PicoKine™ Quick ELISA

Catalog number: FEK0527

For the quantitation of **Mouse Tnf** concentrations in cell culture supernates, cell lysates, serum and plasma (heparin, EDTA).

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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Mouse TNF PicoKine™ Quick ELISA Kit

Catalog Number: FEK0527

Assay Principle

The Boster Quick Picokine™ Mouse Tnf Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Tnf with a 96-well strip plate that is pre-coated with antibody specific for Tnf. The detection antibody is a HRP conjugated antibody specific for Tnf. The capture antibody is monoclonal antibody from hamster, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse Tnf with immunogen: Expression system for standard: E.coli; Immunogen sequence: L80-L235 The kit is analytically validated with ready to use reagents.

To measure Mouse Tnf, add standards and samples to the wells, then add HRP conjugated detection antibody. Wash the wells with PBS or TBS buffer, and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly propotional to Mouse Tnf in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Tnf in the sample. For more information on assay principle, protocols, and troubleshooting tips, see Boster's ELISA Resource Center at https://www.bosterbio.com/elisa-technical-resource-center.

Overview

Product Name	Mouse TNF ALPHA PicoKine™ Quick ELISA Kit
Reactive Species	Mouse
Size	96wells/kit, with removable strips.
Description	The Quick Picokine ELISA kits, assay takes less than 1.5 hours. Detect Mouse Tnf with <2pg/ml sensitivity. Format: 96-well plate with removable strips. Compatible samples: cell culture supernates, cell lysates, serum and plasma (heparin, EDTA). This is a TMB colorimetric sandwich ELISA kit with short assay time and quick experiment set up.
Sensitivity	<2pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	15.6pg/ml-1000pg/ml(cell culture supernates), 7.8pg/ml-500pg/ml(mouse serum, plasma)
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles. (Shipped with wet ice.)
Uniprot ID	P06804

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Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from hamster, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Mouse Tnf
Immunogen	Expression system for standard: E.coli; Immunogen sequence: L80-L235
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.
1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don’t let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don’t reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Mouse Tnf Pre-coated 96-well Strip Microplate	1	12 strips of 8 wells
Mouse Tnf Standard	2	10ng/tube
Mouse Tnf Detection Antibody (Read the label to find dilution ratio)	1	Read the label to find volume
Sample Diluent	1	15ml
TBS-T Wash Buffer (25x)	1	12ml
Detection Antibody Diluent	1	6ml

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Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Plate Sealers	2	Piece

Required Materials That Are Not Supplied

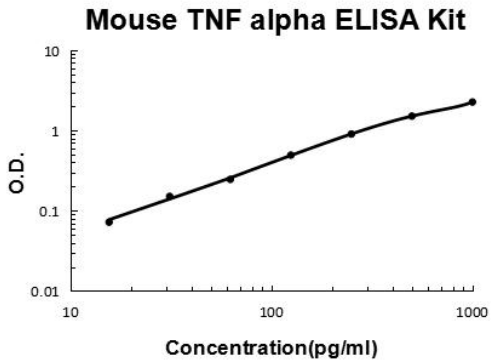
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Mouse TNF Alpha PicoKine™ Quick ELISA Kit (FEK0527) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	15.6	31.2	62.5	125	250	500	1000
O.D.	0.160	0.232	0.311	0.406	0.654	1.065	1.673	2.430

Mouse TNF alpha PicoKine ELISA Kit Standard Curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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Intra/Inter Assay Variability

Boster spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision accross assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	20	55	314	18	63	350
Standard deviation	1.04	3.74	23.55	1.06	4.59	28.7
CV(%)	5.2%	6.8%	7.5%	5.9%	7.3%	8.2%

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	20	22	18	19	19	0.89	4.7%
Sample 2	55	58	64	57	58	3.65	6.3%
Sample 3	314	297	303	287	300	8.1	2.7%

*number of samples for each test n=16.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature prior to use.
Wash Buffer(25x)	Add 10ml of Wash Buffer into 240ml of deionized water.

Mouse Tnf Detection Antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Tnf Detection Antibody using the dilution ratio on the vial label with Detection Antibody Diluent. For instance, if the dilution ratio is 1:50, prepare 50 µl by adding 1 µl of Mouse Tnf Detection Antibody to 49 µl of Detection Antibody Diluent. Mix gently and thoroughly and use within 2 hours of generation.
Mouse Tnf Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Mouse Tnf standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Mouse Tnf Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –1000pg/ml, #2 –500pg/ml, #3 – 250pg/ml, #4 – 125pg/ml, #5 – 62.5pg/ml, #6 – 31.25pg/ml, #7 – 15.625pg/ml, #8 – Sample Diluent serves as the zero standard (0pg/ml).
2. To generate standard #1, add 100µl of the reconstituted standard stock solution of 10ng/ml and 900µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Cell lysates	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 50 µl of the standard, samples, or control per well. Add 50µl of Sample Diluent into the Zero well. And add 50µl of the prepared 1x Mouse Tnf detection antibody per well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 60 minutes at RT.
5. Wash the plate 4 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 90 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
6. Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 15 minutes at RT. (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
7. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
8. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

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Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on Tnf

Tumor necrosis factor-alpha(TNF-alpha, or TNF) is secreted by macrophages in response to inflammation, infection and cancer. Human Tumor Necrosis Factor(TNF) and Lymphotoxin(TNF-beta) are cytotoxic proteins which have similar biological activities and share 30% amino acid homology. TNF-alpha is produced by monocytes, which can stimulate endothelial cells to produce the multilineage growth factor granulocyte- macrophage colony-stimulating factor and extend the role of this immunoregulatory protein to the regulation of hematopoiesis in vitro. TNF is a soluble protein that causes damage to tumor cells but has no effect on normal cells. Human TNF has been purified to apparent homogeneity as a 17.3-kilodalton protein from HL-60 leukemia cells and has showed cytotoxic and cytostatic activities against various human tumor cell lines. The human TNF cDNA is 1585 base pairs in length and encodes a protein of 233 amino acids. The mature protein begins at residue 77, leaving a long leader sequence of 76 amino acids. TNF-alpha has been mapped to human chromosome 6.