Bovine TNF ELISA Kit

Prod. No.: DEIA619
Pkg. Size: 96T

INTENDED USE

Enzyme linked immunosorbent assay (ELISA) for the detection of Bovine TNF-α in cell culture supernatants. Kit contains sufficient components to quantitate Bovine TNF-α protein concentration in up to 40 samples, tested in duplicate.

GENERAL DESCRIPTION

Tumor necrosis factor-alpha (TNF-α), also referred to as tumor necrosis factor (TNF), is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The primary role of TNF is in the regulation of immune cells. TNF is able to induce apoptotic cell death, to induce inflammation, and to inhibit tumorigenesis and viral replication. Dysregulation of TNF production has been implicated in a variety of human diseases, including major depression, Alzheimer's disease and cancer.

TNF was originally thought to be 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. Large amounts of TNF are released in response to lipopolysaccharide, other bacterial products, and interferon-γ. Two receptors, TNF-R1 and TNF-R2, can be bound to by TNF. TNF-R1 is expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, whereas TNF-R2 is found only in cells of the immune system, and respond to the membrane-bound form of the TNF homotrimer.

Upon contact with their ligand, TNF receptors undergo a conformational change, leading to the dissociation of an inhibitory protein from the intracellular death domain. This dissociation enables the adaptor protein TRADD (tumor necrosis factor receptor type 1-associated death domain) to bind to the death domain, serving as a platform for subsequent protein binding. Following TRADD binding, three pathways can be initiated including activation of NF-κB, activation of MAPK pathways, and induction of death signaling.

The myriad and often-conflicting effects mediated by these pathways indicate the existence of extensive cross-talk. Other factors, such as cell type, concurrent stimulation with other cytokines, or the amount of reactive oxygen species (ROS) present can shift the balance in favor of one pathway or another. Such complicated signaling ensures that, whenever TNF is released, various cells with vastly diverse functions and conditions can all respond appropriately to inflammation.

GENE INFORMATION

- **Gene Name:** TNF tumor necrosis factor [Bos taurus]  
- **Official Symbol:** TNF  
- **Synonyms:** TNF; tumor necrosis factor; TNFa; APC1 protein; TNF-α; cachectin; TNFSF2; TNF superfamily, member 2; TNFA; TNF, macrophage-derived; DIF; TNF, monocyte-derived; Tumor necrosis factor ligand superfamily member 2; tumor necrosis factor alpha; TNF-α; tumor necrosis factor-alpha; tumor necrosis factor (TNF superfamily, member 2); Cachectin; tumor necrosis factor, alpha (TNF superfamily, member 2)
- **Gene ID:** 285943  
- **mRNA Refseq:** NM_173966  
- **Protein Refseq:** NP_776391  
- **UniProt ID:** Q06599  
- **Chromosome Location:** 23q22  
- **Pathway:** Adipocytokine signaling pathway; African trypanosomiasis; Allograft rejection; Alzheimer's disease; Amyotrophic lateral sclerosis (ALS); MAPK signaling pathway; Malaria; Natural killer cell mediated cytotoxicity; Pertussis; T cell receptor signaling pathway; TNF signaling; Type 1 diabetes mellitus
- **Function:** cytokine activity; identical protein binding; protease binding; transcription regulatory region DNA binding; tumor necrosis factor receptor binding

REAGENTS PROVIDED

1. Bovine TNF-α Pre-Coated 96-well Strip Plate, 1 each  
2. Bovine TNF-α Standard, 10 ng/vial, 2 each  
3. Bovine TNF-α Detection Antibody, 12 ml  
4. Dilution Buffer A, 25 ml  
5. HRP Solution, 12 ml  
6. TMB Substrate, 12 ml  
7. Stop Solution, 12 ml  
8. 20X Wash Buffer, 50 ml  
9. Sealing Tape, 6 sheets

MATERIALS REQUIRED BUT NOT PROVIDED

1. Ultrapure water  
2. Precision pipettors, with disposable plastic tips  
3. Polypropylene or polyethylene tubes to prepare standard and samples – do not use polystyrene, polycarbonate or glass tubes  
4. A container to prepare 1X Wash Buffer  
5. A wash bottle or an automated 96-well plate washer  
6. Disposable reagent reservoirs  
7. A standard microtiter plate reader for measuring absorbance at 450 nm
**ASSAY PROCEDURE**

