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Product Information

Rat Tumor Necrosis Factor-α (TNF-α) ELISA Kit
for cell and tissue lysates

Catalog Number **RAB0480**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

TNF-α (tumor necrosis factor-α) is secreted by macrophages, monocytes, neutrophils, T cells, and NK cells following their stimulation by bacterial lipopoly-saccharides. Human TNF-α is a non-glycosylated protein of 17.5 kDa with a length of 157 amino acids. TNF-α shows a wide spectrum of biological activities. It causes cytolysis and cytostasis of many tumor cell lines *in vitro*. Within hours after injection TNF-α leads to the destruction of small blood vessels within malignant tumors. TNF-α also enhances phagocytosis and cytotoxicity in neutrophilic granulocytes, and also modulates the expression of many other proteins. In general, TNF-α and TNF-β display similar spectra of biological activities *in vitro*, although TNF-β is often less potent or displays apparent partial agonist activity.

The Rat TNF-α ELSA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of rat TNF-α in cell and tissue lysates. This assay employs an antibody specific for rat TNF-α coated on a 96 well plate. Standards and samples are pipetted into the wells and TNF-α present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat TNF-α antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Rat TNF-α Antibody-coated ELISA Plate (Item A) - RABRTNFAA: 96 wells (12 strips × 8 wells) coated with anti-rat TNF-α.
2. 20x Wash Buffer (Item B) - RABWASH4: 25 mL of 20x concentrated solution.
3. Lyophilized Rat TNF-α Protein Standard (Item C) - RABRTNFAS: 2 vials, recombinant rat TNF-α.
4. ELISA 5x Sample Diluent Buffer (Item D2) - RABDIL6: 10 mL of 5x concentrated buffer. For Standard/Sample (cell lysate/tissue lysate) diluent.
5. ELISA 5x Assay/Sample Diluent Buffer E (Item E2) - RABELADE: 15 mL of 5x concentrated buffer. For Detection Antibody (Item F) and HRP-Streptavidin concentrate (Item G) diluent.
6. Biotinylated Rat TNF-α Detection Antibody (Item F) - RABRTNFAF: 2 vials of biotinylated anti-rat TNF-α (each vial is enough to assay half microplate).
7. HRP-Streptavidin (Item G) - RABHRP5: 200 µL of 200x concentrated HRP-conjugated streptavidin.
8. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3: 12 mL of 3,3′,5,5′-tetra-methylbenzidine (TMB) in buffered solution.
9. ELISA Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.
10. 2x Cell Lysis Buffer (Item J) - RABLYSIS1: 5 mL of 2x cell lysate buffer.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper, or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

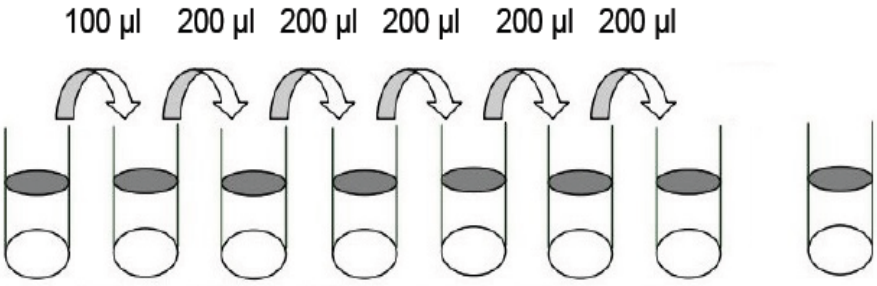
1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

3. Sample dilution: Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1x Sample Diluent Buffer. Generally, a minimum of 1 mg of protein per 1 mL of original lysate solution is recommended. Addition of protease inhibitors (not included) to the lysis buffer prior to use is also recommended.
   Note: Levels of TNF-alpha may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL of 1x Sample Diluent Buffer (Item D, should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 100 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 100 µL of TNF-α standard from the vial of Item C into a tube with 400 µL of Sample Diluent Buffer to prepare a 20,000 pg/mL stock standard solution. Pipette 400 µL of 1x Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/mL).

Figure 1.
Dilution Series for Standards



		Std1	Std2	Std3	Std4	Std5	Std6	Zero Standard
Diluent volume	Item C + 400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Conc.	100 ng/ml	20000 pg/ml	6667 pg/ml	2222 pg/ml	740.7 pg/ml	246.9 pg/ml	82.3 pg/ml	0 pg/ml

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in Procedure, step 5.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 200-fold with 1x Assay Diluent.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 µL of HRP-Streptavidin concentrate into a tube with 10 mL of 1x Assay Diluent to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Storage/Stability
Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8 well strips as appropriate for the experiment.

3. Add 100 µL of each standard (see Preparation Instructions, step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.

4. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with 1x Wash Buffer (300 µL) using a multichannel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µL of 1x prepared biotinylated antibody (Preparation Instructions, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µL of prepared Streptavidin solution (see Preparation Instructions, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Results

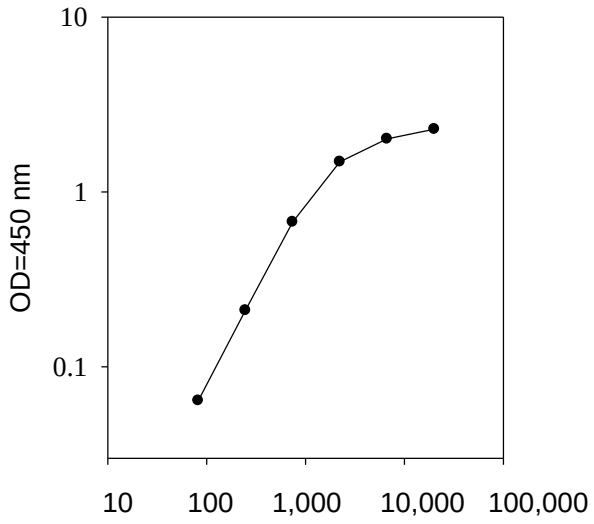
Calculations

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.

Sample Diluent Buffer



Product Profile

Sensitivity: The minimum detectable dose of Rat TNF-α was determined to be 25 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Reproducibility:

Intra-Assay: CV <10%
Inter-Assay: CV <12%

Spiking & Recovery: Recovery was determined by spiking various levels of rat TNF-α into rat tissue lysate and cell lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	92.48	80-104
Cell lysate	93.17	81-105

Linearity:

Sample Type		Tissue Lysate	Cell lysate
1:2	Average % of	90	88
	Expected Range (%)	80-103	76-102
1:4	Average % of	94	92
	Expected Range (%)	84-106	83-104

Specificity

The antibody pair provided in this kit recognizes rat TNF-α.

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested *(e.g.*, rat CINC-2, CINC-3, CNTF, Fractalkine, IL-1α, IL-1β, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, Leptin, Lix, MCP-1, MIP-3α, β-NGF, TIMP-1, and VEGF).

References

1. Bonavida, B., Immunomodulatory effect of tumor necrosis factor. Biotherapy, **3**, 127-33 (1991).
2. Brouckaert, P. et al., Tumor necrosis factor, its receptors and the connection with interleukin 1 and interleukin 6. Immunobiology, **187**, 317-29 (1993).
3. Blankenstein, T. et al., Tumor suppression after tumor cell-targeted tumor necrosis factor alpha gene transfer. Journal of Experimental Medicine, **173**, 1047-52 (1991).

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 3 may be done overnight at 4 °C with gentle shaking. Note: may increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate

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