Bovine TNF-α ELISA Reagent Set

NB-06-1121

FOR RESEARCH USE ONLY

Technical Notes:	This kit is for the quantitative measurement of Bovine TNF-α in cell culture supernatants. If assaying other sample types, an appropriate Sample and Standard Diluent will need to be developed and validated. Any changes to the ELISA protocol may significantly affect the results generated and will require optimization.

Included Components:	**Description**	**Quantity**
	Bovine TNF-α Coated Plate	2 each
	Bovine TNF-α Standard	2 each
	Bovine TNF-α Detection Antibody	2 each
	Streptavidin-HRP	1 each
	Plate Sealer	6 each

Additional Reagents Required:	**Reagent**	**Formulation**
	DPBS	0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4
	Reagent Diluent	4% BSA in DPBS, 0.2 µm filtered
	Standard and Sample Diluent	Complete cell culture medium used to generate cell culture supernatant samples It is critical that this medium contain at least 1% carrier protein. If the medium does not contain carrier protein, use Reagent Diluent to dilute the Standard and samples.
	Wash Buffer	0.05% Tween®-20 in DPBS
	Substrate	3,3',5,5'-tetramethylbenzidine (TMB) Substrate
	Stop Solution	0.18 M Sulfuric Acid

Component Preparation:	**Component**	**Preparation**
	Bovine TNF-α Standard	Reconstitute Standard in 1 mL Standard and Sample Diluent. Dilute 250µL of the reconstituted standard in 250µL of Standard and Sample Diluent. The Standard now has a concentration of **7.2 ng/mL**. Prepare 1:1 serial dilutions of the Standard by mixing 250 µL Standard with 250 µL Standard and Sample Diluent. Repeat 1:1 serial dilutions until reach a final concentration of 0.113 ng/mL. Use Standard and Sample Diluent as a zero standard.
	Bovine TNF-α Detection Antibody Working Solution	Dilute the 500 µL of reconstituted Detection Antibody in 11.5 mL Reagent Diluent.
	Streptavidin-HRP Working Solution	Dilute 500 µL of Streptavidin-HRP in 11.5 mL Reagent Diluent.

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ELISA Procedure:

1. Prepare Standard and cell culture supernatant sample dilutions in Standard and Sample Diluent.
2. Add 100 uL of Standard or sample to appropriate wells.

Note: Run each Standard or sample in duplicate.

3. Cover plate with Plate Sealer and incubate at room temperature (20-25C) for 1 hour.
4. Wash plate FOUR times with Wash Buffer.

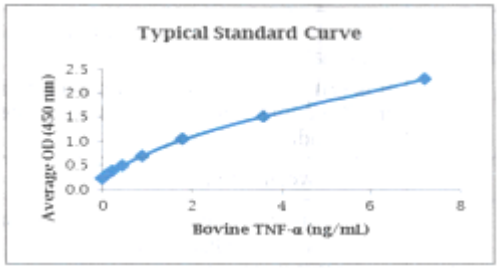
Note: Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.

5. Add 100 uL of Detection Antibody Working Solution to each well.
6. Cover plate with Plate Sealer and incubate at room temperature for 1 hour.
7. Wash plate FOUR times with Wash Buffer as described in step 4.
8. Add 100 uL of Streptavidin-HPR Working Solution to each well.
9. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes.
10. Wash plate FOUR times with Wash Buffer as described in step 4.
11. Add 100 uL of TMB Substrate Solution to each well.
12. Develop the plate in the dark at room temperature for 30 minutes.

Note: Do **NOT** cover plate with Plate Sealer.

13. Stop reaction by adding 100 uL of Stop Solution to each well.
14. Measure absorbance on a plate reader at 450 nm.

Typical Standard Curve:



Data represents a typical standard curve generated using the NeoBiotech Bovine TNF-α ELISA Development Kit.

A standard curve should be generated with each assay.

Representative Data:

Stimulant	Bovine TNF-α (ng/mL)
Unstimulated	<0.113
Staphylococcal enterotoxin B (SEB; 5 µg/mL)	0..409
Phytohemagglutinin (PHA; 10ug/ml)	0.354
Phorbol 12-myristate 13-acetate (PMA; 10 ng/mL) and Ionomycin (500 ng/mL)	1.925

PBMCs harvested by ficoll density gradient from an apparently healthy bovine were suspended in RPMI medium containing 10% serum and stimulated as desired. The cell-free supernatants were harvested following three days stimulation and analyzed in the NeoBiotech Bovine TNF-α ELISA Development Kit.

Country of Origin:

USA