Laminin (Human) ELISA Kit

Catalog Number KA1999

96 assays

Version: 09

Intended for research use only

Table of Contents

Introduction

Intended Use

For the quantitation of human LAMA1; LAMB1; LAMC1 concentrations in cell culture supernates, cell lysates and serum.

Background

Laminin is a large basement membrane glycoprotein composed of three subunits designated the A, B1, and B2. Laminin has diverse biological functions, which include stimulating epithelial cell growth and differentiation. The nucleotide sequence of human laminin A chain has an open reading frame encoding 3075-amino acids. The human laminin A chain is at locus 18p11.3. The nucleotide sequence of the human laminin B1 reveals a 5358-base pair open reading frame that potentially codes for 1786 amino acids, including 20 amino acids of a presumptive signal peptide. The gene for the human laminin-B1 chain has been localized to chromosome 7, band q31. The B2 chain consists of six distinct domains, including two domains with alpha-helical, coiled-coil structures, two domains with cysteine-rich homologous repeats, and two globular domains. The amino acid sequences of the B2 and B1 chains demonstrate considerable homology. The human laminin B2 chain gene maps to the long arm of chromosome 1 in the band q31.

Principle of the Assay

The Laminin (Human) ELISA Kit is a solid phase immunoassay specially designed to measure Human LAMA1; LAMB1; LAMC1 with a 96-well strip plate that is pre-coated with antibody specific for LAMA1; LAMB1; LAMC1. The detection antibody is a biotinylated antibody specific for LAMA1; LAMB1; LAMC1. The capture antibody is polyclonal antibody from rabbit, the detection antibody is polyclonal antibody from rabbit. The kit contains recombinant Human LAMA1; LAMB1; LAMC1 with immunogen: Expression system for standard: from human fibroblasts. The kit is analytically validated with ready to use reagents.
To measure Human LAMA1; LAMB1; LAMC1, 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 Human LAMA1; LAMB1; LAMC1 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 Human LAMA1; LAMB1; LAMC1 in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Anti-Human LAMA1; LAMB1; LAMC1 Pre-coated 96-well strip microplate	96 (8x12) wells
Human LAMA1; LAMB1; LAMC1 Standard	10 ng/tube x 2
Human LAMA1; LAMB1; LAMC1 Biotinylated antibody (100x)	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	130 µL
Sample Diluent	30 mL
Antibody Diluent	12 mL
Avidin-Biotin-Peroxidase Diluent	12 mL
Color Developing Reagent (TMB)	10 mL
Stop Solution	10 mL
Plate Sealers	4 slides

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but 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 large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.
- ✓ Washing buffer

  Prepare standard 1X PBS as wash buffer. Wash buffer can be prepared in-house.

  Preparation of wash buffer: Add 8.5 g NaCl, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 mL distilled water and adjust pH to 7.2-7.6.

Precautions for Use

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

✓ 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.

Assay Protocol

Reagent Preparation

- ✓ 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 (15-25 min) is based on 37°C.
  - Biotinylated Anti-Human LAMA1; LAMB1; LAMC1 antibody
    It is recommended to prepare this reagent immediately prior to use by diluting the Human LAMA1; LAMB1; LAMC1 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.
  - Human LAMA1; LAMB1; LAMC1 Standard
    It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human LAMA1; LAMB1; LAMC1 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 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 Human LAMA1; LAMB1; LAMC1 Standard
  1. Number tubes 1-8. Final Concentrations to be Tube # 1 –10000 pg/mL, #2 –5000 pg/mL, #3 – 2500 pg/mL, #4 – 1250 pg/mL, #5 – 625 pg/mL, #6 – 312.5 pg/mL, #7 – 156.25 pg/mL, #8 –Sample Diluent serves as the zero standard (0 pg/mL).
  2. For standard #1, add 1000 µL of undiluted standard stock solution to tube #1.
  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

✓ 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.

- 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.
- 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 Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation 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 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 minutes 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-Human LAMA1; LAMB1; LAMC1 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 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 plate sealer 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 450 nm.

Data Analysis

Calculation of Results

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.

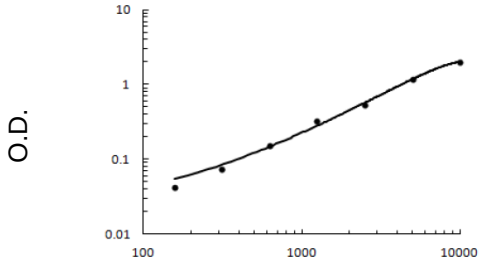
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but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

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

✓ Laminin (Human) ELISA Kit 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	156	312	625	1250	2500	5000	10000
O.D.	0.095	0.137	0.168	0.247	0.425	0.624	1.284	2.110



This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

Performance Characteristics

✓ Detection Range: 156 pg/mL-10000 pg/mL

✓ Sensitivity: < 10 pg/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.

✓ Specificity: Natural and recombinant human LAMA1; LAMB1; LAMC1

✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins

✓ 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			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	318	1051	4462	295	1015	4289
Standard deviation	23.21	47.29	348.03	26.25	58.87	411.74
CV (%)	7.3%	4.5%	7.8%	8.9%	5.8%	9.6%

✓ 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	318	364	314	373	342	26.47	7.7%
Sample 2	1051	1242	1122	1215	1157	75.9	6.5%
Sample 3	4462	4109	4606	4544	4430	192.37	4.3%

*number of samples for each test n=16

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								