Human sTNF-RI ELISA Kit

Catalog Number KAC1761 (96 tests)

Pub. No. MAN0019410 Rev. 1.0

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 sTNF-RI ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human sTNF-RI in serum, plasma, and cell culture medium. The assay recognizes both natural and recombinant human sTNF-RI.

sTNF-RI, also named TBPI or p55, is one of the two receptors of tumor necrosis factors that are present at the surface of many cells. Different processes modulate their presence. IL-2 and the activation of T-lymphocytes, maturation of macrophages, and the presence of protein kinase activators all increase the presence of both TNF-RI and RII. Factors that can decrease the presence include the presence of H_2O_2, epinephrine, insulin, and somatostatin. The molecular weight of TNF-RI is ~55 kDa, which suggests an important glycosylation.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. Store the Wash Buffer Concentrate at room temperature. When stored as indicated, all reagents are stable until the expiration date.

Contents	Cat. No. KAC1761 (96 tests)
anti-sTNF-RI Antibody-Coated Wells, 96-well strip-well plate	1 plate
Standard; 0 ng/mL in buffer with bovine serum	1 vial
Standards 1 to 5; in bovine serum; lyophilized. Refer to vial label for reconstitution volume and range	5 vials
Anti-sTNF-RI-HRP Conjugate; in buffer with proteins	21 mL
Controls 1 and 2; in buffer with human plasma	2 vials
Wash Buffer Concentrate (200X)	10 mL
Chromogenic TMB (tetramethylbenzidine) in DMF	25 mL
Stop Solution (1 N HCl)	25 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm, 490 nm, and 650 nm (polychromatic reading)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Horizontal microplate shaker capable of 700 rpm ± 100 rpm
- Magnetic stirrer

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 2 mL of Wash Solution Concentrate (200X) with 398 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Use a magnetic stirrer to mix the solution.

   Note: Use 1X Wash Buffer on the same day it is prepared. Discard unused 1X Wash Buffer at the end of the day.

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.
- Avoid impurities contained in sampling materials that can stimulate human sTNF-RI production by blood cells and produce a false increase in plasma values for human sTNF-RI.

Reconstitute controls

Note: Controls are stable for 4 days at 2–8°C. For longer term storage, make aliquots and store at –20°C for up to 2 months. Avoid successive freeze thaw cycles.

Reconstitute Controls 1 and 2 by adding 0.5 mL of distilled water to each vial.

If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy can be determined.

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.

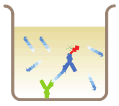
- Dilute the sample as needed.
- If samples generate values higher than the highest standard, dilute samples further and repeat the assay.

Perform Assay (Total assay time: 1.25 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



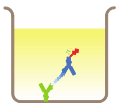
a. Add 50 µL of calibrators, controls, or samples (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 200 µL of anti-sTNF-RI-HRP Conjugate solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 1 hour at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
d. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.

2 Add Chromogenic TMB

a. Add 50 µL of Chromogenic TMB to each well. The substrate solution begins to turn blue.
b. Incubate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm in the dark.

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

3 Add Stop Solution



Add 200 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 3 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 are for illustration only and should never be used in place of a real-time standard curve.

Concentration (ng/mL)	Optical Density (450 nm)
47	5.20
22	3.69
8	1.87
2.5	0.70
1	0.25
0	0.03

Inter-assay precision

Samples were assayed 10 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2
Mean (ng/mL)	1.8	26.8
Standard Deviation	0.10	2.4
% Coefficient of Variation	5.7	8.9

Intra-assay precision

Samples of human sTNF-RI were assayed in replicates of 12 (Sample 1) and 18 (Sample 2) to determine precision within an assay.

Parameters	Sample 1	Sample 2
Mean (ng/mL)	0.99	19.11
Standard Deviation	0.02	1.25
% Coefficient of Variation	1.7	6.5

Expected values

These values are given only for guidance and it is recommended that each laboratory establishes its own normal values.

Sample	Range (ng/mL)	Average (ng/mL)	Standard deviation
Plasma (n=129)	0.30–2.9	1.2	0.6

High-dose hook effect

A sample spiked with human sTNF-RI up to 4,000 ng/mL gives a response higher than that obtained for the last standard point.

Recovery

Sample	Added sTNF-RI (ng/mL)	Recovery sTNF-RI (ng/mL)	Recovery %
Serum	19.62	20.48	98
	4.38	5.56	98
	1.72	2.96	98
	0	1.27	—
Plasma	30.30	34.37	107
	6.99	9.92	115
	2.65	4.11	85
	0	1.85	—
Cell culture medium	31.12	31.65	102
	11.45	12.46	108
	2.52	2.53	99
	0	0.04	—

Sensitivity

The minimum detectable dose of human sTNF-RI is 50 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

Cross reactivity and interference were analyzed by the addition of different analytes to sTNF-RI samples and measuring the apparent sTNF-RI concentration.

Analyte	Results in the Presence of 1.62 ng/mL sTNF-RI (Ratio*)	Results in the Presence of 5.20 ng/mL sTNF-RI (Ratio*)	Results in the Presence of 12.60 ng/mL sTNF-RI (Ratio*)
sTNF-RII (p75) (1500 ng/mL)	1.88 ng/mL (116.1%)	5.65 ng/mL (108.6%)	14.45 ng/mL (114.7%)
TNF-α (400 ng/mL)	1.38 ng/mL (85.2%)	4.80 ng/mL (92.3%)	12.23 ng/mL (97.1%)

Ratio* = [(sTNF-RI measured in the presence of analyte) x 100] / [sTNF-RII added in the absence of analyte]

No significant cross-reaction was observed in the presence of sTNF-RII or TNF-α. This kit is specific for human natural and recombinant human sTNF-RI.

Linearity of dilution

Dilution	Serum			Plasma			Cell Culture Medium		
	Measured conc. (ng/mL)	Theor. conc. (ng/mL)	Recovery (%)	Measured conc. (ng/mL)	Theor. conc. (IU/mL)	Recovery (%)	Measured conc. (ng/mL)	Theor. conc. (ng/mL)	Recovery (%)
1/1	18.09	18.09	—	23.82	23.82	—	33.16	33.16	—
1/2	9.55	9.05	106	10.53	11.91	88	14.66	16.58	88
1/4	4.67	4.52	103	4.85	5.96	81	8.06	8.29	97
1/8	2.56	2.26	113	2.63	2.98	88	4.39	4.15	106
1/16	1.44	1.13	127	1.43	1.49	96	2.43	2.07	117

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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**.

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

Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.