

Bovine TNF- α ELISA Kit

TNF- α , the prototypical member of the TNF protein superfamily, is a homotrimeric type-II membrane protein (1, 2). Membrane bound TNF- α is cleaved by the metalloprotease TACE/ADAM17 to generate a soluble homotrimer (2). Both membrane and soluble forms of TNF- α are biologically active. TNF- α is produced primarily by macrophages, but it is produced also by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue (1). Cellular response to TNF- α is mediated through interaction with receptors TNF-R1 and TNF-R2 and results in activation of pathways that favor both cell survival and apoptosis depending on the cell type and biological context. Activation of kinase pathways (including JNK, ERK (p44/42), p38 MAPK and NF- κ B) promotes the survival of cells, while TNF- α mediated activation of caspase-8 leads to programmed cell death (1,2). TNF- α plays a key regulatory role in inflammation and host defense against bacterial infection, notably *Mycobacterium tuberculosis* (3). TNF- α causes many of the clinical problems associated with autoimmune disorders such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, psoriasis, hidradenitis suppurativa and refractory asthma. The role of TNF- α in autoimmunity is underscored by blocking TNF- α action to treat rheumatoid arthritis and Crohn's disease (1, 2, 4).

References

1. Aggarwal, B.B. (2003) *Nat Rev Immunol* 3, 745-56.
2. Hehlhans, T. and Pfeffer, K. (2005) *Immunology* 115, 1-20.
3. Lin, P.L. et al. (2007) *J Invest Dermatol Symp Proc* 12, 22-5.
4. Brennan, F.M. and McInnes, I.B. (2008) *J Clin Invest* 118, 3537-45.

PRINCIPLE OF THE ASSAY

This kit is for quantification of TNF α in cow, cattle and bull. This is a shorter ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for bovine TNF- α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF- α present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for bovine TNF- α is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of TNF- α bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

Storage

Store at 4°C. The kit should be used in 3 months.

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Conjugate	1	Reagent Diluent	1	DataSheet/Manual	1
Standard	3	Antibody Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

Bovine TNF- α Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C in a manual defrost freezer for up to 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 μ L of sterile 1 x PBS to the antibody vial and vortex briefly and sit for 5 min. If the entire 96-well plate is used, take 200 μ L of detection antibody to 10.5 mL of Antibody Diluent to make **working dilution of Detection Antibody** and mix thoroughly prior to the assay. If a partial antibody is used store the rest at -20°C until use.

Bovine TNF- α Standard (3 vials) – The lyophilized Bovine TNF- α Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. The non-reconstituted standard can be stored at 4°C or -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 μ L of Reagent Diluent to a Standard vial to make the high standard concentration of 2000 pg/ml. Vortex 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in the Reagent Diluent, vortex 20 sec for each of dilution steps.

Conjugate (53 μ L) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 53 μ L Conjugate sufficient for a 96-well plate. If the volume is less than 53 μ L, add sterile 1 x PBS to reach 53 μ L and vortex briefly. Make 1:200 dilution in Reagent Diluent. If the entire 96-well plate is used, add 53 μ L of Conjugate to 10.5 mL of Reagent Diluent to make **working dilution of Conjugate** and mix thoroughly prior to the assay. The rest of undiluted Conjugate can be stored at 4°C for up to 3 months. **DO NOT FREEZE.**

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.

20 x Wash Buffer, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.

Reagent Diluent, 21 mL.

Antibody Diluent, 12 mL.

Substrate Solution, 10.5 mL.

Stop Solution, 5.5 mL.

Assay Procedure

1. Lift the plate cover from the top left corner and cover the wells that are not used. Vortex briefly the samples prior to the assay. Add 100 μ L of **sample** (such as plasma or serum) or **standards** per well and use duplicate wells for each sample. Cover the 96-well plate and incubate 90 min at room temperature.
2. Aspirate each well and wash with **1 x Wash Buffer**, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 μ L) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 μ L of the **working dilution of Detection Antibody** to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 μ L of the **working dilution of Conjugate** to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μ L of **Substrate Solution** to each well. Incubate for 10-20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μ L of **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes

1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A standard curve should be generated for each set of samples assayed and thorough mixing of the Standards at each of dilution steps is critical to ensure a normal standard curve.
3. Plasma or serum sample should be diluted with equal volume of 1 x Wash Buffer and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.
4. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
5. The Stop Solution is an acid solution, handle with caution.
6. This kit should not be used beyond the expiration date on the label.
7. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
8. Use a fresh reagent reservoir and pipette tips for each step.
9. It is recommended that all standards and samples be assayed in duplicate.
10. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

