



RES91-EN.01

# **Human Laminin quantitative ELISA Kit (Enzyme-Linked Immunosorbent Assay)**

**Catalog Number: RES-A091**

**Pack Size: 96 tests**

**IMPORTANT: Please carefully read this manual before performing your experiment.**

**For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure**

## **INTENDED USE**

The kit is developed for the detection and quantitative determination of Laminin in cell culture supernates, serum, and plasma. This kit can specifically identify full length Laminin521, Laminin521 E8 fragment, full length Laminin511 and Laminin511 E8 fragment by testing. It is intended for research use only (RUO).

## **BACKGROUND**

Laminin is a class of Basement membrane (BM) proteins, Laminin affects cell adhesion, migration, phenotypic maintenance, cell survival, and cell differentiation. Laminin is an isomeric trimer with different kinds of chains, five kinds of  $\alpha$  chains, four kinds of  $\beta$  chains, and three kinds of  $\gamma$  chains can form more than 60 combinations. Among them, LN511 and LN521 are the ubiquitous BM protein components, they play important roles in the development of differentiation scheme.

To support the development of cell therapy drugs, ACROBiosystems developed Human Laminin quantitative ELISA Kit with rigorous methodological validation, which is used for the detection and quantitative determination of Laminin in cell culture supernates, serum, and plasma. Besides, this kit can also be used for the quantitative determination of GMP human Laminin 521 Protein (ACROBiosystems, cat#GMP-LA5H24) concentrations.

## **PRINCIPLE OF THE ASSAY**

This assay kit is used to measure the levels of human Laminin by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Laminin Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Laminin Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of human Laminin bound.

## **PRECAUTIONS**

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant and serum samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

## **MATERIALS PROVIDED**

**Table1. Materials provided**

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES091-C01	Pre-coated Anti-Laminin Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES091-C02	Human Laminin 521 Standard	20 µg	Powder	2-8°C	-70°C
RES091-C03	Biotin-Anti-Laminin Antibody	20 µg	Powder	2-8°C	-70°C
RES091-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES091-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES091-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES091-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES091-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

*Note: It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

## **SRORAGE**

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

*Note: a. Do not use reagents past their expiration date.*

*b. Find the expiration date on the outside packaging.*

## **REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED**

Single or multi-channel micropipettes and pipette tips: need to meet 10 µL, 300 µL, 1000 µL injection requirements;

37°C Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

## **REAGENT PREPARATION**

Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 5 µg.

*Note: Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

**Table 2. Preparation method**

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES091-C02	Human Laminin 521 Standard	20 µg	100 µg/mL	200 µL
RES091-C03	Biotin-Anti-Laminin Antibody	20 µg	100 µg/mL	200 µL

## **RECOMMENDED SAMPLE PREPARATION**

### **1. Working Solution Preparation**

#### 1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

#### 1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

#### 1.3 Preparation of Biotin-Anti-Laminin Antibody working fluid:

Dilute Biotin-Anti-Laminin Antibody to 0.5 µg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

#### 1.4 Preparation of Streptavidin-HRP working fluid:

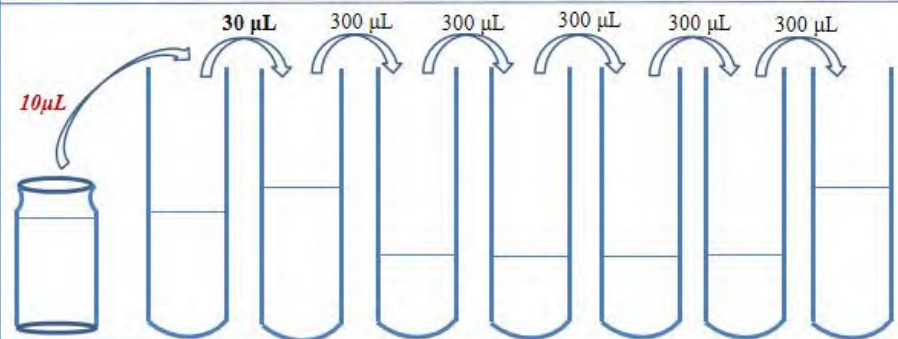
Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

