



Rat TNF- α High Sensitivity ELISA Kit

[Catalog No] EK382HS

[SIZE] 48T/96T

[INTENDED USE] For the quantitative determination of rat tumor necrosis factor alpha (TNF- α) concentrations in cell culture supernates, serum and plasma.

[INTRODUCTION]

Tumor necrosis factor alpha (TNF- α), also known as cachectin and TNFSF1A, is an adipokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. It functions as a multipotent modulator of immune response and further acts as a potent pyrogen. TNF- α circulates throughout the body responding to stimuli (infectious agents or tissue injury), activating neutrophils, altering the properties of vascular endothelial cells, regulating metabolic activities of other tissues, as well as exhibiting tumoricidal activity by inducing localized blood clotting. TNF- α may play a significant role in the pathogenesis of inflammatory disease of the joints and other tissues. Recently, a growing body of information has pointed to a role for TNF- α in the pathogenesis of AIDS. Measurement of TNF- α levels has also been shown to be useful in transplant research.

[PRINCIPLE OF THE ASSAY]

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat TNF- α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and TNF- α present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-linked detect antibody specific for TNF- α is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, streptavidin-HRP is added. After washing, amplification reagent is added to the wells. Following incubation any unbound substances is removed during a wash step and streptavidin-HRP is added. After washing, substrate solution is added to the wells and color develops in proportion to the amount of TNF- α bound in the initial step. The color development is stopped and the intensity of the color is measured.

[MATERIALS PROVIDED]

PART	PART #	EK382HS-48	EK382HS-96
Coated Microplate	EK382HSP	48T	96T
Standard	EK382HSS	1vial	2vials
Detect antibody	EK382HSD	1vial	1vial
Standard Diluent	E0260	5ml	5ml
Streptavidin-HRP	E0290	1vial	2vials
Amplification Reagent Concentrate	E0270	1vial	1vial
Amplification Diluent	E0320	12ml	12ml
Assay Buffer (10 \times)	E0310	5ml	5ml
TMB	E0230	6ml	11ml
Stop Solution	E0300	11ml	11ml
Washing Buffer (20 \times)	E0281	50ml	50ml
Adhesive Film	E0200	10	10

Note: Components from reagent kits of different batch numbers must not be used interchangeably.

OTHER SUPPLIES REQUIRED

- 1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- 2) Pipettes and pipette tips.
- 3) 50 μ l to 300 μ l adjustable multichannel micropipette with disposable tips.
- 4) Multichannel micropipette reservoir.
- 5) Beakers, flasks, cylinders necessary for preparation of reagents.
- 6) Deionized or distilled water.
- 7) Polypropylene test tubes for dilution.

[STORAGE]

Store at 2-8°C; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8°C for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20°C for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8°C for approximately 1 month.

[SAMPLE COLLECTION AND STORAGE]

1) **Cell Culture Supernates** - Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at \leq -20°C for later use. Avoid repeated freeze-thaw cycles.

2) **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 \times g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at \leq -20°C for later use. Avoid repeated freeze-thaw cycles.

3) **Plasma** - Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 \times g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at \leq -20°C for later use. Avoid repeated freeze-thaw cycles.

4) Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay.

Dilution with Assay Buffer may be needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at 2 to 8°C.

For longer storage, aliquot samples and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

[REAGENT PREPARATION]

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1 \times)

Pour entire contents (50 ml) of the **Washing Buffer (20 \times)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25°C. Washing Buffer (1 \times) is stable for 30 days.

Assay Buffer (1 \times)

Pour the entire contents (5 ml) of the **Assay Buffer (10 \times)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8°C. Assay Buffer (1 \times) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a 1: 100 dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1 \times) in a clean plastic tube.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.

Make a **1: 100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Amplification Reagent Concentrate

Mix well prior to making dilutions.

Make a **1: 100** dilution of the concentrated **Amplification Reagent** solution with **Amplification Diluent** in a clean plastic tube as needed.

The diluted Amplification Reagent should be used within 30 minutes after dilution.

