# PicoKine™ Quick ELISA Kit

## Catalog number: FEK0717

For the quantitation of **Mouse Ctla4** concentrations in cell culture supernatants, serum and plasma (heparin).

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 CTLA4 PicoKine® Quick ELISA Kit

**Catalog Number:** FEK0717

## Assay Principle

The Boster Quick Picokine™ Mouse Ctla4 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Ctla4 in cell culture supernatants, serum and plasma (heparin). It uses our proprietary Quick ELISA technology. Quick ELISA technology employs capture antibodies conjugated to an affinity tag that is recognized by the polyclonal antibody used to coat our Quick ELISA plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. The kit includes Mouse Ctla4 protein as standards.

To measure Mouse Ctla4, add standards and samples to the wells, then add antibody cocktail. Wash the wells with PBS or TBS buffer, and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product is linearly proportional to Mouse Ctla4 in the sample. Read the absorbance 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 Ctla4 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 CTLA4 PicoKine® Quick ELISA Kit |
| Reactive Species | Mouse |
| Size | 96 wells/kit, with removable strips. |
| Description | Mouse CTLA4 PicoKine™ Quick ELISA Kit (90 minutes, 96 Tests). Quantitate Mouse CTLA4 in cell culture supernatants, serum and plasma (heparin). Sensitivity: 10 pg/ml. |
| Sensitivity | <10 pg/ml<br>*The sensitivity or the minimum detectable dose (MDD) is the lower limit of the 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 | 31.2 pg/ml - 2,000 pg/ml |
| Storage Instructions | Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.) |
| Uniprot ID | P09793 |

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## Technical Details

| | |
|---|---|
| Capture/Detection Antibodies | The capture antibody is polyclonal antibody from goat and the detection antibody is polyclonal antibody from goat. |
| Specificity | Natural and recombinant Mouse Ctla4 |
| Standard | Expression system for standard: NS0; Immunogen sequence: A37-F162 |
| 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. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don't reuse tips and tubes to avoid cross-contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

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## Kit Components/Materials Provided

| Catalog number | Description | Quantity | Volume |
|---|---|---|---|
| FEK0717-CAP | Anti-tag Pre-coated 96-well Strip Microplate | 1 | 12 strips of 8 wells |
| EK0717-ST | Mouse Ctla4 Standard | 2 | 10 ng/tube |
| FEK0717-A | Mouse Ctla4 Antibody A | 1 | 3 ml |
| FEK0717-B | Mouse Ctla4 Antibody B | 1 | 3 ml |
| AR1106-1 | Sample Diluent | 1 | 15 ml |
| AR1106-7 | TBS-T Wash Buffer (25x) | 1 | 12 ml |
| AR1104 | Color Developing Reagent (TMB) | 1 | 10 ml |
| AR1105 | Stop Solution | 1 | 10 ml |
| PLA-SEA | Adhesive Plate Sealers | 2 | Piece |

## Required Materials That Are Not Supplied

Microplate reader capable of reading absorbance at 450 nm.
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 a large numbers of samples.
Deionized or distilled water.
500 ml graduated cylinders.
Test tubes for dilution.
Horizontal orbital microplate shaker capable of maintaining a speed of 500 rpm, amplitude 3 mm.

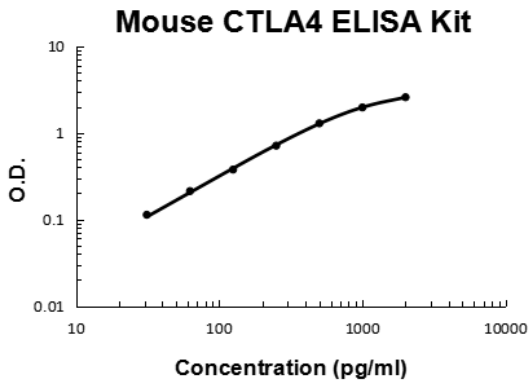
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# Mouse CTLA4 PicoKine® Quick ELISA Kit (FEK0717) Standard Curve Example

The 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 | 31.2 | 62.5 | 125 | 250 | 500 | 1,000 | 2,000 |
|---|---|---|---|---|---|---|---|---|
| O.D. | 0.016 | 0.129 | 0.228 | 0.392 | 0.726 | 1.301 | 1.978 | 2.589 |

**Mouse CTLA4 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.

## Intra/Inter Assay Variability

Boster spend great efforts in documenting lot-to-lot variability and ensuring 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 across 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) | 88 | 302 | 874 | 79 | 274 | 826 |
| Standard deviation | 4.84 | 20.84 | 41.95 | 5.21 | 16.17 | 58.65 |
| CV(%) | 5.5% | 6.9% | 4.8% | 6.6% | 5.9% | 7.1% |

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## Reproducibility

We ensure reproducibility by testing three samples with differing concentrations of Bdnf in ELISA kits from four different production batches/lots.

## Reproducibility

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

| Lots | Lot 1 (pg/ml) | Lot 2 (pg/ml) | Lot 3 (pg/ml) | Lot 4 (pg/ml) | Mean (pg/ml) | Standard Deviation | CV (%) |
|---|---|---|---|---|---|---|---|
| Sample 1 | 88 | 91 | 95 | 99 | 93 | 3.08 | 3.3% |
| Sample 2 | 302 | 299 | 240 | 274 | 279 | 13.66 | 4.9% |
| Sample 3 | 874 | 726 | 864 | 791 | 814 | 42.32 | 5.2% |

*number of samples for each test n=16.

## Preparation Before The Experiment

| Item | Preparation |
|---|---|
| All reagents | Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. We recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C. It is recommended that all reagents be prepared no more than 1 hour prior to performing the experiment. |
| TBS-T Wash Buffer (25x) | Add 12 ml of Wash Buffer into 288 ml of deionized water. |
| Mouse Ctla4 Standard | Use one 10 ng of lyophilized Mouse Ctla4 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions. |
| Mouse Ctla4 Antibody Cocktail | Mix Antibody A and B at dilution ratio of 1:1. |
| Microplate | The included microplate is coated with capture antibodies and is 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 Ctla4 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 2,000.00 pg/ml, # 2: 1,000.00 pg/ml, # 3: 500.00 pg/ml, # 4: 250.00 pg/ml,
   # 5: 125.00 pg/ml, # 6: 62.50 pg/ml, # 7: 31.25 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).
2. To generate standard #1, add 200 µl of the reconstituted standard stock solution of 10 ng/ml and 800 µl of sample diluent to tube #1 for a

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final volume of 1000 µl. 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.

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## 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 as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.<br>*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. |

## Sample Collection Notes

1. Boster recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. Boster is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

## Sample Dilution

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type is necessary. The sample must be mixed thoroughly with Sample Diluent.

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## Assay Protocol

It is recommended that all reagents and materials be equilibrated to 37°C/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. At least two replicates of each standard, sample, or control is recommended.
4. And add 50µl of Mouse Ctla4 Antibody Cocktail per well.
5. Cover with the plate sealer provided and incubate for 60 minutes at room temperature on the shaker.
6. 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.
 iscard the wash buffer 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.
7. 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.)
8. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
9. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

## 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 O.D. 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.

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## Background on Ctla4

CTLA-4(Cytotoxic T-Lymphocyte Antigen 4), also known as CD152(Cluster of differentiation 152), is a protein receptor that down regulates the immune system. CTLA4 is a member of the immunoglobulin superfamily, which is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Blockade of CTLA4 expression in vitro augmented HIV-specific CD4-positive T-cell function.

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