

Speedy™ Human/Cynomolgus Monkey TNF beta One-Step ELISA Kit

Datasheet

Please read it entirely before use

Catalogue Number: SE50234

Size: 96T

Sensitivity: 1.1 pg/mL

Range: 7.8-500 pg/mL

Usage: For the quantitative detection of human/cynomolgus monkey TNF beta concentration in cell culture supernatant.

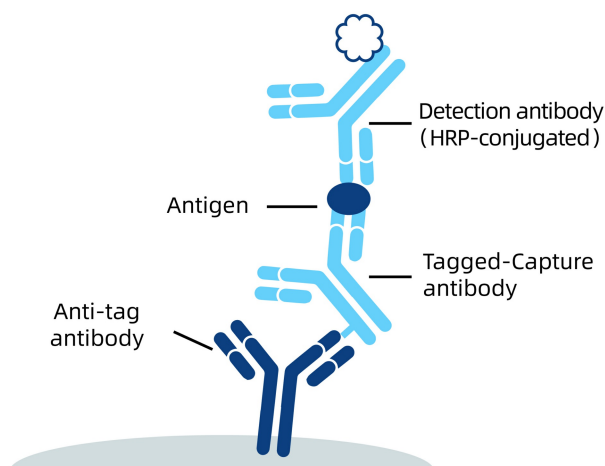
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1. Background

TNF beta also known as lymphotoxin alpha (LTA), is a member of the tumor necrosis factor family, and is a cytokine produced by lymphocytes. TNF beta is highly inducible, secreted, and forms heterotrimers with lymphotoxin-beta which anchor lymphotoxin-alpha to the cell surface. TNF beta also mediates a large variety of inflammatory, immunostimulatory, and antiviral responses, is involved in the formation of secondary lymphoid organs during development and plays a role in apoptosis.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, <https://www.ptgcn.com/products/elisa-kits/>.
- 3.8 Microplate thermostatic shaker.

4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 1000 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Sample Diluent PT 3 - 30 mL/bottle	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

- 6.1 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500×g and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 μ L 100X capture antibody + 50 μ L 100X Detection Antibody, HRP-conjugated + 4,900 μ L Detection Diluent. Mix gently but thoroughly.

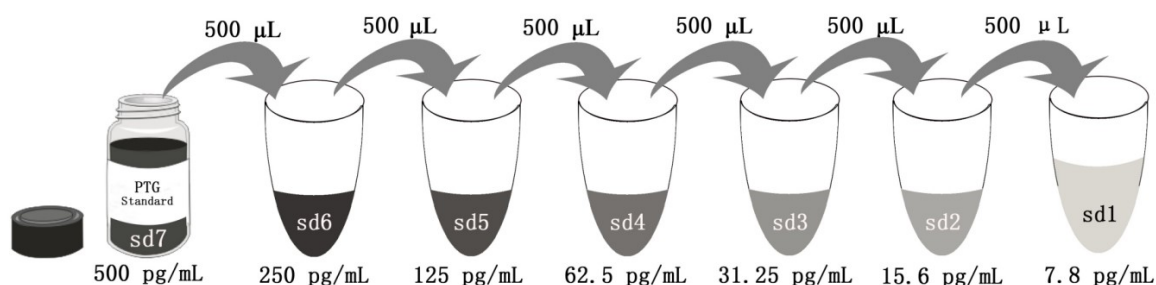
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 μ L of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 to 1:20 is recommended for cell culture supernatant.

7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 3 in protein standard.



Add # μ L of Standard diluted in the previous step	—	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L
# μ L of Sample Diluent PT 3	2000 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available).

