**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, NK cells following their stimulation by bacterial lipopolysaccharides. Human TNF-α is a non-glycosylated protein of 17.5 kDa with a length of 157 amino acid. 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 lysate and tissue lysate. 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 x 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'-tetramethylbenzidine (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.
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 R&D use only, not for drug, household, or other uses. Please consult the Material 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 dilution: Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1x Sample Diluent Buffer.

3. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.

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).

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 4.

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. 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 overnight at 4 °C with gentle shaking.

3. 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.

4. 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.

5. Discard the solution. Repeat the wash as in step 3.

6. 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.

7. Discard the solution. Repeat the wash as in step 3.

8. 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.

9. 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

![Sample Diluent Buffer Graph](image)

Rat TNF-alpha concentration (pg/ml)

<table>
<thead>
<tr>
<th>OD=450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rat TNF-alpha concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
</tr>
<tr>
<td>1,000</td>
</tr>
<tr>
<td>100,000</td>
</tr>
</tbody>
</table>
Product Profile

Sensitivity: The minimum detectable dose of TNF-α is typically less than 25 pg/ml.

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

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue lysate</td>
<td>92.48</td>
<td>80-104</td>
</tr>
<tr>
<td>Cell lysate</td>
<td>93.17</td>
<td>81-105</td>
</tr>
</tbody>
</table>

Linearity:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Tissue Lysate</th>
<th>Cell Lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>Average % of Expected Range (%)&lt;br&gt;90</td>
<td>88&lt;br&gt;80-103</td>
</tr>
<tr>
<td>1:4</td>
<td>Average % of Expected Range (%)&lt;br&gt;94</td>
<td>92&lt;br&gt;84-106</td>
</tr>
</tbody>
</table>

Specificity

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
## Appendix
### Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standard dilution</td>
<td>Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.</td>
</tr>
<tr>
<td>Low signal</td>
<td>Too brief incubation times</td>
<td>Ensure sufficient incubation time; Procedure, step 2 may change to over night</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Large CV</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td>High background</td>
<td>Plate is insufficiently washed</td>
<td>Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store the standard at (&lt;-20 , ^\circ \text{C}) after reconstitution, others at 4 , ^\circ \text{C}). Keep substrate solution protected from light</td>
</tr>
<tr>
<td></td>
<td>Stop solution</td>
<td>Stop solution should be added to each well before measurement.</td>
</tr>
</tbody>
</table>

©2013 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.