Human TNF-α ELISA Kit

Catalog Number KHC3011 (96 tests), KHC3012 (2 × 96 tests), and KHC3011C (5 × 96 tests)

Pub. No. MAN0003931 **Rev.** 5.0 (32)

CAUTION! This kit contains materials with small quantities of sodium azide and Proclin™ 300. 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. In case of contact, rinse affected area with plenty of water. Proclin™ 300 is toxic, corrosive, and a skin irritant. 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™ Human 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 human TNF-α in human serum, plasma, buffered solutions, or cell culture medium. The assay recognizes both natural and recombinant human TNF-α.

Human tumor necrosis factor alpha (Hu TNF-α), also called cachectin, is a 157 AA non-glycosylated polypeptide cytokine produced by activated macrophages. Lipopolysaccharide (LPS) is a potent stimulus for TNF-α production in macrophages, and TNF-α is an important mediator of the *in vivo* effects of LPS. The biological activities of TNF-α may be classified as:

- Immunomodulating and proinflammatory: TNF-α regulates the production of antibodies by B cells and stimulates cytotoxic T cells.

- Metabolic: TNF-α strongly inhibits lipoprotein lipase and adipocyte gene expression.

Contents and storage

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

Contents	Cat. No. KHC3011 (96 tests)
Hu TNF-α Standard, lyophilized; contains 0.1% sodium azide	2 vials
Standard Diluent Buffer; contains 8 mM sodium azide	25 mL
Incubation Buffer; contains 8 mM sodium azide	11 mL
Antibody-Coated Wells, 96-well plate	1 plate
Hu TNF-α Biotin Conjugate; contains 8 mM sodium azide	11 mL
Streptavidin-HRP, (100X); contains 1.3 mM thymol	0.125 mL
Streptavidin-HRP Diluent; contains 1.3 mM thymol and 0.05% Proclin™ 300	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Materials required but not supplied

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

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 25 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

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

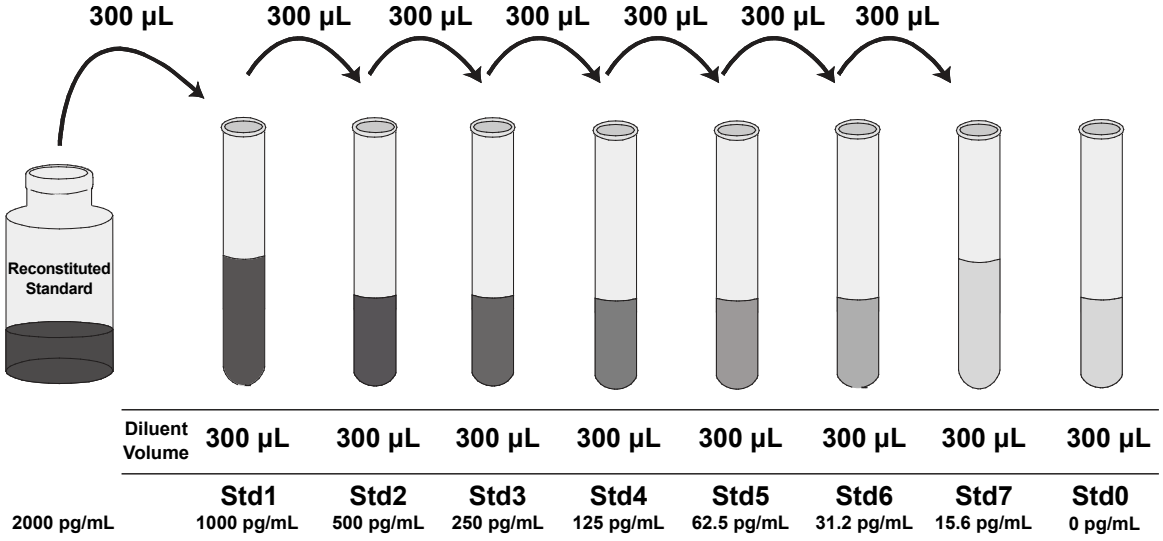
Perform sample dilutions with Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant).

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This assay has been calibrated against the International Standard preparation (87/650) for Hu TNF-α (NIBSC, Hertforshire, UK, EN6 3QG). One microgram equals 40,000 International Units.

1. Reconstitute Hu TNF-α Standard to 2000 pg/mL with Standard Dilution 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 2000 pg/mL human TNF-α. **Use the standard within 1 hour of reconstitution**.
2. Add 300 µL Reconstituted Standard to one tube containing 300 µL Standard Diluent Buffer and mix. Label as 1000 pg/mL human TNF-α.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, 15.6, and 0 pg/mL human TNF-α.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard all remaining reconstituted and diluted standards after completing assay. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

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

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

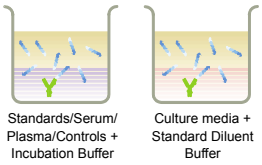
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (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 50 µL of **Incubation Buffer** to wells for serum or plasma samples, standards, or controls; or 50 µL of **Standard Diluent Buffer** to the wells for cell culture samples. Leave the wells for chromogen blanks empty.
b. 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.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Hu 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

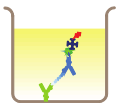
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

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 four 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 (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant) 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 0–1000 pg/mL human TNF-α.

Standard Human TNF-α (pg/mL)	Optical Density (450 nm)
1000	3.38
500	1.97
250	1.18
125	0.75
62.5	0.38
31.2	0.22
15.6	0.15
0	0.08

Inter-assay precision

Samples were assayed 18 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	47.0	170.0	438.0
Standard Deviation	4.0	14.0	26.0
% Coefficient of Variation	8.5	8.2	5.9

Intra-assay precision

Samples of known human 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)	58.0	167.0	459.0
Standard Deviation	3.0	6.9	18.0
% Coefficient of Variation	5.2	4.1	3.9

Expected values

Human PBMCs or whole blood were stimulated from 4 to 72 hours with lipopolysaccharide (LPS), phytohaemagglutinin (PHA), or ionomycin and phorbol myristate acetate (PMA) then evaluated for the presence of human TNF-α in this assay. Whole blood samples were pre-diluted 10-fold for the assay.

Stimulation condition	Human TNF-α (pg/mL)
LPS (0.1 ng/mL)	1800–6000
PHA (1 µg/mL)	1800–6000
LPS (25 µg/mL) + PHA (5 µg/mL)	5000–11,000
Ionomycin (0.1 µg/mL) + PMA (3 ng/mL)	1600–9000

Linearity of dilution

Human serum containing 806 pg/mL of measured human TNF-α was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Recovery

The average recovery of Human TNF-α ELISA Kit added to a variety of samples is listed in the following table.

Sample	Average % Recovery
EDTA plasma	96
Heparin plasma	88
Citrate plasma	93
Serum	92
10% fetal bovine serum	107

Sensitivity

The analytical sensitivity of ths assay is 1.7 pg/mL human TNF-α. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times.

Specificity

Buffered solutions of The following panel of substances were assayed at 50 ng/mL and were found to have no cross-reactivity: **human** IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IFN-α, IFN-β, IFN-γ, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, TGF-β, RANTES; **swine** TNF-α; **rat** TNF-α; **mouse** TNF-α.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

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