Mouse Legumain (total) ELISA Kit

Catalog Number:
HLE11-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Mouse Legumain (total) ELISA Kit is intended for the quantitation of Mouse LGMN concentrations in cell culture supernates, cell lysates, serum and plasma (heparin, EDTA). The Eagle Biosciences Mouse Legumain (total) ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

INTRODUCTION

Legumain (asparaginyl endopeptidase, citvac, proteinase B, hemoglobinase, PRSC1 gene product or LGMN (Homo sapiens), vicilin peptidohydrolase, bean endopeptidase) is an enzyme that in humans is encoded by the LGMN gene (previous symbolPRSC1). Using fluorescence in situ hybridization, the LGMN gene was mapped to chromosome 14q32.1. This enzyme may be involved in the processing of bacterial peptides and endogenous proteins for MHC class II presentation in the lysosomal/endosomal systems. Enzyme activation is triggered by acidic pH and appears to be autocatalytic. Protein expression occurs after monocytes differentiate into dendritic cells. A fully mature, active enzyme is produced following lipopolysaccharide expression in mature dendritic cells. Overexpression of this gene may be associated with the majority of solid tumor types.

PRINCIPLE OF THE ASSAY

The Mouse Lgmn Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Lgmn with a 96-well strip plate that is pre-coated with antibody specific for Lgmn. The detection antibody is a biotinylated antibody specific for Lgmn. The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse Lgmn with immunogen: Expression system for standard: NSO; Immunogen sequence: V18-Y435. The kit is analytically validated with ready to use reagents. To measure Mouse Lgmn, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP 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 proportional to Mouse Lgmn 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 Lgmn in the sample.

Reactive Species	Mouse
Size	96 wells/kit, with removable strips
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Mouse Legumain (total). 96wells/kit, with removable strips.
Sensitivity	<10pg/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	31.2pg/ml-2000pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

TECHNICAL DETAILS

Capture/Detection Antibodies The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat.

Specificity Natural and recombinant Mouse LGMN

Immunogen Expression system for standard: NSO; Immunogen sequence: V18-Y435

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 LGMN Precoated 96-well strip microplate	1	12 strips of 8 wells
Mouse LGMN Standard	2	10ng/tube
Mouse LGMN Biotinylated antibody (100x)	1	130 µl
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Wash Buffer Powder	1	Pack
Plate Sealers	4	Piece

REQUIRED MATERIALS THAT ARE NOT SUPPLIED

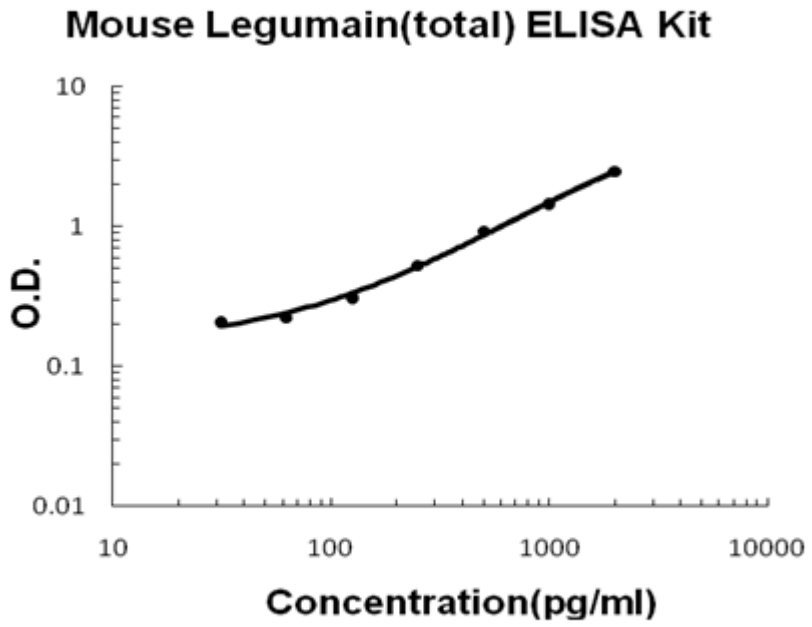
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml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

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	2000	2000	2000	2000	2000	2000	2000
O.D.	0.157	0.207	0.222	0.308	0.522	0.921	1.448	2.465

Mouse Legumain (total) 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

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

Sample	1	2	3
N	16	16	16
Mean(pg/ml)	84	270	1001
Standard Deviation	3.94	12.42	68.06
CV (%)	4.7%	4.6%	6.8%

Inter-Assay Precision

Sample	1	2	3
N	24	24	24
Mean(pg/ml)	90	264	954
Standard Deviation	4.32	15.31	65.82
CV (%)	4.8%	5.8%	6.9%

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	84	80	93	92	87	5.44	6.2%
Sample 2	270	260	234	241	251	14.41	5.7%
Sample 3	1001	921	978	997	974	31.94	3.2%

*number of samples for each test n=16.

PREPARATION BEFORE THE EXPIREMENT

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.
Wash Buffer	Dissolve the wash buffer powder in 1000ml of water to make 1X PBS wash buffer.
Biotinylated Anti-Mouse LGMN antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Mouse LGMN Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Mouse LGMN Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 100ng of lyophilized Mouse LGMN standard for each experiment. Gently spin the vial prior to use.

	Reconstitute the standard to a stock concentration of 100ng/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 LGMN STANDARD

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –2000pg/ml, #2 –1000pg/ml, #3 – 500pg/ml, #4 – 250pg/ml, #5 – 125pg/ml, #6 –62.5pg/ml, #7 – 31.25pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 200µl of the reconstituted standard stock solution of 10ng/ml and 800µl 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.
7. Tube #8 is a blank standard to be used with every experiment.

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 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 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. 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.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Mouse LGMN antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 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 60 seconds between each wash).
    c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 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 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (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.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.

13. 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-logisticcurve.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.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.