Mouse Tumor Necrosis Factor alpha (TNF-alpha) ELISA Kit

Catalog No.: IRKTAH2622

Lot No.: Sample

Mouse TNF-alpha ELISA kit is designed for detection of TNF-alpha in mouse plasma, serum or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TNF-alpha in less than 5 hours.

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Assay Principle

Mouse TNF-alpha ELISA kit is designed for detection of TNF-alpha in mouse plasma, serum or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TNF-alpha in less than 5 hours. A polyclonal antibody specific for mouse TNF-alpha has been pre-coated onto a microplate. TNF-alpha in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for mouse TNF-alpha, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Reagents Provided

• TNF-alpha Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against TNF-alpha.

• Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.

• TNF-alpha Standard: Mouse TNF-alpha in a buffered protein base (2 ng, lyophilized).

• Biotinylated Mouse TNF-alpha Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against mouse TNF-alpha (80 µl).

• MIX Diluent Concentrate (10x): A 10-fold buffered protein base (30 ml).

• Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).

• Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µl).

• Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

• Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).

Storage and Stability

• Store components of the kit at 2-8C or -20C upon arrival up to the expiration date.

• Store SP Conjugate and Biotinylated Antibody at -20C

• Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8C

• Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

• Diluent (1x) may be stored for up to 1 month at 2-8C.

• Store Standard at 2-8C before reconstituting with Diluent and at -20C after reconstituting with Diluent.

Reagents and Equipment Required

• Microplate reader capable of measuring absorbance at 450 nm

• Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel)

• Deionized or distilled reagent grade water

Sample Collection

• Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Store the remaining samples at -20C or below. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)

• Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes and assay. The undiluted samples can be stored at -20C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

• Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

MIX Diluent Concentrate (20x): Dilute the MIX Diluent Concentrate 1:20 with reagent grade water. Store for up to 1 month at 2-8C.

Standard Curve: Reconstitute the 2 ng of mouse TNF-alpha Standard with 1 ml of MIX Diluent to generate a 2 ng/ml of standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the TNF-alpha standard solution (2ng/ml) twofold with equal volume of MIX Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625 and 0.0313 ng/ml. MIX Diluent serves asthe zero standard (0 ng/ml). Any remaining solution should be frozen at -20C.

Biotinylated Mouse TNF-alpha Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20C.

Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20C.

Assay Procedure

Standard Point	Dilutions	TNF Alpha (ng/ml)
P1	1 part Standard (2ng/ml)	2.000
P2	1 part P1 + 1 part MIX Diluent	1.000
P3	1 part P2 + 1 part MIX Diluent	0.500
P4	1 part P3 + 1 part MIX Diluent	0.250
P5	1 part P4 + 1 part MIX Diluent	0.125
P6	1 part P5 + 1 part MIX Diluent	0.063
P7	1 part P6 + 1 part MIX Diluent	0.031
P8	Mix Diluent	0.000

• Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30C).

• Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

• Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

• Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.

• Add 50 µl of Biotinylated Mouse TNF-alpha Antibody to each well and incubate for two hours.

• Wash a microplate as described above.

• Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

• Wash a microplate as described above.

• Add 50 µl of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

• Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.

• Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.

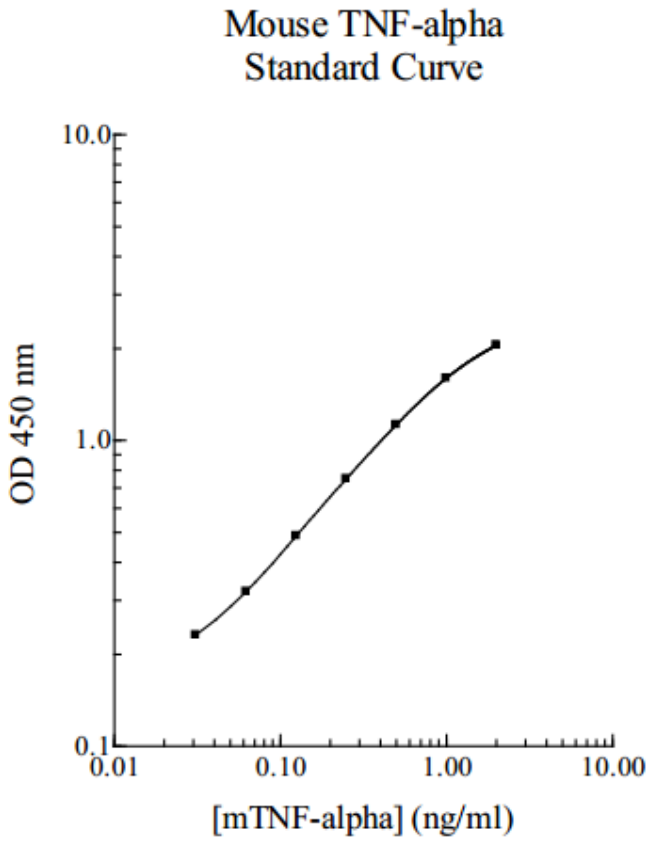
Expected Values

• Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

• To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

• Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve Examples



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Performance Characteristics

- The minimum detectable dose of TNF-alpha is typically ~ 30 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5% and 7.1% respectively.
- This assay recognizes both natural and recombinant mouse TNF-alpha.

Standard Added Value	0.025-0.5 ng/ml
Recovery %	85-111%
Average Recovery%	97%

	Average % of Expected Value	
Sample Dilution	Plasma	Serum
No Dilution	99%	97%
1:2	101%	98%
1:4	100%	99%

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