL	135.5
	% Expected value	NL	107
16	pg/mL	NL	55.6
	% Expected value	NL	88

NL – Non-Linear

Recombinant mouse TNF alpha was spiked into mouse serum and mouse heparin plasma and diluted in a 2-fold dilution series in Sample Diluent 25BS. Recombinant mouse TNF alpha was spiked into mouse citrate and mouse EDTA plasmas and diluted in a 2-fold dilution series in Sample Diluent 10BS.

Dilution Factor	Interpolated value	100% Mouse Serum	100% Mouse Plasma (Citrate)	100% Mouse Plasma (EDTA)	100% Mouse Plasma (Heparin)
Undiluted	pg/mL	1,724.5	1,750.2	2,019.5	1,544.2
	% Expected	**100**	**100**	**100**	**100**
2	pg/mL	832.3	832.8	922.0	705.4
	% Expected	**97**	**95**	**91**	**91**
4	pg/mL	384.8	424.9	444.4	357.7
	% Expected	**89**	**97**	**88**	**93**
8	pg/mL	191.5	206.8	214.3	178.8
	% Expected	**89**	**95**	**85**	**93**
16	pg/mL	104.6	101.1	104.4	94.4
	% Expected	**97**	**92**	**83**	**98**

PRECISION –

Mean coefficient of variations of interpolated values of TNF alpha from three concentrations of RAW 264.7 LPS stimulated media within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	6.7	9.8

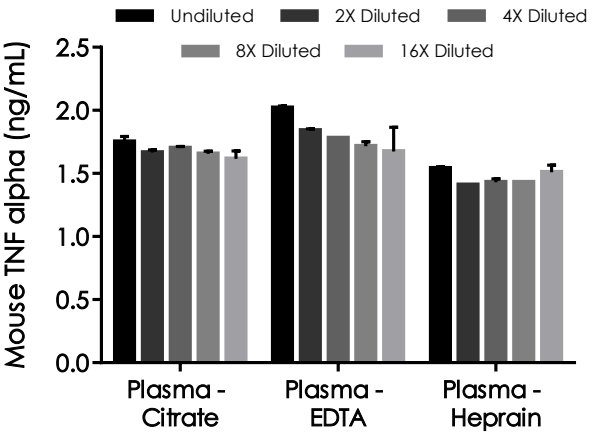


Figure 2. Interpolated concentrations of spiked TNF alpha in mouse plasma samples. The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curves and corrected for sample dilution. Undiluted samples are as follows: plasma (citrate) 100%, plasma (EDTA) 100%, 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).

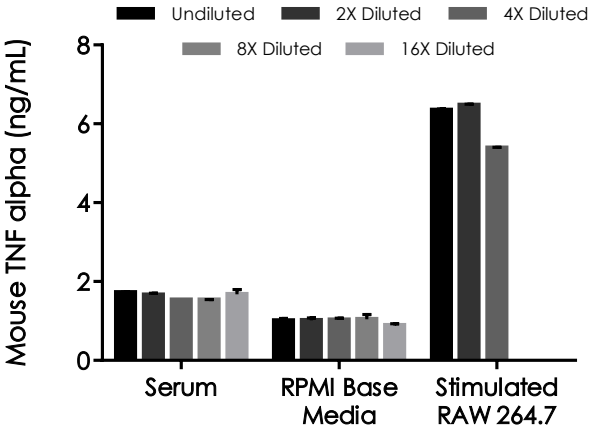


Figure 3. Interpolated concentrations of spiked mouse TNF alpha in mouse serum and cell culture samples and native mouse TNF alpha in mouse RAW 264.7 LPS stimulated cell culture supernatants. The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curves 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.

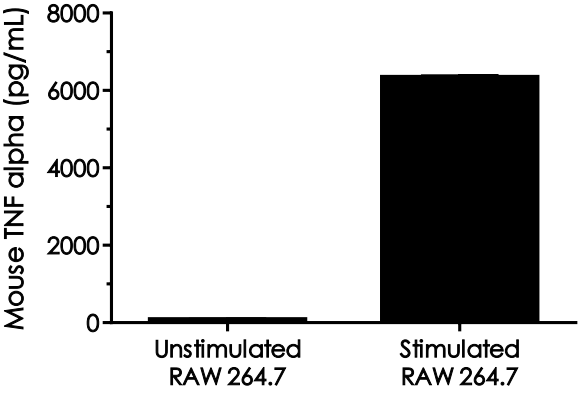


Figure 4. Interpolated concentrations of native TNF alpha in mouse RAW 264.7 LPS stimulated and unstimulated cell culture supernatant samples. The concentrations of TNF alpha were measured in duplicates, interpolated from the TNF alpha standard curves and corrected for sample dilution. Undiluted samples 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 HGDMEM 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.

18. 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.

19. Species Reactivity

This kit recognizes mouse TNF alpha protein.

Please contact our Technical Support team for more information.

20. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with CatchPoint HRP Development Solution too brief	Read plate again after longer incubation time
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep Stoplight Red Substrate protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)