#### 1.5 Sample preparation

If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

### **2. Preparation of Standard curve**

The concentration of the reconstituted human Laminin 521 Calibrator (RES091-C02) is 100 µg/mL , prepare (Std.-0) by diluting 10 µL the reconstituted human Laminin 521 Calibrator into 490 µL Sample Dilution Buffer, mix gently well. Then prepare the highest concentration of standard curve, Std.-1 (100 ng/mL), by diluting 30 µL Std.- 0 into 570 µL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

Tubes/ Solution Code	Laminin 521 Standard stock solution	Std.-0	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Operating								
Solution Con.	100µg/mL	2000 ng/mL	100 ng/mL	50 ng/mL	25 ng/mL	12.5 ng/mL	6.25 ng/mL	3.125 ng/mL
Dilution Buffer Vol.		490 µL	570 µL	300 µL	300 µL	300 µL	300 µL	300 µL

### 3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

*Note: It is recommended to set double holes for samples and standard curves to be tested.*

### 4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

### 6. Add Biotin-Anti-Laminin Antibody

For all wells, add 100 µL Biotin-Anti-Laminin Antibody (dilute to 0.5 µg/mL) working solution. Please prepare it for one-time use only.

### 7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 8. Washing

Repeat step 5.

## 9. Add Streptavidin-HRP

For all wells, add 100  $\mu$ L Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

## 10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

## 11. Washing

Repeat step 5.

## 12. Substrate Reaction

Add 100  $\mu$ L Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

## 13. Termination

Add 50  $\mu$ L Stop Solution to each well and tap the plate gently to allow thorough mixing.

*Note: The color in the wells should change from blue to yellow.*

## 14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

*Note: To reduce the background noise, subtract the value read at  $OD_{450nm}$  with the value read at  $OD_{630nm}$ .*

## CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve:  $R^2 \geq 0.9900$ .
4. Detection range: 3.125 ng/mL-100 ng/mL. If the OD value of the sample to be tested is higher than 100 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 3.125 ng/mL, the sample should be reported.

## QUICK GUID

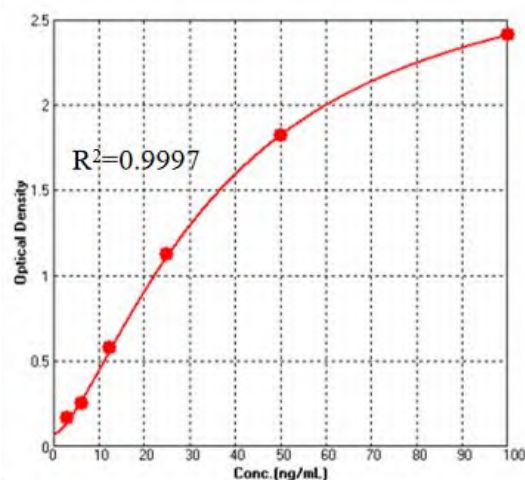




## TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
100	2.480	2.440	2.460	2.411
50	2.037	1.701	1.869	1.820
25	1.186	1.158	1.172	1.123
12.5	0.526	0.725	0.626	0.576
6.25	0.307	0.296	0.302	0.252
3.125	0.218	0.210	0.214	0.165
0	0.048	0.051	0.050	/



## SENSITIVITY

The minimum detectable concentration of Laminin 521 is 0.472 ng/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## PRECISION

### 1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

### 2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	10	10	10	3	3	3

Mean (ng/mL)	77.646	21.770	5.268	76.716	20.951	5.230
SD	4.027	1.324	0.305	2.898	0.732	0.129
CV (%)	5.2	6.1	5.8	3.8	3.5	2.5

*Note: The example data is for reference only.*

## **RECOVERY**

Three Laminin 521 with different concentrations were tested to calculate the recovery rate.

