Human TNF-α Ultrasensitive ELISA Kit

Catalog Number KHC3014 (96 tests), KHC3013 (2 × 96 tests), and KHC3014C (5 × 96 tests)

Pub. No. MAN0014853 **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™ Human TNF-α Ultrasensitive 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.

Contents and storage

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

Contents	Cat. No. KHC3014 (96 tests)
Hu TNF-α US Standard, lyophilized ; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Incubation Buffer; contains 8 mM sodium azide	12 mL
Antibody Coated Wells; 96-well plate	1 plate
Hu TNF-α US Biotin Conjugate (Biotin-labeled anti-TNF-α); contains 0.1% sodium azide	6 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
Plate Covers, adhesive strips	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)
- 37°C Incubator

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

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.

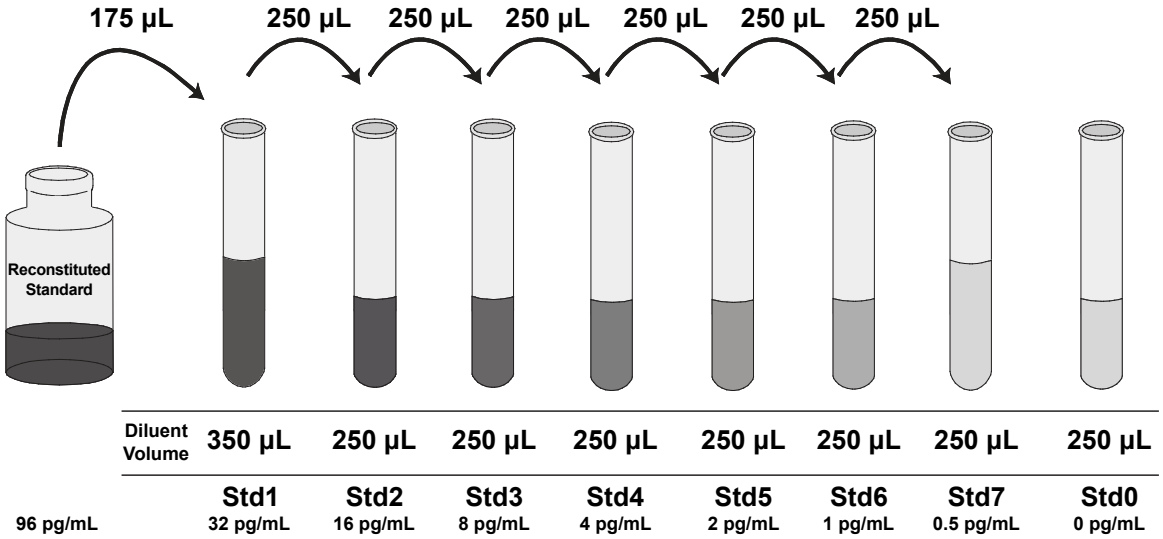
- Dilute samples >32 pg/mL with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: One microgram of recombinant human TNF-α equals 237,000 International Units of WHO reference preparation 88/786 (NIBSC, Hertfordshire, UK, EN6 3QG).

1. Reconstitute Hu TNF-α US Standard to 96 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 96 pg/mL human TNF-α. **Use the standard within 1 hour of reconstitution.**
2. Add 175 µL Reconstituted Standard to one tube containing 350 µL Standard Diluent Buffer and mix. Label as 32 pg/mL human TNF-α.
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 16, 8, 4, 2, 1, 0.5 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.

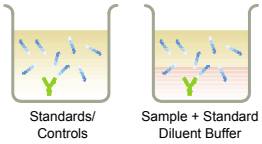
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 3 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 the Incubation Buffer to all wells except the chromogen blanks.
b. Add 100 µL of standards, or controls to the appropriate wells. For serum, plasma, buffered solution and cell culture medium wells, add 50 µL of **Standard Diluent Buffer** followed by 50 µL of sample (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
c. Add 50 µL Hu TNF-α US Biotin Conjugate solution into each well except the chromogen blanks.
d. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at 37°C.
e. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP

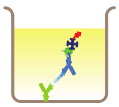
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 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.

4 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 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 32 pg/mL human TNF-α.

Standard Human TNF-α US (pg/mL)	O.D. (450 nm)
32	2.34
16	1.24
8	0.72
4	0.46
2	0.34
1	0.29
0.5	0.24
0	0.19

Inter-assay precision

Samples were assayed 42 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	6.1	11.6	18.6
Standard Deviation	0.5	0.89	1.8
% Coefficient of Variation	8.2	7.7	9.7

Intra-assay precision

Samples of known human TNF-α concentration were assayed in replicates of 14 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	6.3	12.3	19.0
Standard Deviation	0.42	0.7	1.0
% Coefficient of Variation	6.7	5.7	5.3

Expected values

A limited number of sera (n = 8) and plasma (n = 8) samples were assayed with the Human TNF-α Ultrasensitive ELISA Kit. The values for sera ranged from 0 to 2.1 pg/mL. The values for plasma ranged from 0 to 3.8 pg/mL.

Linearity of dilution

A normal human serum pool containing 24.49 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 1.00.

Recovery

The average recovery of human TNF-α from samples assayed with the Human TNF-α Ultrasensitive ELISA Kit is shown in the following table.

Sample	Average % Recovery
Serum	104
Plasma	103
1% fetal bovine serum	111
10% fetal bovine serum	117

Sensitivity

The analytical sensitivity of the assay is <0.09 pg/mL human TNF-α. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard.

Specificity

Buffered solutions of a panel of substances at 500 pg/mL were assayed with the Human TNF-α Ultrasensitive ELISA Kit. The following substances were tested and found to have no cross-reactivity: **human** IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, sIL-2R, sIL-6R, IFN-γ, GM-CSF, SCF, RANTES; **mouse** IL-1β, IL-3, IL-4, IL-6, IL-10, TNF-α; **rat** IL-1β, IL-4, IL-6, IL-10, MIP-2, TNF-α.

Limited product warranty

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Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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