Laminin (Rat) ELISA Kit

Catalog Number KA0379

96 assays

Version: 08

Intended for research use only

Table of Contents

Introduction

Intended Use

Sandwich ELISA kit for quantitative detection of rat Laminin in cell culture supernates, serum and plasma (heparin, EDTA).

Background

Laminin is a large basement membrane glycoprotein composed of three subunits designated the A, B1, and B2.[1] Laminin has diverse biological functions, which include stimulating epithelial cell growth and differentiation.[2] The nucleotide sequence of human laminin A chain has an open reading frame encoding 3075-amino acids.[1] The human laminin A chain is at locus 18p11.3.[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.[2] The gene for the human laminin-B1 chain has been localized to chromosome 7, band q31.[4] 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.[5] The human laminin B2 chain gene maps to the long arm of chromosome 1 in the band q31.[6]

Principle of the Assay

The Laminin (Rat) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for Laminin has been precoated onto 96-well plates. Standards(Expression system for standard: from rat sarcoma basement membrane) and test samples are added to the wells, a biotinylated detection antibody from goat specific for Laminin is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Rat Laminin amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
96-well Plate precoated with anti- rat Laminin antibody	96 (8x12) wells
Lyophilized rat Laminin standard	10 ng/tube x 2
Biotinylated anti- rat Laminin antibody, dilution 1:100	130 µL
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µL
Sample diluent buffer	30 mL
Antibody diluent buffer	12 mL
ABC diluent buffer	12 mL
TMB color developing agent	10 mL
TMB stop solution	10 mL
Adhesive cover	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 in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).
    - Preparation of 0.01 M TBS:
      Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1000 mL H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
    - Preparation of 0.01 M PBS:
      Add 8.5 g sodium chloride, 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. Finally, adjust the total volume to 1 L.

Precautions for Use

Please read the following instructions before starting the experiment.

- ✓ 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.
- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
- ✓ Duplicate well assay is recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ Avoid using the reagents from different batches together.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Assay Protocol

Reagent Preparation

✓ Reconstitution of the rat Laminin standard: Laminin standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of Laminin standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10,000 pg/mL of rat Laminin standard solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
- 5000 pg/mL → 156 pg/mL of rat Laminin standard solutions: Label 6 Eppendorf tubes with 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL, respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 10,000 pg/mL Laminin standard solution into 1st tube and mix. Transfer 0.3 mL from 1st tube to 2nd tube and mix. Transfer 0.3 mL from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/mL standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

✓ Preparation of biotinylated anti-rat Laminin antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1 mL well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- Biotinylated anti-rat Laminin antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-rat Laminin antibody to 99 µL antibody diluent buffer.)

✓ Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume.)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1µL ABC to 99 µL ABC diluents buffer.)

Sample Preparation

✓ Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernate: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1500 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 min at 2000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as the anticoagulant.

✓ Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- High target protein concentration (100-1000 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL sample diluent buffer.
- Medium target protein concentration (10-100 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL sample diluent buffer.
- Low target protein concentration (156-10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL sample diluent buffer.
- Very Low target protein concentration (0-156 pg/mL). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard Laminin detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of Laminin amount in samples.

1. Aliquot 0.1 mL per well of the 10,000 pg/mL, 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL rat Laminin standard solutions into the precoated 96-well plate. Add 0.1 mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of rat cell culture supernates, serum or plasma (heparin, EDTA) to each empty well. See “Sample Dilution Guideline” above for details. It is recommended that each rat Laminin standard solution and each sample be measured in duplicate.
2. Seal the plate with a new adhesive cover provided and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 mL of biotinylated anti-rat Laminin antibody working solution into each well, seal the plate with a new adhesive cover provided and incubate the plate at 37°C for 60 min.

5. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
   (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 mL PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. *Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.*)
6. Add 0.1 mL of prepared ABC working solution into each well, seal the plate with a new adhesive cover provided and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new adhesive cover provided and incubate at 37°C in dark for 15-20 min *(Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated rat Laminin standard solutions; the other wells show no obvious color).*
9. Add 0.1 mL of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

✓ Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 15-20 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat Laminin concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Data Obtained from Rat Laminin

Concentration (pg/mL)	0	156	312	625	1250	2500	5000	10,000
O.D	0.077	0.127	0.174	0.244	0.449	0.771	1.206	2.108

(TMB reaction incubate at 37°C for 15-20 min)

Performance Characteristics

✓ Range: 156 pg/mL-10,000 pg/mL

✓ Sensitivity: < 10 pg/mL

✓ Specificity: Natural rat Laminin

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

✓ Precision

- 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 between 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)	1476	4258	6993	1689	4437	7147
Standard deviation	88.56	234.2	342.7	120	275.1	407.4
CV (%)	6	5.5	4.9	7.1	6.2	5.7

Resources

References

1. Haaparanta, T.; Uitto, J.; Ruoslahti, E.; Engvall, E. Molecular cloning of the cDNA encoding human laminin A chain. Matrix 11: 151-160, 1991.
2. Sasaki, M.; Kato, S.; Kohno, K.; Martin, G. R.; Yamada, Y. Sequence of the cDNA encoding the laminin B1 chain reveals a multidomain protein containing cysteine-rich repeats. Proc. Nat. Acad. Sci. 84: 935-939, 1987.
3. Nagayoshi, T.; Mattei, M.-G.; Passage, E.; Knowlton, R.; Chu, M.-L.; Uitto, J. Human laminin A chain (LAMA) gene: chromosomal mapping to locus 18p11.3. Genomics 5: 932-935, 1989.
4. Jaye, M.; Modi, W. S.; Ricca, G. A.; Mudd, R.; Chiu, I.-M.; O'Brien, S. J.; Drohan, W. N. Isolation of a cDNA clone for the human laminin-B1 chain and its gene localization. Am. J. Hum. Genet. 41: 605-615, 1987.
5. Sasaki, M.; Yamada, Y. The laminin B2 chain has a multidomain structure homologous to the B1 chain. J. Biol. Chem. 262: 17111-17117, 1987.
6. Mattei, M.-G.; Weil, D.; Pribula-Conway, D.; Bernard, M. P.; Passage, E.; Van Cong, N.; Timpl, R.; Chu, M.-L. cDNA cloning, expression and mapping of human laminin B2 gene to chromosome 1q31. Hum. Genet. 79: 235-241, 1988.

Plate Layout

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