MULTI SCIENCES

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

【Catalog No】EK182EG

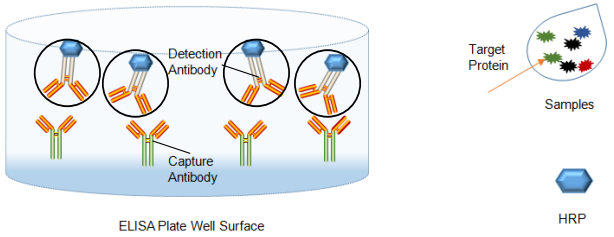
【SIZE】48T/96T

【INTENDED USE】For the quantitative determination of Human Tumor Necrosis Factor alpha (TNF-α) concentrations in cell culture supernates,serum and plasma.

【INTRODUCTION】

Interferon gamma (TNF-α) is a dimerized soluble cytokine that is the only member of the type II class of interferons. TNF-α is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops. TNF-α, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. TNF-α is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression. Aberrant TNF-α expression is associated with a number of autoinflammatory and autoimmune diseases. The importance of TNF-α in the immune system stems in part from its ability to inhibit viral replication directly, and most importantly from its immunostimulatory and immunomodulatory effects. The role of TNF-α as a disease marker has been demonstrated for a number of different pathological situations including infections, autoimmune diseases, transplant rejection, allergy and diabetes.

【PRINCIPLE OF THE ASSAY】



This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human 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 in the incubation. 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 and the intensity of the color is measured by microplate reader.

【MATERIALS PROVIDED】

PART	PART #	EK182EG-48	EK182EG-96
Coated Microplate	EK182EGP	48T	96T
Standard	EK182EGS	1 vial	2 vials
Assay Buffer (10×)	E0310	5 mL	5 mL
TMB	E0230	6 mL	11 mL
Stop Solution	E0300	11 mL	11 mL
Washing Buffer (20×)	E0281	11 mL	11 mL

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) **Cell Culture Supernates** - Remove particulates by centrifugation at 300 × g for 10 minutes and assay immediately or aliquot and store samples at ≤ -20℃.

2) **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.

3) **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.

4) Other biological samples might be suitable for use in the assay. Serum and plasma were tested with this assay. Dilution with Assay Buffer (1×) 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.

Assay Buffer (1×)

Pour the entire contents (5 mL) of the Assay Buffer (10×) 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℃. Assay Buffer (1×) is stable for 30 days.

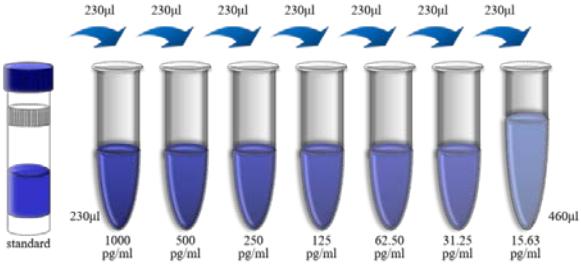
Sample Dilution: If your samples have high **TNF-α** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Human TNF-α Standard: Reconstitute **Human TNF-α Standard** by

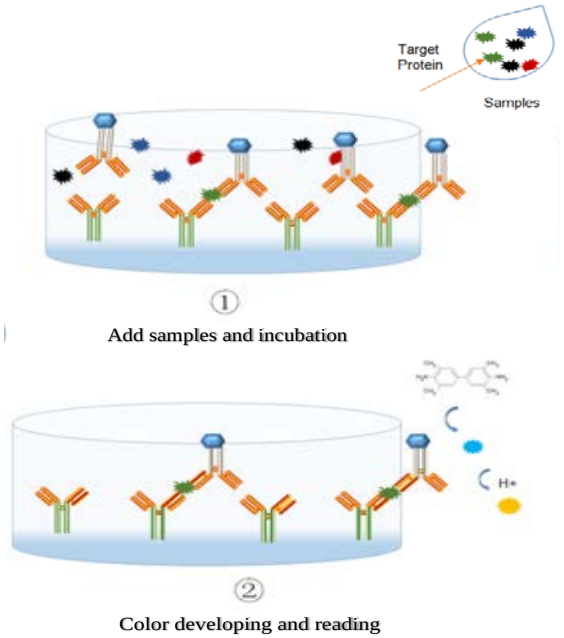
addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 2,000 pg/mL).

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

- **For serum/plasma samples,** mixing concentrated Human TNF-α standard (230 µL) with 230 µL of Assay Buffer (1×) creates the high standard (1,000 pg/mL). Pipette 230 µL of Assay Buffer (1×) into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Assay Buffer (1×) serves as the zero standard (0 pg/mL).
- **For cell culture supernates,** mixing concentrated Human IL-8 standard (230 µL) with 230 µL of cell culture medium creates the high standard (1,000 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. ***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 Assay Buffer (1×) to Blank well. ***The standards/samples can be added directly from the middle opening of the plate adhesive film.***

4) **Adding Samples**: ***Serum/Plasma*** - Add 80 µL of Assay Buffer (1×) and 20 µL sample to the sample well.***Cell culture supernates*** - Add 100 µL cell culture supernates 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.

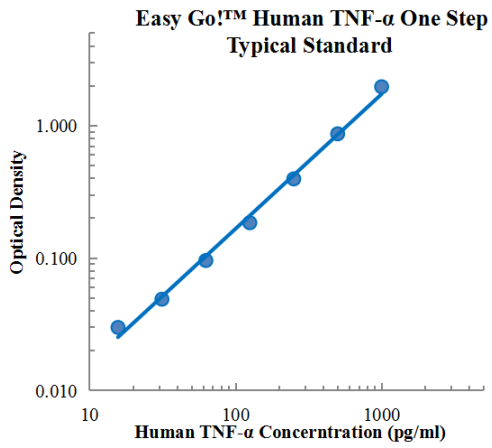
【TYPICAL DATA】

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

Note: The finally concentration of top standard is 1,000 pg/mL.

If Serum/Plasma samples have been diluted following the instruction, the final dilution factor is 5. If sample have been diluted by other means, the concentration read from the standard curve must be multiplied by the appropriate dilution factor.

pg/mL	O.D.		Average	Corrected
0.00	0.011	0.013	0.012	
15.63	0.040	0.044	0.042	0.030
31.25	0.063	0.059	0.061	0.049
62.50	0.108	0.108	0.108	0.096
125.00	0.198	0.196	0.197	0.185
250.00	0.399	0.418	0.409	0.397
500.00	0.902	0.858	0.880	0.868
1,000.00	2.026	1.931	1.979	1.967



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