invitrogen PRODUCT INFORMATION SHEET

Swine TNF-α ELISA Kit

Catalog Number KSC3011 (96 tests), KSC3012 (2 x 96 tests)

Pub. No. MAN0014387 **Rev.** 3.0 (31)

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™ Swine 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 swine TNF-α in swine serum, EDTA plasma, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant swine TNF-α.

Tumor Necrosis Factor Alpha (TNF-α), also called cachectin, is a 157 amino acid nonglycosylated polypeptide cytokine mainly produced by activated macrophages. Lipopolysaccharide (LPS) is a potent stimulus for TNF-α production in macrophages.

Contents and storage

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

Contents	Cat. No. KSC3011 (96 tests)
Sw TNF-α Standard; lyophilized.	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
Sw TNF-α Biotin Conjugate (100X concentrate); contains 8 mM sodium azide	0.125 mL
Sw TNF-α Biotin Conjugate Diluent Buffer; contains 8 mM sodium azide	12.5 mL
Streptavidin-Peroxidase (HRP) (100X)	0.125 mL
Streptavidin-Peroxidase (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	4

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)

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

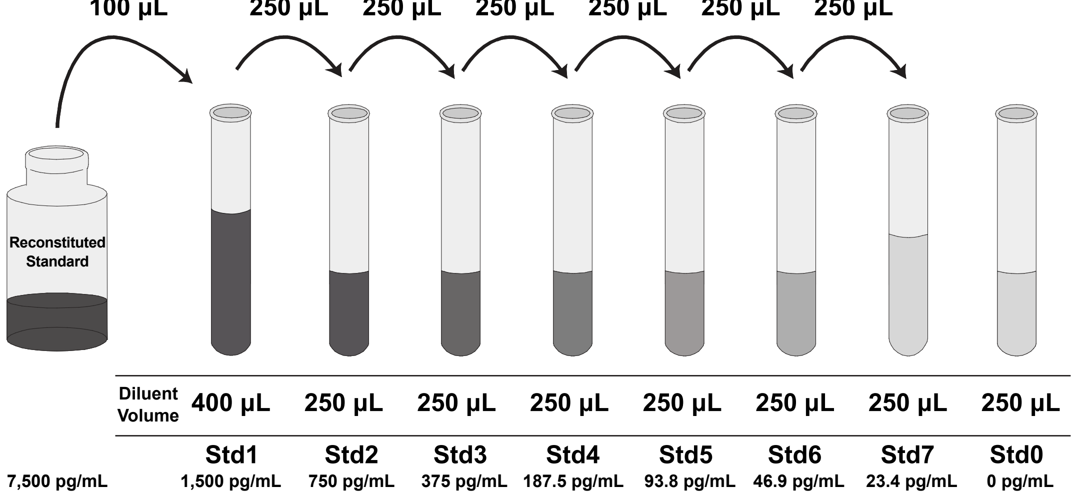
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Perform sample dilutions with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Sw TNF-α Standard to 7,500 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 7,500 pg/mL swine TNF-α. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 400 µL Standard Diluent Buffer and mix. Label as 1,500 pg/mL swine TNF-α.
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 750, 375, 187.5, 93.8, 46.9, 23.4, and 0 pg/mL swine TNF-α.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Biotin Conjugate solution

Note: Prepare 100 µL 1X Biotin Conjugate solution for each well used in the assay. Use the 1X Biotin Conjugate within 15 minutes of preparation.

1. Dilute appropriate volume of Biotin Conjugate (100X) by 1:100 in 1X Biotin Conjugate Diluent Buffer.
2. Return unused Biotin Conjugate (100X) and Biotin Conjugate Diluent Buffer to the refrigerator. Discard 1X Biotin Conjugate solution after use.

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 1X Assay Buffer. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

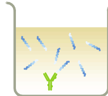
Perform ELISA (Total assay time: 5 hours)

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.