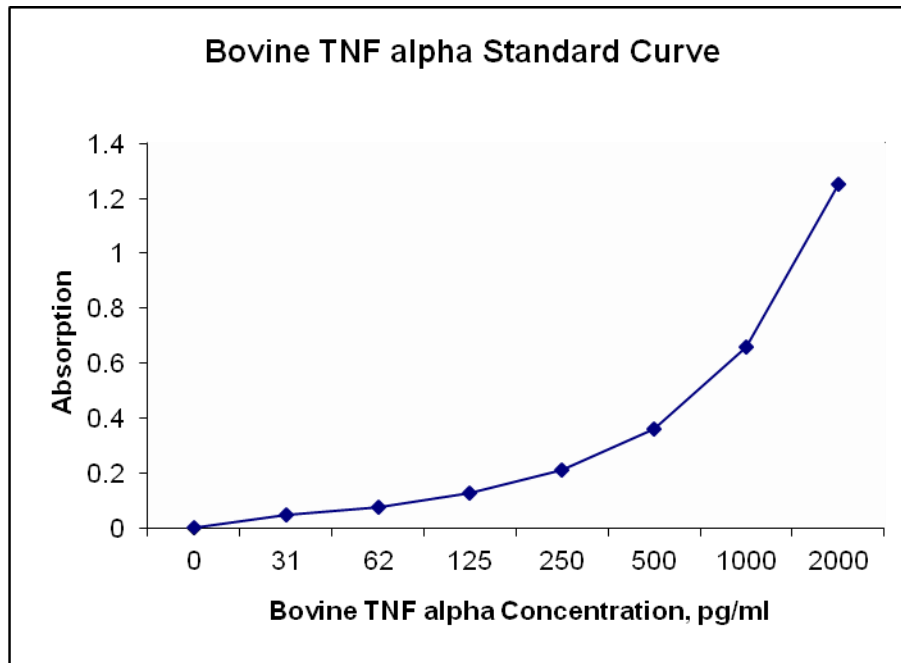
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TNF- α concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The Standard Curve

The graph below represents typical data generated when using this bovine TNF- α ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve.



Specificity

The following recombinant bovine proteins prepared at 1 ng/ml were tested and exhibited no cross-reactivity or interference.

BMP1, BMP2, BMP4, HGF, IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IFN γ , MMP-2, TGF β 1, TGF β 2, TGF β 3, TLR1, TLR2, TLR3, VEGF.

Calibration

This kit is calibrated against a highly purified yeast-expressed recombinant bovine TNF- α .

Detection Range

31-2000 pg/ml

Assay Sensitivity

6 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 8

For Research Use Only

Related products

- 20 x Sample Diluent
- 20 x PBS
- 20 x ELISA Wash Buffer
- 10 x ELISA Reagent Diluent
- Universal Blocking Buffer
- 2 x Recombinant Protein Stabilizer
- 5 x Recombinant Protein Stabilizer
- ELISA G-Blue Substrate Solution
- Bovine TNF α Standard
- Bovine TNF α detection antibody

Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	<ul style="list-style-type: none"> Inaccurate pipetting Improper standard curve 	<ul style="list-style-type: none"> Check pipettes Check and use the correct dilution buffer Vortex 30 sec for each of standard dilution steps
Low signal	<ul style="list-style-type: none"> Improper preparation of standard, samples, detection antibody, and/or conjugate Too brief incubation times Inadequate reagent volume or improper dilution 	<ul style="list-style-type: none"> Briefly spin down vials before opening. Reconstitute the powder thoroughly. Ensure sufficient incubation time. Check pipettes and ensure correct preparation.
Large CV	<ul style="list-style-type: none"> Inaccurate pipetting and mixing Improper standard/sample dilutions. Air bubbles in wells. 	<ul style="list-style-type: none"> Check pipettes and ensure thorough mixing. Use the correct dilution buffers Remove bubbles in wells.
High background	<ul style="list-style-type: none"> Plate is insufficiently washed. Contaminated wash buffer 	<ul style="list-style-type: none"> Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed. Make fresh wash buffer
No signal detected	<ul style="list-style-type: none"> The procedure was misconducted. 	<ul style="list-style-type: none"> Ensure the step-by-step protocol was correctly followed and no misstep was conducted.
Low sensitivity	<ul style="list-style-type: none"> Improper storage of the ELISA kit Stop solution 	<ul style="list-style-type: none"> Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light. Adding stop solution to each well before reading plate