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antibody and ELISA experts

PicoKine® Quick ELISA Kit

Catalog number: FEK0854

For the quantitation of **Mouse Lcn2** concentrations in cell culture supernatants, serum, plasma (heparin, EDTA) and urine

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 Lipocalin-2/NGAL PicoKine® Quick ELISA Kit

Catalog Number: FEK0854

Assay Principle

The Boster Quick Picokine® Mouse Lcn2 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Lcn2 with a 96-well strip plate that is pre-coated with antibody specific for Lcn2. The detection antibody is a HRP linked antibody specific for Lcn2. The kit includes Mouse Lcn2 protein as standards.

To measure Mouse Lcn2, add standards and samples to the wells, then add HRP linked detection antibody. 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 Lcn2 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 Lcn2 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.

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Overview

Product Name	Mouse Lipocalin-2/NGAL PicoKine® Quick ELISA Kit
Reactive Species	Mouse
Size	96 wells/kit, with removable strips.
Description	Mouse Lipocalin-2/NGAL PicoKine® Quick ELISA Kit (90 minutes, 96 Tests). Quantitate Mouse Lcn2 in cell culture supernatants, serum, plasma (heparin, EDTA) and urine . Sensitivity: 10pg/ml. The brand Picokine indicates this is a premium quality ELISA kit. Each Picokine kit delivers precise quantification, high sensitivity, and excellent reproducibility. Only our most reliable and effective kits qualify as Picokine, guaranteeing top-tier results for your assays.
Sensitivity	<10 pg/ml *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	78 pg/ml - 5,000 pg/ml
Storage Instructions	Store at 4°C for 6 months. (Ships with gel ice, can store for up to 3 days in room temperature. Refrigerate upon receipt.)

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Technical Details

Specificity	Natural and recombinant Mouse Lcn2
Standard Protein	Expression system for standard: NS0; Immunogen sequence: Q21-N200
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
FEK0854-CAP	Anti-Mouse Lcn2 Pre-coated 96-well Strip Microplate	1	12 strips of 8 wells
EK0854-ST	Mouse Lcn2 Standard	2	10 ng/tube
FEK0854-H	HRP Linked Anti-Mouse Lcn2 Antibody	1	6 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

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.

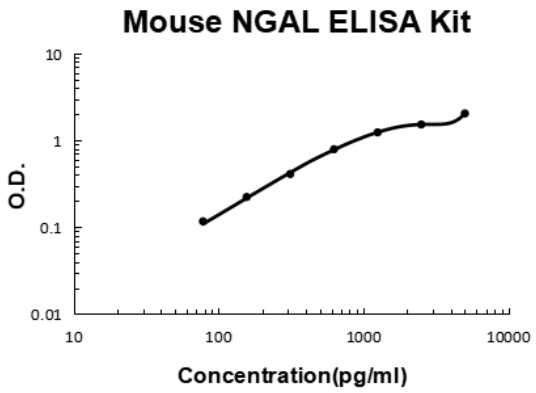
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Mouse Lipocalin-2/NGAL PicoKine® Quick ELISA Kit (FEK0854) Standard Curve Example

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	78	156	312	625	1250	2500	5000
O.D.	0.014	0.131	0.237	0.425	0.807	1.253	1.541	2.061

Mouse Lipocalin-2 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 spends 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)	139	827	2674	144	858	2755
Standard deviation	7.5	48.79	117.65	8.35	57.48	143.26
CV (%)	5.4%	5.9%	4.4%	5.8%	6.7%	5.2%

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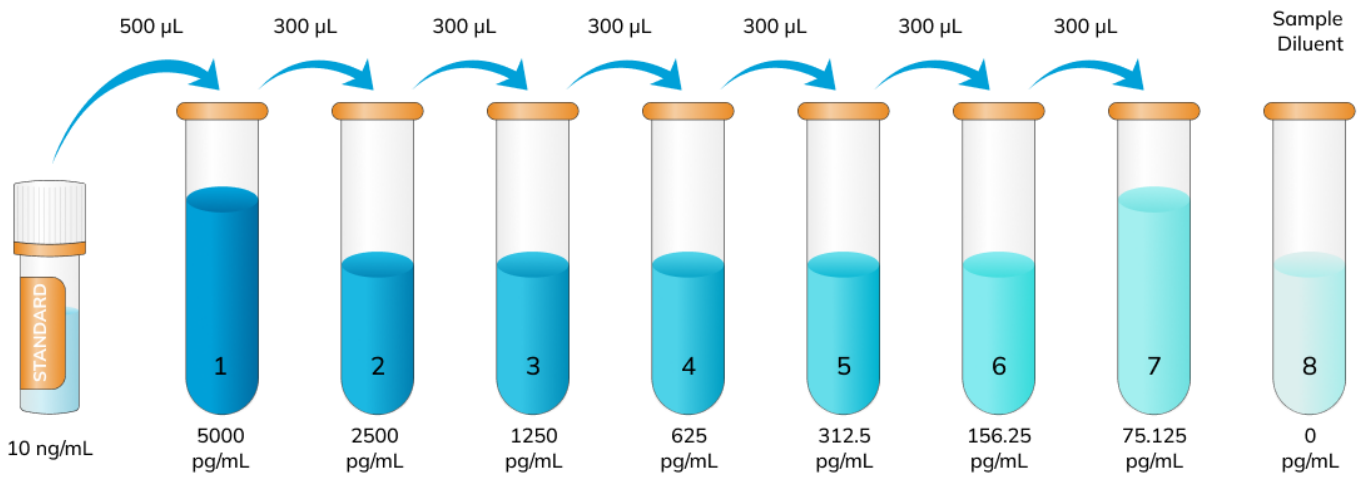
Preparation Before The Experiment

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 Lcn2 Standard	Use one 10 ng of lyophilized Mouse Lcn2 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1 ml 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 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.

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Dilution of Mouse Lcn2 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 5,000.00 pg/ml, # 2: 2,500.00 pg/ml, # 3: 1,250.00 pg/ml, # 4: 625.00 pg/ml,
   # 5: 312.50 pg/ml, # 6: 156.25 pg/ml, # 7: 78.13 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).
2. To generate standard #1, add 500 µl of the reconstituted standard stock solution of 10 ng/ml and 500 µl of sample diluent to tube #1 for a
   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, 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.
Urine	Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

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 are 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 room temperature (18-25°C) 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 50 µl of Sample Diluent into the Zero well. At least two replicates of each standard, sample, or control is recommended.
4. And add 50 µl of Mouse Lcn2 detection antibody 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.
 d. Discard 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 450 nm.

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 Lcn2

Lipocalin-2 (LCN2), also known as NGAL, is a protein associated with neutrophil gelatinase.1 The LCN2 gene is located at 9q34 and contains 7 exons.2 The 25-kD LCN2 protein is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharide (LPS) and formylpeptides and may function as a modulator of inflammation. NGAL tightly binds bacterial catecholate-type ferric siderophores through a cyclically permuted, hybrid electrostatic/cation-pi interaction and is a potent bacteriostatic agent in iron-limiting conditions.3 The primary LCN2 transcript is 3,696 nucleotides long, and the processed transcript is 809 nucleotides long.4 LCN2 expression in adult bone marrow, uterus, prostate, salivary gland, stomach, appendix, colon, trachea, and lung, and in fetal spleen and lung. The standard product used in this kit is recombinant mouse NGAL, consisting of 179 amino acids with the molecular mass of 22KDa.

Mouse Lipocalin-2/NGAL ® Quick ELISA Kit

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