Capture antibody Antigen Biotin conjugate Streptavidin-HRP

1 Bind antigen



a. Add 50 µL of **Incubation Buffer** to wells corresponding to serum, plasma, buffered solution and cell culture samples and standards. Leave the wells for chromogen blanks empty.

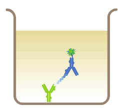
b. Add 100 µl of the Standard Diluent Buffer to the zero standard wells. Leave the wells for chromogen blanks empty.

c. Add 100 µL of standards or samples to the appropriate wells. For swine serum, plasma, cell culture medium or buffered solutions, add 50 µL of sample followed by 50 µL of Standard Diluent Buffer (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.

d. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 3 hours at room temperature.

e. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

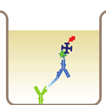


a. Add 100 µL Sw TNF-α Biotin Conjugate solution into each well except the chromogen blanks.

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

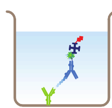


a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.

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

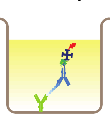


a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.

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 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards and unknowns prior to plotting.
3. Read the concentrations for unknown samples 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

Specificity

Buffered solutions of a panel of substances at 50 ng/mL were assayed with the Swine TNF-α ELISA Kit. The following substances were tested and found to have no cross-reactivity: **swine** IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-15; **mouse** TNF-α; rat TNF-α; **human** TNF-α.

Sensitivity

The analytical sensitivity of this assay is <3 pg/mLswine TNF-α. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard.

Standard curve example

The following data were obtained for the various standards over the range of 0 to 1500 pg/mL swine TNF-α.

Standard Swine TNF-α (pg/mL)	Optical Density (450 nm)
1,500	3.54
750	1.77
375	0.97
187.5	0.51
93.8	0.29
46.9	0.15
23.4	0.11
0	0.05

Inter-assay precision

Samples were assayed 16 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	63	168	695
Standard Deviation	5.2	11	50
% Coefficient of Variation	8.2	6.5	7.2

Intra-assay precision

Samples of known swine 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)	56	149	614
Standard Deviation	3.2	9.2	37
% Coefficient of Variation	5.7	6.2	6.0

Linearity of dilution

Swine serum or cell culture samples containing swine TNF-α were serially diluted over the range of the assay in Standard Diluent Buffer or RPMI containing 10% fetal bovine serum, respectively. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

Dilution	Serum			Cell Culture		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
1/2	886	—	—	1/20	—	—
1/4	444	443	100	1/40	497	98.6
1/8	222	222	100	1/80	249	98.8
1/16	105	111	94.6	1/160	124	109
1/32	53	55	96.4	1/320	62	93.5

Expected values

Serum/Plasma

Each laboratory must establish its own normal values. For guidance, the mean of 20 normal sera was 53 pg/mL (range: from 16 to 138 pg/mL). The mean of 20 normal EDTA plasma samples was 65 pg/mL (range: from undetectable to 189 pg/mL).

Cell culture supernatants were evaluated in this assay.

Swine whole blood (WB) cells were cultured for 24, 48 or 72 hours in RPMI supplemented, or not, with a blend of LPS (25 mg/mL) and PHA (5 mg/mL), or ionomycin (100 ng/mL) and PMA (100 ng/mL). Results are shown below.

	Sw TNF-α(pg/mL)		
Stimulus	24 hours	48 hours	72 hours
Neat	ND	ND	ND
LPS+PHA	212	181	168
PMA+ionomycin	27,040	17,080	12,520

Recovery

The recovery of swine TNF-α added to swine serum and EDTA plasma averaged 91% or 81%, respectively. The recovery of swine TNF-α added to cell culture medium containing 1% fetal bovine serum averaged 102%, while the recovery of swine TNF-α added to cell culture medium containing 10% fetal bovine serum averaged 98%.

Sample	Average % Recovery
Swine serum and EDTA plasma	91.0 or 81.0
Cell culture medium (1% fetal bovine serum)	102.0
Cell culture medium (10% fetal bovine serum)	98.0

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

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1 June 2018