novex
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Rat TNF-α Chemiluminescence ELISA Kit

Catalog. no. KRC3019 **Quantity:** 96 tests

Pub. Part no. MAN0006616 **Rev 1.0**

Description

The Rat TNF-α Chemiluminescence 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 cell culture supernatants, serum, plasma or other body fluids. The assay will recognize both natural and recombinant rat TNF-α.

Tumor necrosis factor alpha (TNF-α) is a key inflammatory mediator that is implicated in a number of pathologies (including septic shock and autoimmune diseases) as well as normal cell processes (cell growth and homeostasis, immune system function, and angiogenesis). Rat TNF-α bears a high degree of homology to mouse and human TNF-α (94% and 76% for the soluble forms, respectively), as well as other members of the TNF superfamily. TNF-α is synthesized as a type II transmembrane protein with a receptor binding motif located at the C terminus. In the rat, the transmembrane form is a 26 kDa protein containing 253 amino acid residues. Cleavage of the membrane-associated form by the metalloprotease TNF-α converting enzyme releases soluble TNF-α, a 17 kDa protein containing 165 amino acid residues. Both the membrane-associated and the soluble forms are biologically active.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2 to 8°C.

Components	Quantity
Rat TNF-α Antibody Coated Wells. 96 well plate.	1 plate
Rat TNF-α Detection Antibody (100X).Contains 0.1% sodium azide.	0.125 mL
Detection Antibody Diluent. Contains 0.1% sodium azide.	11 mL
Rat TNF-α Standard. Lyophilized.Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Rat TNF-α High and Low Controls, recombinant Rat TNF-α, lyophilized. Contains 0.1% sodium azide. Refer to the vial label for reconstitution volume and range.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL
Novobright CSPD-Emerald II Substrate (clear and greenish)	15 mL
Adhesive Plate Covers	2

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.

Materials required but not provided

- Distilled or deionized water
- Luminescence microtiter plate reader with software
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before starting

Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.

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

Dilute wash buffer

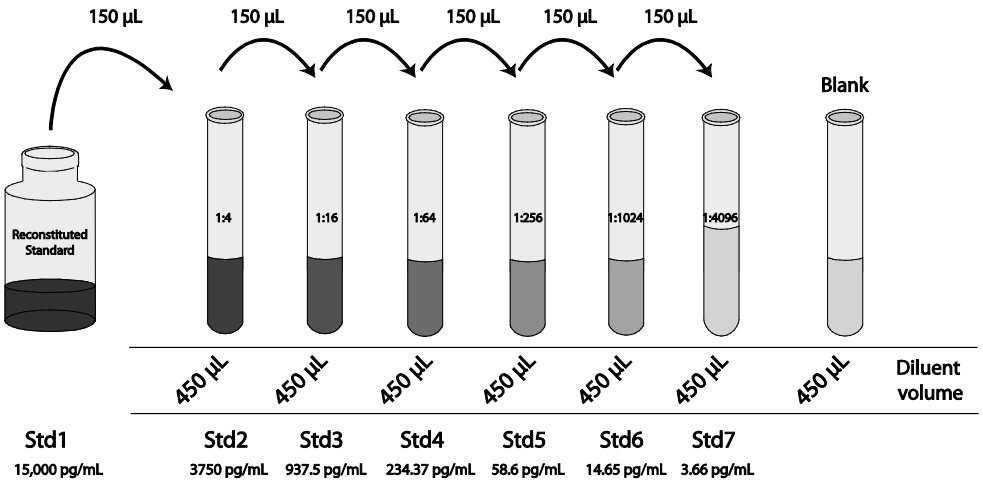
- Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
- Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Wash Buffer (1X).
- Store the concentrate and Wash Buffer (1X) in the refrigerator. Use the diluted buffer within 14 days.

Dilute the standards

Note: Use glass or plastic tubes for diluting standards.

- Reconstitute Rat TNF-α Standard to 15,000 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 15,000 pg/mL rat TNF-α. Use the standard within 1 hour of reconstitution.
- Add 450 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 3750, 937.5, 234.37, 58.6, 14.65, 3.66, and 0 pg/mL rat TNF-α.
- Make 1:4 serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.

Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare Detection Antibody solution

Note: Prepare the Rat TNF-α Detection Antibody solution within 15 minutes of usage.

The Rat TNF-α Detection Antibody (100X) is conjugated to alkaline phosphatase (AP) and is in 50% glycerol, which is viscous. To ensure accurate dilution:

- For each 8-well strip used in the assay, pipette 9 µL Rat TNF-α Detection Antibody (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution to a tube containing 891 µL of Detection Antibody Diluent for a total volume of 900 µL.
- Return any unused Rat TNF-α Detection Antibody (100X) solution to the refrigerator.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be frozen at –80°C if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute sample

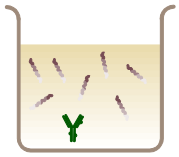
Cell culture supernatant, serum, and plasma samples should be diluted 2-fold in Standard Diluent Buffer.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours and 30 minutes.**

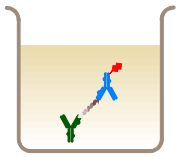
IMPORTANT! Perform a standard curve with each assay.

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 to 8°C for future use.



