Rat TNF-α ELISA Kit

Catalog Number KRC3011 (96 tests), KRC3012 (2 × 96 tests), KRC3011C (5 × 96 tests)

Pub. No. MAN0003966 **Rev.** 5.0 (32)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Rat TNF-α ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of rat TNF-α in rat serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant rat TNF-α.

Contents and storage

Upon receipt, store the kit at 2 to 8°C.

Contents	Cat. No. KRC3011 (96 tests)
Rt TNF-α Standard, lyophilized; contains 0.1% sodium azide	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Wells, 96-well plate	1 plate
Rt TNF-α Biotin Conjugate; contains 0.1% sodium azide	11 mL
Incubation Buffer	12 mL
Streptavidin-HRP (100X)	0.15 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	3

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

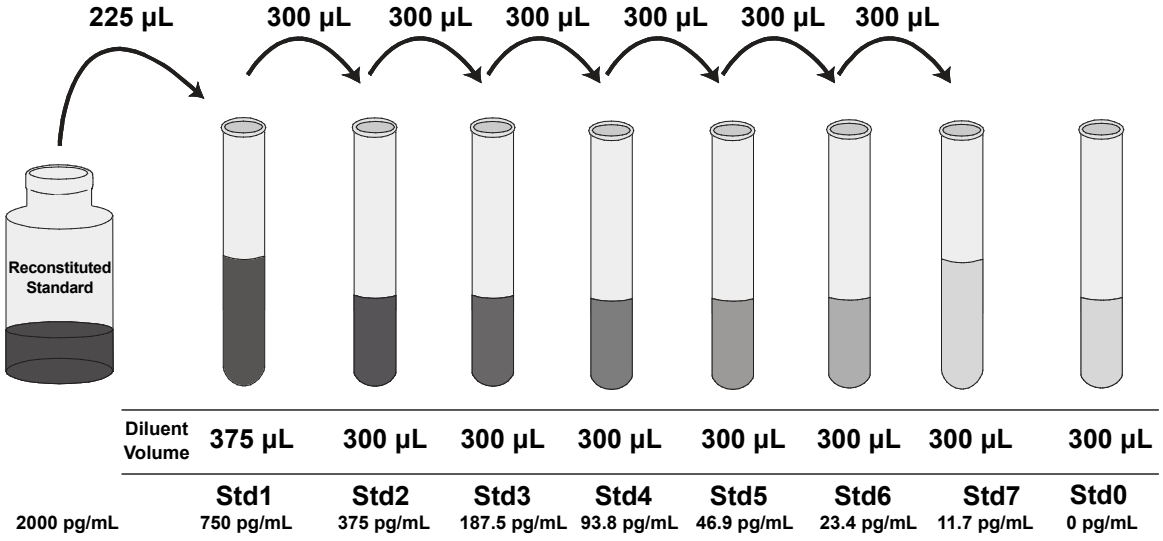
Because conditions may vary, we recommend that each investigator determine the optimal dilution for each application.

- Dilute serum and plasma samples 1:2 in Incubation Buffer.
- Dilute tissue culture supernatants 1:2 in Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Rt TNF-α Standard to 2,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 2,000 pg/mL rat TNF-α. **Use the standard within 1 hour of reconstitution.**
2. Add 225 µL Reconstituted Standard to one tube containing 375 µL Standard Diluent Buffer and mix. Label as 750 pg/mL rat TNF-α.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 375,187.5, 93.8, 46.9, 23.4, 11.7, and 0 pg/mL rat TNF-α.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

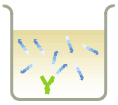
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Rt TNF-α Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 750 pg/mL rat TNF-α.

Standard Rat TNF-α (pg/mL)	Optical Density (450 nm)
750	3.29
375	1.93
187.5	1.03
93.8	0.59
46.9	0.32
23.4	0.18
11.7	0.13
0	0.05

Inter-assay precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	135	259.2	970.9
Standard Deviation	12.1	20.7	75.4
% Coefficient of Variation	9.0	8.0	7.8

Intra-assay precision

Samples of known rat TNF-α concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	130.7	241.8	912.1
Standard Deviation	9.0	14.9	39.1
% Coefficient of Variation	6.9	6.2	4.3

Cross-reactivity
Recombinant mouse TNF-α protein demonstrated 100% cross-reactivity with this kit.

Expected values
Twenty rat serum and plasma samples were evaluated for detectable levels of Rat TNF-α ELISA Kit in this assay. The mean value of the serum and plasma samples measured less than the lowest Rat TNF-α ELISA Kit standard, 11.7 pg/mL. Rat splenocytes supernatants, from rats stimulated for 72 hours with 0.5 mg of LPS, cultured in the presence of 50 ng/mL PMA and 250 ng/mL calcium ionophore for 72 hours were assayed for Rat TNF-α ELISA Kit and measured an average of 725 pg/mL.

High-dose hook effect
No hook effect was observed with concentrations up to 1 µg/mL.

Linearity of dilution
Rat serum,EDTA plasma, and heparinized plasma samples (all spiked with natural rat TNF-α) were serially diluted in Incubation Buffer, and supernatant from stimulated rat splenocytes was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded the average correlation coefficients in the following table:

Sample	Correlation Coefficient
Serum	1.00
EDTA plasma	0.97
Heparin plasma	0.99
Splenocyte supernatant	0.98

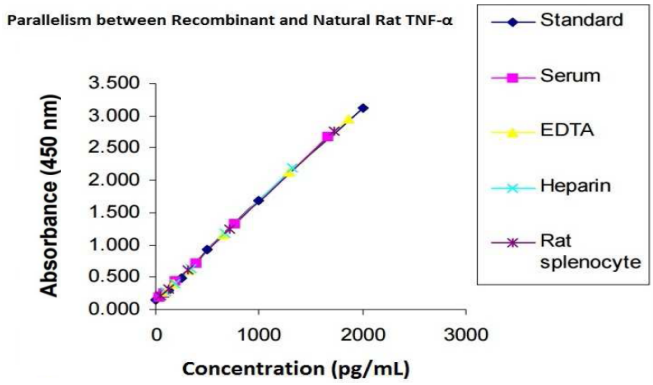
Recovery
The recoveries of rat TNF-α added to rat serum, EDTA plasma, heparinized plasma, cell culture media containing 1% fetal bovine serum, and cell culture media containing 10% fetal bovine serum were measured with the Rat TNF-α ELISA Kit.

Sample	Range %	Avg % Recovery
Serum	83–97	92
EDTA plasma	101–115	106
Heparin plasma	106–122	115
RPMI+1% fetal bovine serum	97–113	105
RPMI+10% fetal bovine serum	108–110	109

Sensitivity
The analytical sensitivity for rat TNF-α is <4 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Specificity
Buffered solutions of a panel of substances ranging in concentrations from 1.5 to 9.0 ng/mL were assayed with the Rat TNF-α ELISA Kit and found to have no cross-reactivity: **rat** GM-CSF, IFN-γ, IL-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, MCP-1, MIP-1α, MIP-2, and RANTES; **human** TNF-α; and **swine** TNF-α.

Parallelism
Supernatants from stimulated rat splenocytes were serially diluted in Standard Diluent Buffer, while rat serum and plasma samples spiked with natural rat TNF-α were serially diluted in Incubation Buffer. The optical density of each dilution was plotted against the rat TNF-α standard curve. The standard accurately reflects the rat TNF-α content in natural samples.



Limited product warranty
Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.