1. **Reagent And Sample Preparation**
   1) **Standard Preparation**
      a. Reconstitute 10 ng standard vial with 1 ml of Dilution Buffer A to achieve a concentration of 10 ng/ml. Mix well. Dilute 500 µl of reconstituted standard in 500 µl Dilution Buffer A. Mix well. This is the top standard with a final concentration of 5 ng/ml.
      b. Label seven (7) tubes, one for each additional standard curve point: 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.313 ng/ml, 0.156 ng/ml, 0.078 ng/ml, and 0 pg/ml.
      c. Pipette 250 µl of Dilution Buffer A into tubes.
      d. Serial dilute the 5 ng/ml standard 1:1 with Dilution Buffer A. Perform dilution by mixing 250 µl of the previous standard with 250 µl of Dilution Buffer A. Continue until reach standard value of 0.078 ng/ml.
      e. Use Dilution Buffer A only as the zero standard value.
      f. **Sample Handling**
         a. Cell culture supernatants may be tested in this ELISA.
         b. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
         c. 100 µl of sample or standard is required per well.
         d. Samples must be assayed in duplicate each time the assay is performed.
         e. Store samples to be assayed within 24 hours at 2-8ºC. Leave these strips for one (1) hour at room temperature, 20-25ºC.
         f. **Sample Preparation**
            If it is suspected that the concentration of the sample exceeds the highest point of the standard curve, prepare one or more dilutions of the sample in Dilution Buffer A until the desired concentration is obtained. For example, a 1:5 dilution could be prepared by diluting 50 µl of sample in 200 µl of Dilution Buffer A in a clean, fresh tube and mixing well.
      g. **Detection Antibody Incubation**
         a. Add 100 µl of Detection Antibody to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.
         b. Carefully attach a new adhesive plate cover. Incubate plate for one (1) hour at room temperature, 20-25ºC.
         c. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.
      2) **HRP Solution Incubation**
         a. Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame.
         b. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.
      3) **Detection Antibody Incubation**
         Only remove the required amount of Detection Antibody reagent for the number of strips being used.
         a. Add 100 µl of Detection Antibody to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.
         b. Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at room temperature, 20-25ºC.
         c. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.
      4) **HRP Solution Incubation**
         Only remove the required amount of HRP Solution for the number of strips being used.
         a. Add 100 µl of HRP Solution to each well containing sample or blank.
         b. Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at room temperature, 20-25ºC.
         c. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.
      5) **TMB Substrate Incubation and Reaction Stop**
         Only remove the required amount of TMB Substrate Solution and Stop Solution for the number of strips being used. Do NOT use a glass pipette to measure the TMB Substrate Solution. Do NOT cover the plate with aluminum foil or metalized mylar. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate Solution. If the solution is blue before use, DO NOT USE IT!
         a. Add 100 µl of TMB Substrate Solution into each well.
         b. Allow the enzymatic color reaction to develop at room temperature (20-25ºC) in the dark for 30 minutes. Do NOT cover plate with a plate sealer. The substrate reaction yields a blue solution.
         c. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should change from blue to yellow.
      6) **Absorbance Measurement**
         a. Wipe underside of wells with a lint-free tissue.
         b. Measure the absorbance on an ELISA plate reader set at 450 nm.

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**CALCULATION**

Duplicate absorbance values should be within 10% of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10%.

1. Prepare a standard curve to determine the amount of Bovine TNF-α in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding Bovine TNF-α concentration (X axis) using graph paper or curve-fitting software.

2. Calculate the Bovine TNF-α concentration in unknown samples using the prepared standard curve. Determine the amount of Bovine TNF-α in each unknown sample by noting the Bovine TNF-α concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.

3. If the sample was diluted, multiply the Bovine TNF-α concentration obtained by the dilution factor to determine the amount of Bovine TNF-α in the undiluted sample.

**ASSAY CHARACTERISTICS**

1. Assay Range: 0.078 - 5 ng/ml

Suggested standard curve points are 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.313 ng/ml, 0.156 ng/ml, 0.078 ng/ml, and 0 pg/ml.

2. Representative Data

PBMCs were harvested by ficoll density gradient from day old whole blood collected from an apparently healthy cow. The PBMCs were suspended at 1 x 10⁶ cells/ml in RPMI medium containing 10% serum. PBMCs were stimulated with phytohemagglutinin (PHA; 10 µg/ml), staphylococcal enterotoxin B (SEB; 5 µg/ml) or phorbol 12-myristate 13-acetate (PMA; 10 ng/ml) and inomycin (500 ng/ml). Cell-free supernatants were harvested following three days stimulation and run in the Bovine TNF-α ELISA. The levels of Bovine TNF-α detected are as follows:

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Bovine TNF-α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>&lt;0.078</td>
</tr>
<tr>
<td>PHA</td>
<td>0.409</td>
</tr>
<tr>
<td>SEB</td>
<td>0.354</td>
</tr>
<tr>
<td>PMA/Ionomycin</td>
<td>1.925</td>
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</tbody>
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**REFERENCES**


