Rat TNF-alpha ELISA kit (RF879018)

MATERIALS PROVIDED & STORAGE CONDITIONS

PART	Format	STORAGE CONDITIONS
Pre-coated Microplate	1 plate	Store in sealed at -20℃.
Rat TNF-alpha Standard	2 bottles	Store at -20℃.
Detection A	1 vial	Store at -20℃.
Detection B	1 vial	Store at -20℃.
Standard Diluent	1 bottle	Store at 4℃.
Assay Diluent	1 bottle	Store at 4℃.
20 × Wash Buffer	1 bottle	Store at 4℃.
Color Reagent	1 bottle	Store at 4℃.
Stop Solution	1 bottle	Store at 4℃.
Plate Sealers	4 strips	Store at RT.

* Provided this is within the expiration date of the kit.

INTENDED USE

Used for the quantitative determination of Rat TNF-alpha concentration in rat cell culture supernates, serum, and plasma.

PRINCIPLE OF THE ASSAY

This kit is based on a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). Standards and samples are added to the pre-coated microplate wells, where Rat TNF-alpha participates in a format-specific immunoreaction. After the required incubation and washing steps, enzyme-conjugated reagent and substrate solution are added sequentially to generate a measurable colorimetric signal.

The optical density (OD) value is positively correlated with the concentration of Rat TNF-alpha in the sample. The concentration of Rat TNF-alpha is calculated by interpolation from the standard curve.

OTHER SUPPLIES REQUIRED

• Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 620 nm.

• Pipettes and pipette tips.

• Deionized or distilled water.

• 500 mL graduated cylinder.

• Squirt bottle, manifold dispenser, or automated microplate washer.

• Test tubes for dilution of standards.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Handle all blood and serum as if capable of transmitting infectious agents. The NCCLS provides recommendations for handling and storing cell culture supernates, serum, and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

20-fold Wash Buffer Concentrate - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of **Wash Buffer**.

Cell culture supernates, serum, and plasma - Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 10 µL of sample + 90 µL of Standard Diluent (diluted 1:9). If the sample value is outside the range of the standard curve, the dilution can be adjusted appropriately and the assay can be redetermined. If the antibody concentration in the sample can be estimated and the assay can be performed simultaneously by diluting several gradients prior to the experiment.

Standard - Reconstitute with 1 mL **Standard Diluent**, this reconstitution produces a **stock solution**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. The appropriate **Standard Diluent** serves as the zero standard (0 ng/mL).

Detection A (working solution) - Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the **Detection A** 1: 100 times to the working concentration with **Assay Diluent**.

Detection B (working solution) - Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the **Detection B** 1: 100 times to the working concentration with **Assay Diluent**.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 100 µL standards or samples to each well. Cover with the adhesive strip provided. Incubate for 1 hour at 37℃.

4. Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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 100 µL of Detection A working solution to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37℃.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of Detection B working solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at 37℃.

8. Aspirate each well and wash, repeating the process five times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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.

9. Add 100 µL of Color Reagent to each well. Incubate for 15 minutes at 37℃. Protect from light.

10. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm or 620 nm. If wavelength correction is not available, subtract readings at 630 nm or 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample. 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. The data may be linearized by plotting the log of the Rat TNF-alpha 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.

ASSAY RANGE

The assay range of this kit is provided in the Certificate of Analysis (COA) included with the product.

SENSITIVITY

The minimum detectable dose (MDD) for Rat TNF-alpha is reported in the COA included with each kit.

PRECISION

Intra-Assay Precision (Precision within an assay): <15%

Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): <15%

Three samples of known concentration were tested in twenty four separate assays to assess inter-assay precision.

STABILITY

When the kit was stored at the recommended temperature for 12 months, the signal intensity decreased by less than 30%.

For unopened kits, if you want to prolong the storage time, please store the Standard, Detection A, Detection B and Microplate at - 20 ℃, the rest reagents should be store at 4℃.

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Bubbles in the wells	Tap plate gently to disperse bubbles
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
High background	Plate is not washed properly	Make sure the wash apparatus works properly
	Incorrect incubation times and/or temperatures	The OD value increased gradually along with the time. Reduce the color developing time properly
Weak/No Signal	Pipetting errors	Make sure the pipette is calibrated
	The working solution not be prepared immediately before use	The working solution should be prepared immediately before use and should not be stored
	Volumes errors	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	Detection A working solution is not completely mixed with the samples	After adding the Detection A into the wells, make sure the detection A and the samples are mixed thoroughly