Version 7c, Last updated 25 July 2025

ab119599
Human Laminin ELISA Kit

With Wash Buffer 25x

For quantitative detection of human Laminin in cell culture supernatants, plasma, serum and cell lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Human Laminin *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of human Laminin in cell culture supernatants, plasma, serum and cell lysates.

A Laminin specific rabbit polyclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from rabbit specific for Laminin is then added followed by washing with 1X Wash Buffer. Avidin-Biotin-Peroxidase Complex is added, and unbound conjugates ware washed away with 1X Wash Buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the Human Laminin amount of sample captured in plate.

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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to each well used.

Incubate at room temperature.

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human Laminin Antibody Microplate (12 x 8 wells)	96 wells	-20°C
Lyophilized Human Laminin standard	2 x 10 ng	-20°C
Biotinylated anti-Human Laminin antibody	100 µL	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	100 µL	-20°C
Sample Diluent Buffer	30 mL	-20°C
Antibody Diluent Buffer	12 mL	-20°C
ABC Diluent Buffer	12 mL	-20°C
TMB Color Developing Agent	10 mL	-20°C
TMB Stop Solution	10 mL	-20°C
Plate Seal	4 units	-20°C
Wash Buffer (25X)	20 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader capable of measuring 450nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- 100 mL and 1 liter graduated cylinders.
- Eppendorf tubes.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects 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 be pre-warmed in 37°C for 30 minutes before using

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Biotinylated Anti-Human Laminin

Biotinylated anti-Human Laminin antibody must be diluted in 1:100 with the antibody Diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated Anti-Human Laminin antibody to 99 µL antibody Diluent buffer.) The total volume should be: 0.1 mL/well x (the number of wells). (Allow 100 µL - 200 µL extra for pipetting error).

9.2 1X Avidin-Biotin-Peroxidase Complex

Before use, briefly centrifuge the tubes in case any of the contents are trapped in the lid or sticking to the tube walls.

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL extra for pipetting error).

9.3 Wash Buffer (25X)

Prepare 500 mL of working 1X Wash Buffer by diluting 20 ml of the supplied Wash Buffer (25X) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Reconstitute the human Laminin standard 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.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the Laminin standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes.

Δ Note: This 10 ng/mL Standard #1 can be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

10.2 Label seven tubes with #2 - 8.

10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.

10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.

10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.

10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #5 through #7.

10.8 **Standard #8** contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Step 10.1			10,000
2	300 µL Standard #1	300	10,000	5,000
3	300 µL Standard #2	300	5,000	2,500
4	300 µL Standard #3	300	2,500	1,250
5	300 µL Standard #4	300	1,250	625
6	300 µL Standard #5	300	625	312
7	300 µL Standard #6	300	312	156
8 (Blank)	N/A	300		0

11. Sample Preparation

11.1 Cell Culture Supernatants:

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 Serum:

Rinse the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

11.3 Plasma:

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge at approximately 1,000 x g for 15 minutes. Analyze the plasma immediately or aliquot and store samples at -20°C.

Δ 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.

11.4 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.

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 – 1,000 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 (≤ 312 pg/mL). No dilution necessary.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL of prepared standards and diluted samples to appropriate wells.

13.3 Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.

13.4 Remove the cover, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.

13.5 Add 100 µL of 1X Biotinylated anti-human Laminin antibody into each well, seal with a new plate seal and incubate the plate at 37°C for 60 minutes.

13.6 Wash the plate three times with 300 µL 1X Wash Buffer, and each time let the wash buffer stay in the wells for one minute. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.

Δ Note For automated washing, aspirate all wells and wash three times with 1X Wash Buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

13.7 Add 100 µL of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal with a new plate seal and incubate the plate at 37°C for 30 minutes.

13.8 Wash plate five times with 1X Wash Buffer, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 13.6 for plate washing method).

13.9 Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate plate at 37°C in dark for 25 - 30 minutes

Δ Note The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated human Laminin standard solutions; the other wells show no obvious color.

13.10 Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.
13.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

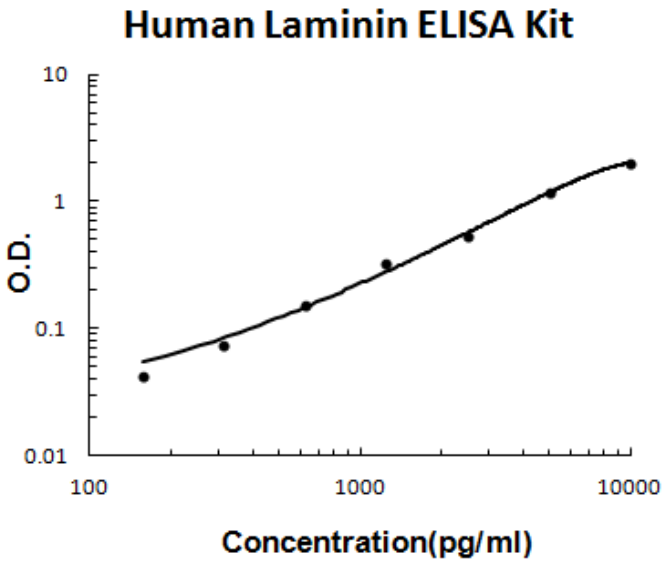
14. Calculations

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 human Laminin concentration of the samples can be interpolated from the standard curve.

