INSTRUCTIONS

Porcine TNFα ELISA Reagent Kit

ESS0003

2470.0

Number	Description
ESS0003	**Porcine TNFα ELISA Reagent Kit,** pre-titered coating and detection antibodies, recommended buffers and specific assay protocol optimized for the quantitative measurement of porcine TNFα in cell culture supernatants

Kit provides sufficient reagents for approximately five 96-well plates, provided the Porcine TNFA ELISA Reagent Kit Protocol is followed.

Kit Contents	Size	Assay Dilution
Anti-Human TNFα Coating Antibody	0.625mL	1:100
Lyophilized Recombinant Porcine TNFα Standard	5 vials	See vial label
Anti-Human TNFA Detection Antibody	0.625mL	1:100
Streptavidin-HRP	0.25mL	1:400
Substrate Solution	55mL	Ready to use
Stop Solution, 0.16M Sulfuric Acid	55mL	Ready to use

For research use only. Not for use in diagnostic procedures.

Storage: Immediately upon receipt, aliquot and freeze the coating and detecting antibodies at ≤ -20°C in a manual defrost freezer (125µL/tube). Avoid repeated freeze-thaw cycles. Store all other components at 2-8ºC. Kit is shipped on dry ice.

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Introduction

The Thermo Scientific™ Porcine TNFα ELISA Reagent Kit is for measuring porcine TNFα in serum; EDTA, heparin and sodium citrate plasma; and culture supernatants. This kit is a sandwich ELISA that uses anti-human TNFα antibodies that recognize porcine TNFα. The microplate provided is coated with an antibody that captures TNFα in standards and samples when added to the plate. After non-bound proteins are removed, the biotinylated detecting antibody is added and binds to a second site on the TNFα. The plate is then washed and streptavidin-horseradish peroxidase is added. The enzyme-substrate reaction generates a colorimetric signal that is measured on a plate reader at 450 nm minus the absorbance at 550 nm. The absorbance is proportional to the amount of porcine TNFα in the standard or sample.

Materials Required

- 8-well strip plates, clear, corner-notched (Product No. 15031)
- Plate sealers for 96-well plates (Product No. 15036)
- Reagent reservoir, sterile, 50mL capacity, 40pk (Product No. 15075)

ELISA Reagent Kit Buffers

- D-PBS**:** 0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4, 0.2µm filtered (e.g., Thermo Scientific™ BupH™ Modified Dulbecco's Phosphate Buffered Saline Packs, Product No. 28374)
- Blocking Buffer**:** 4% bovine serum albumin (BSA), 5% sucrose in D-PBS, 0.2µm filtered OR ELISA Blocker Blocking Buffer, Product No. N502
- Reagent Diluent**:** 4% BSA in D-PBS (pH 7.4), 0.2µm filtered
- Wash Buffer: 0.05% Tween™-20 Detergent (e.g., 0.5% Thermo Scientific™ Surfact-Amps™ 20 Detergent Solution, Product No. 28320) in D-PBS, pH 7.4 OR ELISA Wash Buffer (30X), Product No. N503
- **Note:** Mix new solution daily.

Assay Protocol

Kit components are titered to give optimal results using the Porcine TNFα ELISA Reagent Kit Protocol for cell culture supernatants. Any change, including component concentration, volumes, incubation times or temperatures, buffer content or number of wash steps may significantly affect the ELISA results and require optimization to give the best results.

Note: Allow all reagents and buffers to equilibrate to room temperature (22-25°C) before use. Thaw one aliquot of coating and detecting antibody for each plate. Do not use a water bath.

A. Plate Preparation

1. Dilute the Coating Antibody 1:100 in D-PBS buffer by adding 110µL Coating Antibody to 10.89mL of D-PBS buffer.
2. Add 100µL of diluted Coating Antibody to each well. Cover plate with plate sealer and incubate overnight at room temperature.
3. Aspirate Coating Antibody solution and add 300µL of Blocking Buffer to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
4. Aspirate Blocking Buffer and proceed to assay or allow to dry overnight at room temperature. When sealed with dessicant, plates can be stored at 2-8°C for 6 months.

B. Assay Procedure

1. Reconstitute standard with Reagent Diluent with volume stated on vial label. The concentration of the reconstituted standard is 4000pg/mL.
2. Dilute reconstituted standard 1:2 in Reagent Diluent to prepare top Standard (2000pg/mL). Using Reagent Diluent, prepare 1:2 serial dilutions of top Standard and dilute any supernatant expected to read above the top standard. Add 100µL of sample or Standard to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
3. Aspirate and wash three times with Wash Buffer using 300µL per well.
4. Dilute the Detection Antibody 1:100 in Reagent Diluent by adding 110µL of Detection Antibody to 10.89mL of Reagent Diluent.
5. Add 100µL of Detection Antibody to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
6. Aspirate and wash three times with Wash Buffer, using 300µL per well.
7. Dilute Streptavidin-HRP 1:400 in Reagent Diluent by adding 30µL of Streptavidin-HRP to 12mL of Reagent Diluent.

8. Add 100µL of diluted Streptavidin-HRP reagent to each well. Cover plate with plate sealer and incubate for 30 minutes at room temperature.
9. Aspirate and wash three times with Wash Buffer, using 300µL per well.
10. Add 100µL of Substrate Solution to each well. Cover plate with plate sealer and incubate in the dark for 20 minutes at room temperature.
11. Stop the reaction by adding 100µL of Stop Solution to each well.
12. Measure the absorbance at A_{450} minus A_{550}.

C. Absorbance Measurement

Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is not available, measure the absorbance at 450nm only.

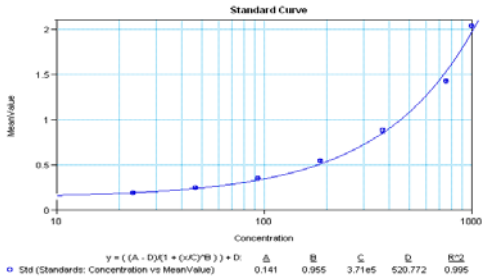
Note: When the 550nm measurement is omitted, absorbance values will be higher.

Note: Evaluate the plate within 30 minutes of stopping the reaction.

D. Calculation of Results

- The standard curve is used to determine porcine TNFα amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding porcine TNFα concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the porcine TNFα amount in each sample by interpolating from the absorbance value (Y-axis) to porcine TNFα concentration (X-axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/mL of porcine TNFα in the sample.

Standard Curve Example



Standard curve based on data obtained using the Human TNFα ELISA Reagent Kit Protocol.

NOTE: This standard curve is for demonstration only. A standard curve must be run with each assay.

- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

Performance Characteristics

Specificity: This ELISA uses antibodies raised against human TNFα. This ELISA reacts with porcine and human TNFα at 100%.

The following cytokines, tested at 1 µg/ml, did not interfere or cross-react: human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12p70, IL-12p40, IL-13, IL-15, IL-16, IL-17, IL-18, Eotaxin, GCSF, GMCSF, GROα, GROβ, IFNγ, IFNα, MCP-1, MCP-2, MCP-3, MCP-4, VEGF, MIP-1α, MIP-1β, TGFβ, TNFβ or RANTES; mouse TNFα; rat TNFα; bovine TNFα.

The following substances, tested at a concentration of 20µg/mL, did not interfere with this ELISA: Concanavalin A (ConA), phytohemagglutinin (PHA), phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), aprotinin, hemoglobin (HGB), β-mercaptoethanol (β-ME).

General Reference

Immunoassay: A Practical Guide. Chan and Perlstein, Eds. (1987). Academic Press: New York. p.71.

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