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

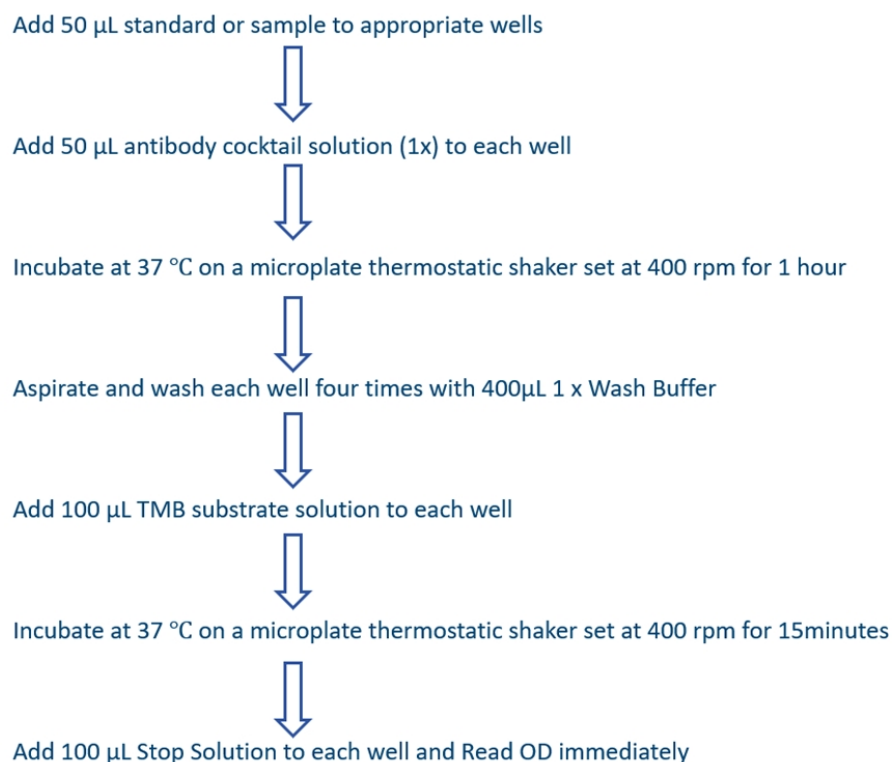
8.5 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve-fit (4-PL) analysis is recommended. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

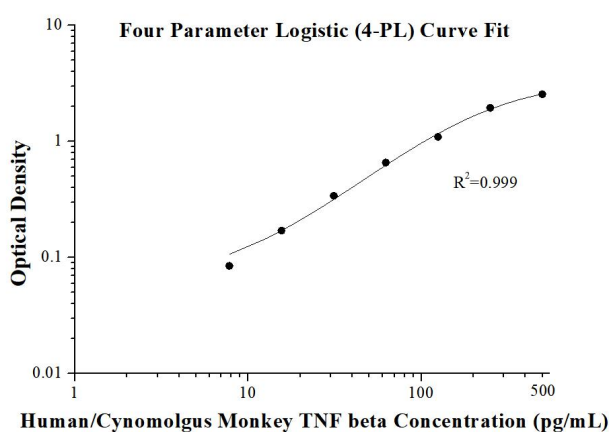
Procedure summary



9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0365 0.02	0.0283	-
7.8	0.1154 0.1102	0.1128	0.0846
15.6	0.2007 0.1963	0.1985	0.1703
31.25	0.3449 0.3919	0.3684	0.3402
62.5	0.6779 0.6963	0.6871	0.6589
125	1.1832 1.0646	1.1239	1.0957
250	1.9834 1.9671	1.9753	1.9470
500	2.5738 2.5839	2.5789	2.5506

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	8	245.0	2.5	1.0	1	16	246.5	3.6	1.5
2	8	76.0	1.3	1.7	2	16	75.3	1.5	2.0
3	8	43.6	1.0	2.3	3	16	42.7	1.4	3.3

9.3 Recovery

The recovery of human/cynomolgus monkey TNF beta spiked to three different levels throughout the range of the assay in cell culture supernatant was evaluated.

Sample Type		Average% of Expected	Range (%)
Human cell culture supernatant	1:2	104	103-106
	1:4	104	104-105
Cynomolgus monkey cell culture supernatant	1:2	97	94-103
	1:4	99	96-101

9.4 Sample values

Cell culture supernatant:

Human peripheral blood mononuclear cells (PBMC) (1×10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 5 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA for 1 day and 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of TNF beta.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)
Unstimulated	ND	ND
Stimulated	1,419.8	9,855.2

Cynomolgus monkey peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 5 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA for 1 day and 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of TNF beta.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)
Unstimulated	ND	ND
Stimulated	518.2	764.7

ND*=Non-detectable

9.5 Sensitivity

The minimum detectable dose of human/cynomolgus monkey TNF beta is 1.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The human cell culture supernatant was initially diluted 1:10. The cynomolgus monkey cell culture supernatant was initially diluted 1:2.)

		Human cell culture supernatant	Cynomolgus monkey cell culture supernatant
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	108	105
	Range (%)	106-110	101-108
1:8	Average% of Expected	114	104
	Range (%)	111-118	100-108
1:16	Average% of Expected	126	102
	Range (%)	122-131	101-103

9.7 Specificity

This kit specifically recognizes native and recombinant human/cynomolgus monkey TNF beta.

10. References

1. Nedwin, G E et al. Nucleic acids research vol. 13,17 (1985): 6361-73.
2. Gruss, H J, and S K Dower. Blood vol. 85,12 (1995): 3378-404.
3. Körner, H, and J D Sedgwick. Immunology and cell biology vol. 74,5 (1996): 465-72.
4. Vujanovic, Nikola L. Immunologic research vol. 50,2-3 (2011): 159-74.