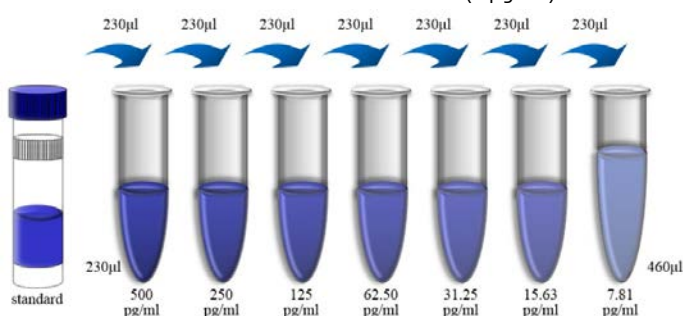
Sample Dilution: If your samples have high **TNF-α** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Rat TNF-α Standard: Reconstitute Rat TNF-α Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 1,000 pg/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

- For serum/plasma samples, mixing concentrated Rat TNF- α standard (230 µl) with 230 µl of Standard Diluent creates the high standard (500 pg/ml). Pipette 230 µl of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).
- For cell culture supernates, mixing concentrated Rat TNF- α standard (230 µl) with 230 µl of cell culture medium creates the high standard (500 pg/ml). Pipette 230 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



[ASSAY PROCEDURE]

Bring all reagents and samples to room temperature before use.

- 1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
- 2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
- 3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. **Do not allow wells to dry.**
- 4) Add 100 µl 2-fold diluted Standard to Standard well. Add 100 µl

Standard Diluent/ culture medium to Blank well.

5) **Serum/Plasma:** Add 80 µl Assay Buffer (1×) and 20 µl sample to the sample well. **Cell culture supernates:** Add 100 µl cell culture supernates to the sample well. Ensure reagent addition in step 4 and 5 is uninterrupted and completed within 15 minutes.

6) Cover with an Adhesive Film. Incubate at room temperature (25°C±3°C) for 1.5 hours on a microplate shaker set at 300 rpm.

7) Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300 µl Washing Buffer (1×). 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.

8) Add 100 µl of diluted Detect Antibody to each well. Cover with a new Adhesive Film. Incubate at room temperature (25°C±3°C) for 30 minutes on a microplate shaker set at 300 rpm.

9) Repeat aspiration/wash as in step 7.

10) Add 100 µl of diluted Streptavidin-HRP to each well. Cover with a new Adhesive Film. Incubate at room temperature (25°C±3°C) for 30 minutes on a microplate shaker set at 300 rpm.

11) Repeat aspiration/wash as in step 7.

12) Add 100 µl of diluted Amplification Reagent to each well. Cover with a new Adhesive Film. Incubate at room temperature (25°C±3°C) for exactly 15 minutes on a microplate shaker set at 300 rpm.

13) Repeat aspiration/wash as in step 7.

14) Add 100 µl of diluted Streptavidin-HRP to each well. Cover with a new Adhesive Film. Incubate at room temperature (25°C±3°C) for exactly 15 minutes on a microplate shaker set at 300 rpm.

15) Repeat aspiration/wash as in step 7.

16) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature (25°C±3°C). **Protect from light.**

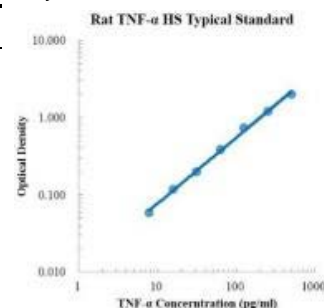
17) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

18) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

[TYPICAL DATA]

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

pg/ml	O.D.	Average	Corrected
0.00	0.015	0.016	0.016
7.81	0.075	0.073	0.074
15.63	0.138	0.129	0.134
31.25	0.220	0.208	0.214
62.50	0.409	0.394	0.402
125.00	0.766	0.730	0.748
250.00	1.239	1.201	1.220
500.00	2.087	1.955	2.021



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