Human Laminin ELISA Kit

Cat. No.:DEIA1124
Pkg.Size:96T

Intended use

The Human Laminin ELISA kit is designed to detect and quantify the level of human Laminin in serum.

General Description

Laminins are major proteins in the basal lamina (one of the layers of the basement membrane), a protein network foundation for most cells and organs. The laminins are an important and biologically active part of the basal lamina, influencing cell ifferentiation, migration, adhesion as well as phenotype and survival.Laminins are trimeric proteins that contain an α-chain, a β-chain, and a γ-chain, found in five, four, and three genetic variants, respectively. The laminin molecules are named according to their chain composition. Thus, laminin-511 contains α5, β1, and γ1 chains.Fourteen other chain combinations have been identified in vivo. The trimeric proteins intersect to form a cross-like structure that can bind to other cell membrane and extracellular matrix molecules.The three shorter arms are particularly good at binding to other laminin molecules, which allows them to form sheets. The long arm is capable of binding to cells, which helps anchor organized tissue cells to the membrane.The laminins are a family of glycoproteins that are an integral part of the structural scaffolding in almost every tissue of an organism. They are secreted and incorporated into cell-associated extracellular matrices. Laminin is vital for the maintenance and survival of tissues. Defective laminins can cause muscles to form improperly, leading to a form of muscular dystrophy, lethal skin blistering disease (junctional epidermolysis bullosa) and defects of the kidney filter (nephrotic syndrome).

Principle Of The Test

A monoclonal antibody specific for human Laminin has been coated onto the wells of the microtiter strips provided. Standards and Samples are pipetted into the wells and human Laminin binds to the immobilized antibody. After incubation, unbound substances is removed during a wash step, a detected antibody is added and binds to human Laminin captured by the first antibody. After incubation, unbound detected antibody is removed during a wash step. Streptavidin-HRP is added and binds to the detected antibody. After incubation, unbound streptavidin-HRP is removed during a wash step, and a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of Laminin bound in the initial step. A colored product is formed and the reaction is terminated by the addition of stop solution. The intensity of the color is measured spectrophotometrically at 450 nm.

Reagents And Materials Provided

Human Laminin Ab Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate
Human Laminin Standard(A-E, 50,100,200,400,600ng/mL: 0.5mL per vial with preservatives: 5 vials
Detected Antibody (Rabbit anti-Human Laminin PolyAntibody), 5 mL per vial with preservatives: 1 vial
Streptavidin-horseradish peroxidase (HRP) Concentrate, 5 mL per vial with preservatives: 2 vials
Wash Solution Concentrate (25×), 20 mL with preservatives: 1 bottle
Chromogen A, 5 mL per bottle: 1 bottle
Chromogen B, 5 mL per bottle: 1 bottle
Stop Solution, 5 mL per bottle: 1 bottle

Materials Required But Not Supplied

1. A standard ELISA plate reader for absorbance at 450 nm.

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2. Calibrated adjustable precision pipettes (single channel and multi channel), preferably with disposable plastic tips.
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software or graph paper.
6. Polypropylene tubes.
7. Graduated cylinders and calibrated beakers in various sizes.

Storage

Store all reagents at 2 to 8℃.

Specimen Collection And Handling

1. The serum must be separated from the clot as early as possible as to avoid hemolysis. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters.
2. Avoid repeated freeze-thaw cycles.
3.It is recommended that all samples be diluted in Assay Solution, and the exact dilution must be determined empirically.

Reagent Preparation

Wash Solution: Make a 1:25 dilution of Wash Solution Concentrate (25X) with deionized or distilled water in a clean plastic tube as needed.

Assay Steps

Allow all reagents and samples to warm up to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.
1. Determine the number of strips needed for the assay and remove excess microplate strips from the plate frame, return them to the foil pouch, and reseal.
2. Prepare standards and samples with appropriate diluents.
3. Add 50 µL of standards or dilutions of samples in duplicate to each well. Add 50 µL detected antibody to each well except for the blank. Seal the plate with Plate Covers and incubate at 37℃ for 60 min.
4. Aspirate each well and wash 5 times.
5. Add 100 µL of Streptavidin-HRP Solution to each well except for the blank. Seal the plate with Plate Covers and incubate at 37℃ for 30 min.
6. Aspirate each well and wash according to Step 4.
7. Dispense 50µl of Chromogen A and 50µl Chromogen B solution into each well including the Blank and mix by tapping the plate gently. Incubate the plate at 37℃ for 15 minutes avoiding light.
8. Add 50 µL of Stop Solution to each well.
11. Read the absorbance of each well at 450 nm within 10 minutes after adding the Stop Solution.

Quality Control

The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.

Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.
Manual Plotting: Plot on graph paper the absorbance of the standards against the standard concentration. Known concentrations of Human Laminin are plotted on the X-axis and the corresponding absorbances on the Y-axis. The standard curve should result

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in a straight line that shows a direct relationship between Human Laminin concentrations and the corresponding absorbances. The concentration of Human Laminin in samples may be determined by plotting the sample absorbances on the Y-axis, then drawing a horizontal line to intersect with the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Human Laminin concentration.
Note: Samples producing signals greater than that of the highest standard should be diluted in Assay Solution and reanalyzed. Multiply the measured concentration by the appropriate dilution factor.
Plate Reader: An alternative approach is to use an ELISA curve fitting software. A linear curve plot is expected to produce the best fit of the resulting sample concentrations.

Sensitivity

The minimum detectable dose of human Laminin is < 50 ng/mL.

Specificity

No detectable cross-reactivity.

Linearity

Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.98.

Reproducibility

Intra-Assay: CV≤15%
Inter-Assay: CV≤20%

Precautions

1. This kit is for research use only, not for diagnostic or therapeutic procedures.
2. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Take care not to contaminate the Chromogen Solution. If the solution is blue before use, DO NOT USE.
3. This kit is intended ONLY for testing of individual serum samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC.

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