Sample(n=5)	Average% Recovery	Range %
High	101.8-116.3	107.4
Middle	97.1-115.2	104.8
Low	85.1-113.8	101.1

## **LINEARITY**

To assess the linearity of the assay, samples spiked with high concentrations of Laminin 521 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)	Cell culture medium (mTESR-plus)
1:2	Average Recovery (%)	94.1	94.8	96.0
	Range (%)	85.7-101.9	90.9-98.8	89.9-107.8
1:4	Average Recovery (%)	93.8	94.8	101.2
	Range (%)	87.3-102.6	86.6-105.4	89.4-109.4
1:8	Average Recovery (%)	94.2	94.2	95.5
	Range (%)	82.9-101.8	83.2-108.1	91.9-102.4
1:16	Average Recovery (%)	95.0	91.0	94.7
	Range (%)	89.3-102.5	88.5-95.0	91.4-97.8

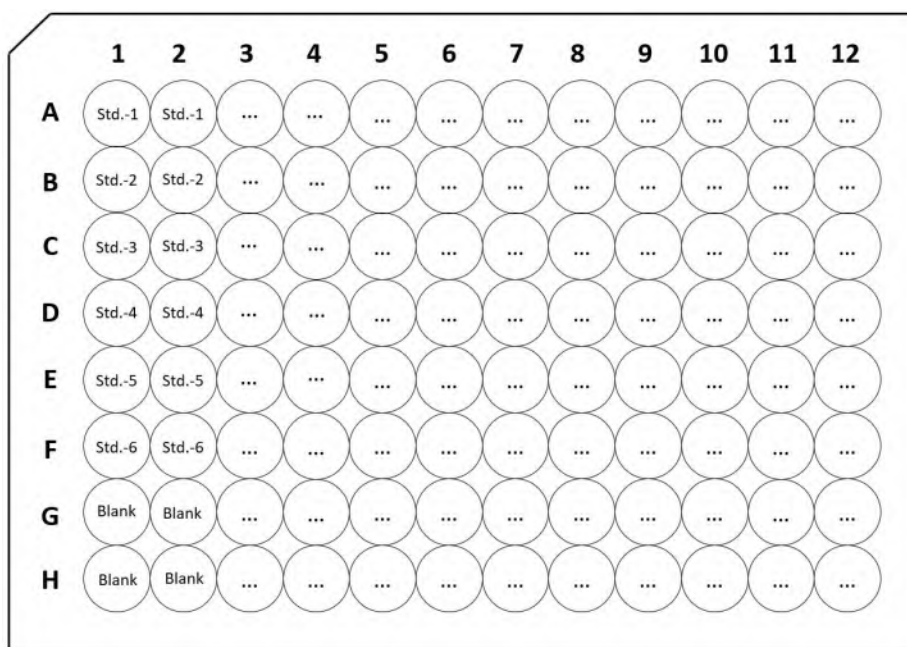
*Note: The example data is for reference only.*

## SPECIFICITY

This assay recognizes natural and recombinant human Laminin 521 and Laminin 511. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

Human		
Laminin 411	Laminin 211	Laminin 121
Laminin 221	Laminin 111	

## PLATE LAYOUT



*Note: Blank is a Blank Dilution Buffer hole.*

## TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed	* Review the manual for proper wash.

	<ul style="list-style-type: none"> <li>* Contaminated wash buffer</li> </ul>	<ul style="list-style-type: none"> <li>* Make fresh wash buffer</li> </ul>
<b>Very low readings across the plate</b>	<ul style="list-style-type: none"> <li>* Incorrect wavelengths</li> <li>* Insufficient development time</li> </ul>	<ul style="list-style-type: none"> <li>* Check filters/reader</li> <li>* Increase development time</li> </ul>
<b>Samples are reading too high, but standard curve looks fine</b>	<ul style="list-style-type: none"> <li>* Samples contain cytokine levels above assay range</li> </ul>	<ul style="list-style-type: none"> <li>* Dilute samples and run again</li> </ul>
<b>Drift</b>	<ul style="list-style-type: none"> <li>* Interrupted assay set-up</li> <li>* Reagents not at room temperature</li> </ul>	<ul style="list-style-type: none"> <li>* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay</li> <li>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts</li> </ul>