Performance characteristics, continued

Intra-assay precision

Samples of known Ms 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)	50.0	149.5	629.2
SD	2.9	9.5	51.1
%CV	5.9	6.4	8.1

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	53.8	152.0	650.6
SD	4.7	9.54	57.3
%CV	8.7	6.3	8.8

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recoveries of Ms TNF-α added to mouse serum, citrate plasma, heparinized plasma, and tissue culture media containing 10% fetal bovine serum were measured with the Ms TNF-α ELISA Kit.

Sample	Range	Average % Recovery
Serum	84.9–99.4%	92.8
Citrate plasma	74.8–104.5%	88.3
Heparin plasma	74.2–118%	91.3
RPMI+10% fetal bovine serum	104.1–112.1%	109

Linearity of dilution

Mouse serum, citrate plasma, heparinized plasma, and tissue culture medium spiked with natural Ms TNF-α were 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
Serum	0.999
Citrate plasma	0.993
Heparin plasma	0.999
Tissue culture medium	0.999

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 found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.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

For support visit thermofisher.com/supportor contact **techsupport@lifetech.com**.

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24 September 2015

invitrogen

Mouse TNF-α ELISA Kit

Catalog nos. KMC3011
KMC3012
KMC3011C

Quantity: 96 tests
192 tests
480 tests

Pub. No. MAN0003948 **Rev** 1.0

Description

The Mouse 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 mouse TNF-α in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant mouse TNF-α.

Contents and storage

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

Components	KMC3011 96 tests	KMC3012 192 tests	KMC3011C 480 tests
Ms TNF-α Antibody Coated Wells. 96 well plate.	1 plate	2 plates	5 plates
Ms TNF-α Biotin Conjugate. Contains 0.1% sodium azide.	6 mL	2 × 6 mL	5 × 6 mL
Ms TNF-α Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Wash Buffer Concentrate (25X).	100 mL	100 mL	2 × 100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL	2 × 25 mL	5 × 25 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL	2 × 0.125 mL	5 × 0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL	25 mL	3 × 25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL	3 × 25 mL
Stop Solution.	25 mL	25 mL	3 × 25 mL
Adhesive Plate Covers.	3	6	15

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
- 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

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/support** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- 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.

Dilute samples

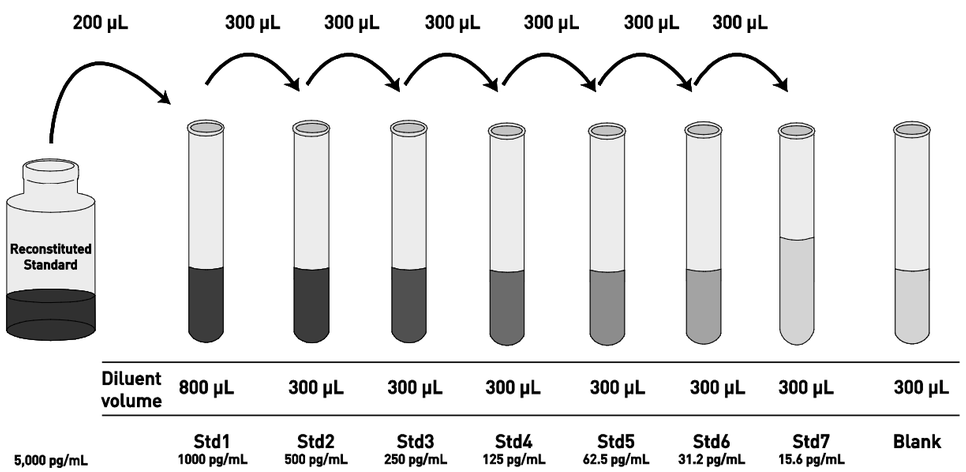
- Dilute **serum, plasma, and cell culture medium** samples 2-fold in Standard Diluent Buffer.
- Alternatively, samples may be diluted directly in the microtiter wells by adding 50 µL of Standard Diluent Buffer to each well, followed by 50 µL of serum, plasma or tissue culture sample.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Ms TNF-α Standard to 5,000 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 5,000 pg/mL Ms TNF-α. **Use the standard within 1 hour of reconstitution**.
2. Add 200 µL Reconstituted Standard to one tube containing 800 µL Standard Diluent Buffer and mix. Label as 1,000 pg/mL Ms 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 Ms 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.

Note: 1 µg of the recombinant Ms TNF-α used in the standard equals 624,000 arbitrary units of WHO reference preparation 88/532 (NIBSC, Hertfordshire, UK, EN6 3QG).



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. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
3. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 2 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

1. Add 100 µL of standards, background controls, or samples (see page 2) to the appropriate wells. Leave wells for chromogen blanks empty.
2. Add 50 µL Ms TNF-α Biotin Conjugate solution into each well except chromogen blanks.
3. Tap side of plate to mix. Cover the plate with the plate cover and incubate for 90 minutes at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Streptavidin-HRP

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

Add chromogen

8. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
9. Incubate for 30 minutes at room temperature **in the dark**.

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

Add stop solution

10. Add 100 µL of 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 30 minutes 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 that of the highest standard 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 0–1000 pg/mL Ms TNF-α.

Standard Ms TNF-α (pg/mL)	Optical Density (450 nm)
1000	3.382
500	2.643
250	1.789
125	1.064
62.5	0.617
31.2	0.405
15.6	0.260
0	0.087

Specificity

Buffered solutions of a panel of substances ranging in concentration from 0.5–30 ng/mL were assayed with the Ms TNF-α kit and found to have no cross-reactivity: **Mouse** EGF, FGF basic, G-CSF, GM-CSF, KC, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IP-10, IFN-γ, MCP-1, MIG, MIP-1α, MIP-1β, MIP-3β, PDGF-BB, RANTES, VEGF; **Human** TNF-α; **Swine** TNF-α; Recombinant Rat TNF-α protein demonstrated 30% cross-reactivity with this kit.

Sensitivity

The minimum detectable dose of Ms TNF-α is <3 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.