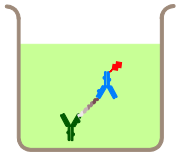
Bind antigen

- Add 100 µL of standards or samples to the appropriate microtiter wells.
- Cover the plate with plate cover and incubate for 2 hours at room temperature.
- Thoroughly aspirate the solution and wash wells 5 times with diluted Wash Buffer.



Add detector antibody

- Add 100 µL Rat TNF-α Detection Antibody (1X) solution into each well.
- Cover the plate with plate cover and incubate for 1 hour at room temperature.
- Thoroughly aspirate the solution from the wells and wash wells 5 times with diluted Wash Buffer.



Add substrate

- Add 100 µL Novobright CSPD-Emerald II Substrate to each well.
- Cover the plate with plate cover and incubate for 30 minutes at room temperature in the dark.

Note: Protect Novobright CSPD-Emerald Substrate from prolonged exposure to light.

Read the plate and generate the standard curve

- Read the luminescence (RLU) 30 minutes after the addition of the CSPD-Emerald Substrate with a 1000 msec integration time. For best results, keep the plate covered in the dark. Plates should be read as soon as possible after the 30 minutes of substrate incubation.
- Use curve-fitting software to generate the standard curve. A five parameter algorithm with weighting provides the best standard curve fit.
- 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.

Standard curve (example)

The following data were obtained for the various standards over the range of 3.66–15000 pg/mL rat TNF-α.

Standard Rat TNF-α (pg/mL)	Luminescence (RLU nm)
15000	229778
3750	129700
938	42653
234	11545
59	3529
15	1000
4	348
0	159

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

Sensitivity

The analytical sensitivity of rat TNF-α is <2.0 pg/mL. This was determined by adding 2 standard deviations to the mean RLU obtained when the zero standard was assayed 40 times

The functional sensitivity defines the assay's ability to accurately quantify the lowest amount of protein associated with %CV <20%. The typical functional sensitivity of this assay is <4.0 pg/mL.

Performance characteristics

Intra-assay precision

Samples of known rat TNF-α concentration were assayed in replicates of 24 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	3201.8	269.0	69.4
SD	242.6	15.4	3.2
%V	7.6	5.7	4.6

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

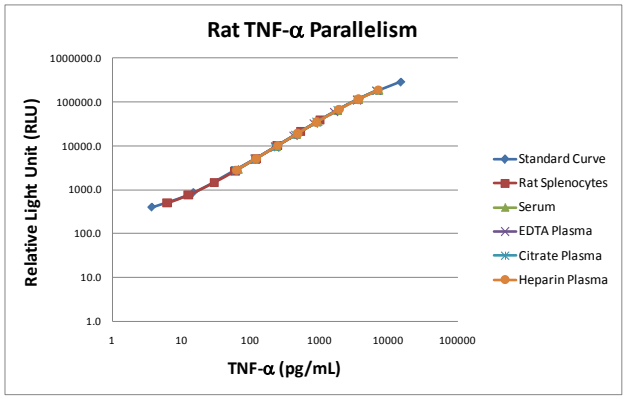
Samples were assayed 72 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	3420.9	243.0	61.3
SD	312.2	14.9	3.6
%CV	9.0	6.1	6.0

SD = Standard Deviation; CV = Coefficient of Variation

Parallelism

Natural sample from LPS-treated rat splenocytes, recombinant standard reconstituted with serum, plasma (EDTA), plasma (citrate), and plasma (heparin), were serially diluted in Standard Diluent Buffer. The luminescence (RLU) of each dilution was plotted against the rat TNF-α standard curve. Parallelism demonstrated by the figure below indicated that the standard accurately reflects rat TNF-α content in samples.



Linearity of dilution

The recombinant standard was reconstituted with either serum, plasma (EDTA), plasma (Heparin) or plasma (Citrate) (data not shown), serially diluted with Standard Diluent Buffer over the range of the assay, and measured for rat TNF-α. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99 for both serum and plasma samples.

Rat splenocytes were grown in tissue culture medium containing 10% fetal bovine serum and treated with 10 µg/mL LPS for 72 hours. Supernatant was collected and diluted in Standard Diluent Buffer. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

	Serum			Supernatant		
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
1/2	7206	7578	105	1012	939	93
1/4	3677	3789	103	536	469	88
1/8	1822	1894	104	245	235	96
1/16	951	947	100	120	117	98
1/32	467	474	101	59	59	100
1/64	240	237	99	30	29	99
1/128	117	118	101	13	15	118
1/256	67	59	89	6	7	119

	EDTA Plasma			Heparin Plasma		
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
1/2	6742	7017	104	6997	7568	108
1/4	3471	3508	101	3639	3784	104
1/8	1639	1754	107	1915	1892	99
1/16	833	877	105	906	946	104
1/32	424	439	103	481	473	98
1/64	229	219	96	247	237	96
1/128	116	110	94	121	118	98
1/256	60	55	91	63	59	94

Recovery

Recombinant rat TNF-α was spiked into serum and plasma to determine percent recovery.

Sample	% Recovery
Serum	108
Plasma (EDTA)	105
Plasma (Heparin)	104
Plasma (Citrate)	97
RPMI + 10% FBS	86

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Product label explanation of symbols and warnings

REF	Catalog Number		Lot/Batch code		Protect from light		Use by	Manufacturer
RUO	Research Use Only		Temperature limitation		Consult accompanying documents		European Community authorized representative	Directs the user to consult instructions for use (IFU), accompanying the product.