Rat TNF-α ELISA Kit

Cat No. ELI-M-09-96
Size: 96 tests

MOLEQULE-ON®

Components	Quantity
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 Plate
Standard - lyophilized, 2000 pg/ml upon reconstitution	2 Vials
Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial	1 Vial
Concentrated Streptavidin-HRP solution (100X) - 120 µl/vial	1 Vial
Standard /sample Diluent - 16 ml/vial	1 Bottle
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 Bottle
Streptavidin-HRP Diluent - 16 ml/vial	1 Bottle
Wash Buffer Concentrate (20x) - 30 ml/vial	1 Bottle
Substrate Solution - 12 ml/vial	1 Bottle
Stop Solution - 12 ml/vial	1 Bottle
Plate Cover Seals	4 pieces

Reagent Preparations

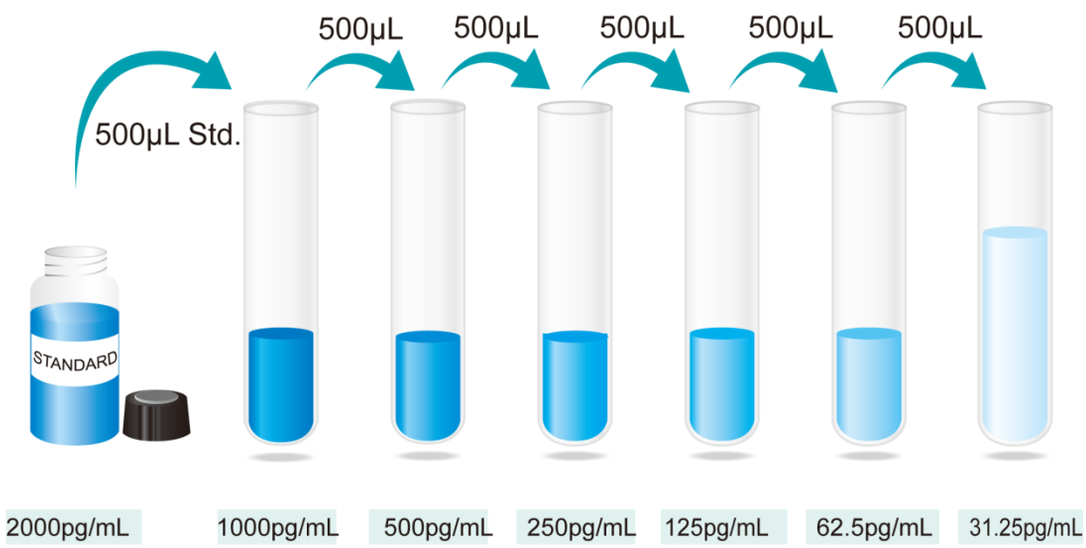
Temperature returning - Bring all kit components and specimen to room temperature (20-25℃) before use.

Wash Buffer - Dilute 10mL of Wash Buffer Concentrate with 190mL of deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Standard/Specimen - Reconstitute the Standard with 1.0mL of Standard /Specimen Diluent. This reconstitution produces a stock solution of 2000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Specimen Diluent into the 2000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2000 pg/mL standard serves as the high standard. The Standard/specimen Diluent serves as the zero standard (0 pg/mL). **If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

Working solution of Biotin-Conjugate anti-rat TNF-α antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube. **The working solution should be used within one day after dilution.**

Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube. **The working solution should be used within one day after dilution.**



Storage

All components of Rat TNF-α ELISA Kit should be store at 2-8°C except reconstituted standard that should be store at -20°C.

Description

Tumor necrosis factor (TNF-α) is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up 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. The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumorigenesis and viral replication and respond to sepsis via IL1 & IL6 producing cells.

Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for TNF-α is added to detect the captured TNF-α protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by Tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Sample Collection And Storage

1. Cell Culture Supernates - Centrifuge cell culture media at 1000 x g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
2. Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately for 15 minutes at 1000 x g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
3. Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Procedure

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8°C sealed tightly.
3. Add 100µl of standard or samples to each well, keep on shaking with micro-oscillator (100rpm/min) and incubate for 120 minutes at 25°C.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µl) using a multichannel pipette, manifold dispenser or auto-washer. 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.
5. Add 100µl working solution of Biotin-Conjugate anti-rat TNF-α antibody to each well, incubate for 60 minutes at 25°C.
6. Repeat step 4.
7. Add 100µl working solution of Streptavidin-HRP to each well, incubate for 30 minutes at 25°C.
8. Repeat step 4 with an additional wash.
9. Add 100µl of Substrate Solution to each well. Incubate for 5-20 minutes at 25°C. Avoid placing the plate in direct light.
10. Add 50µl Stop solution to each well. Gently tap the plate to ensure thorough mixing.
11. Read at 450nm within 30 minutes.

Technical Note

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation. Do NOT subtract the O.D. of stand zero.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the TNF-α concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.065	0.053	0.059	--
31.25	0.211	0.205	0.208	0.149
62.5	0.273	0.283	0.278	0.219
125	0.39	0.395	0.3925	0.3335
250	0.626	0.623	0.6245	0.5655
500	1.02	1.027	1.0235	0.9645
1000	1.709	1.706	1.7075	1.6485
2000	2.51	2.505	2.5075	2.4485

Sensitivity, Specificity And Repeatability

SENSITIVITY: The minimum detectable dose was 15pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant rat TNF-α. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY： The recovery of TNF-α spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

References

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