EasyGo!™ Mouse TNF-α One-Step ELISA Kit

【Catalog No】 EK282EGA

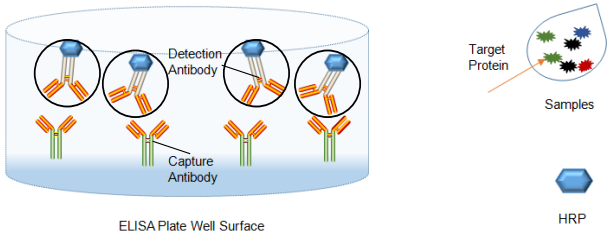
【SIZE】 48T/96T

【INTENDED USE】 For the quantitative determination of mouse Tumor Necrosis Factor alpha (TNF-α) concentrations in 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】



Mouse TNF-α ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of Mouse TNF-α in the samples. A monoclonal antibody specific for Mouse TNF-α has been immobilized onto microwells, and one pellet of the HRP-linked detect antibody specific for TNF-α (light yellow) is pre-placed in the microwells, sealed by the adhesive film. Standard or samples are pipetted into the wells, then TNF-α present is bound by the immobilized antibody and detect antibody. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of TNF-α bound by the immobilized antibody. The color development is stopped by addition of acid and the optical density value is measured by microplate reader.

【MATERIALS PROVIDED】

PART	PART #	EK282EGA-48	EK282EGA-96
Coated Microplate	EK282EGAP	48T	96T
Standard	EK282EGAS	1vial	2vials
Standard Diluent	E0260	5mL	10mL
TMB	E0230	6mL	11mL
Stop Solution	E0300	11mL	11mL
Washing Buffer (20×)	E0281	11mL	11mL

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 μl to 300 μl 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℃; refer to the kit label for expiration date.

For opened kits:

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

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

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

【SAMPLE COLLECTION AND STORAGE】

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

2) **Plasma** – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

3) Other biological samples might be suitable for use in the assay. Serum and plasma were tested with this assay. Dilution with Standard Diluent 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℃. For longer storage, aliquot samples and store frozen at -20℃. 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×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** 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℃. Washing Buffer (1×) is stable for 30 days.

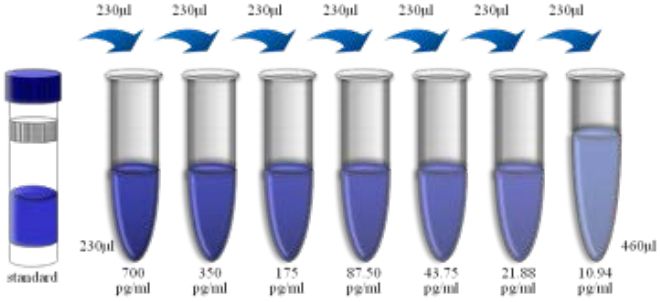
Sample Dilution: If your samples have high **TNF-α** content, dilute serum/plasma samples with Standard Diluent.

Mouse TNF-α Standard: Reconstitute **Mouse 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 = 1,400 pg/ml).

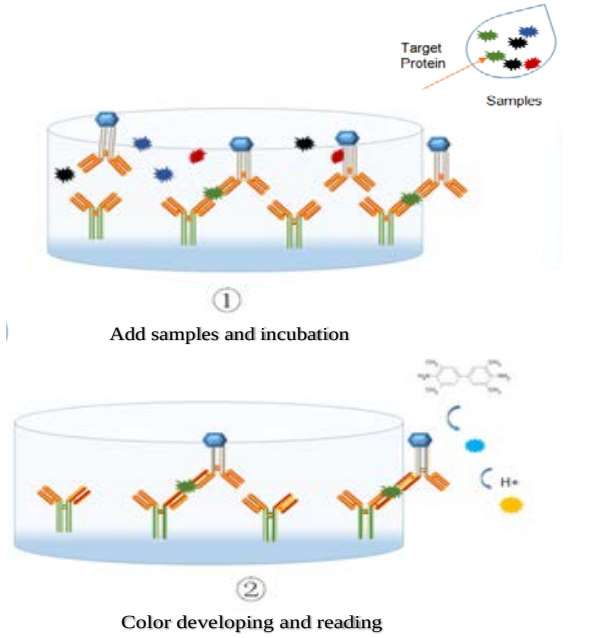
Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.Use polypropylene tubes.

- **For serum/plasma samples,** mixing concentrated Mouse TNF-α standard (230 μl) with 230 μl of Standard Diluent creates the high standard (700 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).



【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. **In any case, avoid touching the inner surface of the microwells and gently tap the plate to ensure that the pellets on the bottom of the microwells. Do not discard the pellets.**

3) Adding Standard: Add 100 μL of 2-fold diluted Standard to Standard well. Add 100 μL of Standard Diluent to Blank well. **The standards/samples can be added directly from the middle opening of the plate adhesive film.**

4) Adding Samples: Add 80 μL of Standard Diluent and 20 μL sample to the sample well.

5) Incubation: Incubate at 37℃ for **1** hour, or at room temperature (25℃ ±3℃) for **2** hours, on a microplate shaker set at 300-500 rpm.

6) Washing: Removing the plate adhesive film. Aspirate each well and wash by filling each well with 300μL of Washing Buffer (1×), repeating the process 3 times for a total four washes with 60 seconds interval. 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.

7) Adding Substrate Solution: Add 100 μL of Substrate Solution to each well. Incubate for 10±5 minutes at room temperature (25±3℃). **Protect from light.**

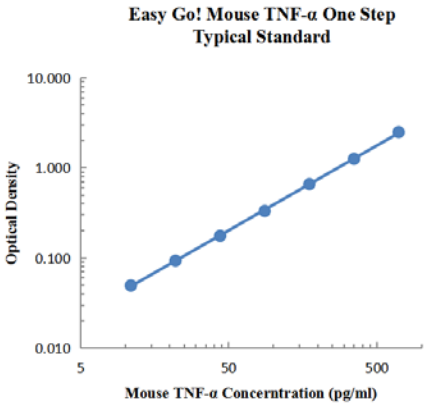
8) Stopping: Add 100 μL of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9) Reading: 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	0.029	0.032	0.031	
10.938	0.077	0.083	0.080	0.050
21.875	0.123	0.125	0.124	0.094
43.75	0.203	0.211	0.207	0.177
87.50	0.363	0.363	0.363	0.333
175.00	0.682	0.701	0.692	0.661
350.00	1.332	1.247	1.290	1.259
700.00	2.545	2.502	2.524	2.493



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