Δ Note If the samples measured were diluted, make sure to account for this in your calculations.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0	0.095
156	0.137
312	0.168
625	0.247
1,250	0.425
2,500	0.624
5,000	1.284
10,000	2.110

Figure 1. Example of Laminin standard curve. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

RANGE – 156 - 10,000 pg/mL

SENSITIVITY – < 10 pg/mL

Precision –

Intra-assay precision: (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Sample	Number of measures	Mean (pg/mL)	Standard Deviation	CV%
1	16	318	23.21	7.3
2	16	1,051	47.29	4.5
3	16	4,462	348.03	7.8

Inter-assay precision: (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Number of assays	Mean (pg/mL)	Standard Deviation	CV%
1	24	295	26.25	8.9
2	24	1,015	58.87	5.8
3	24	4,289	411.74	9.6

17. Assay Specificity

No detectable cross-reactivity with other relevant proteins.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

Version 7c, Last updated 25 July 2025

ab119599
Human Laminin ELISA Kit

Without Wash Buffer (25x)

For quantitative detection of human Laminin in cell culture supernatants, serum and cell lysates.

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1. Overview

Human Laminin *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of human Laminin in cell culture supernatants, plasma, serum and cell lysates.

A Laminin specific rabbit polyclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from rabbit specific for Laminin is then added followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added, and unbound conjugates ware washed away with PBS or TBS buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the Human Laminin amount of sample captured in plate.

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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to each well used.

Incubate at room temperature.

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human Laminin Antibody Microplate (12 x 8 wells)	96 wells	-20°C
Lyophilized Human Laminin standard	2 x 10 ng	-20°C
Biotinylated anti-Human Laminin antibody	100 µL	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	100 µL	-20°C
Sample Diluent Buffer	30 mL	-20°C
Antibody Diluent Buffer	12 mL	-20°C
ABC Diluent Buffer	12 mL	-20°C
TMB Color Developing Agent	10 mL	-20°C
TMB Stop Solution	10 mL	-20°C
Plate Seal	4 units	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader capable of measuring 450nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- 100 mL and 1 liter graduated cylinders.
- Eppendorf tubes.
- Washing buffer, either neutral PBS or TBS (see Section 9 for recipes).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects 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 be pre-warmed in 37°C for 30 minutes before using

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Biotinylated Anti-Human Laminin

Biotinylated anti-Human Laminin antibody must be diluted in 1:100 with the antibody Diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated Anti-Human Laminin antibody to 99 µL antibody Diluent buffer.) The total volume should be: 0.1 mL/well x (the number of wells). (Allow 100 µL - 200 µL extra for pipetting error).

9.2 1X Avidin-Biotin-Peroxidase Complex

Before use, briefly centrifuge the tubes in case any of the contents are trapped in the lid or sticking to the tube walls.

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL extra for pipetting error).

9.3 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 distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

9.4 0.01 M PBS

Add 8.5 g NaCl, 1.4 g Na2HPO4 and 0.2 g NaH2PO4 to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Reconstitute the human Laminin standard 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.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

Prepare a 10 ng/mL **Standard #1** by reconstituting the Laminin standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes.

Δ Note: This 10 ng/mL Standard #1 can be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

11.1 Label seven tubes with #2 - 8.

11.2 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.

11.3 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.

11.4 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.

11.5 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.

11.6 Using the table below as a guide, repeat for tubes #5 through #7.

11.7 **Standard #8** contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Step 10.1			10,000
2	300 µL Standard #1	300	10,000	5,000
3	300 µL Standard #2	300	5,000	2,500
4	300 µL Standard #3	300	2,500	1,250
5	300 µL Standard #4	300	1,250	625
6	300 µL Standard #5	300	625	312
7	300 µL Standard #6	300	312	156
8 (Blank)	N/A	300		0

11. Sample Preparation

11.1 Cell Culture Supernatants:

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 Serum:

Rinse the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

11.3 Plasma:

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge at approximately 1,000 x g for 15 minutes. Analyze the plasma immediately or aliquot and store samples at -20°C.

Δ 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.

11.4 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.

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 – 1,000 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 (≤ 312 pg/mL). No dilution necessary.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

a. Prepare all reagents, working standards, and samples as directed in the previous sections.
b. Add 100 µL of prepared standards and diluted samples to appropriate wells.
c. Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.
d. Remove the cover, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
e. Add 100 µL of 1X Biotinylated anti-human Laminin antibody into each well, seal with a new plate seal and incubate the plate at 37°C for 60 minutes.
f. Wash the plate three times with 300 µL 0.01 M TBS or 0.01 M PBS, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

Δ Note For automated washing, aspirate all wells and wash three times with PBS or TBS buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

g. Add 100 µL of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal with a new plate seal and incubate the plate at 37°C for 30 minutes.
h. Wash plate five times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 13.6 for plate washing method).
i. Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate plate at 37°C in dark for 25 - 30 minutes

Δ Note The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated human Laminin standard solutions; the other wells show no obvious color.

j. Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.
k. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

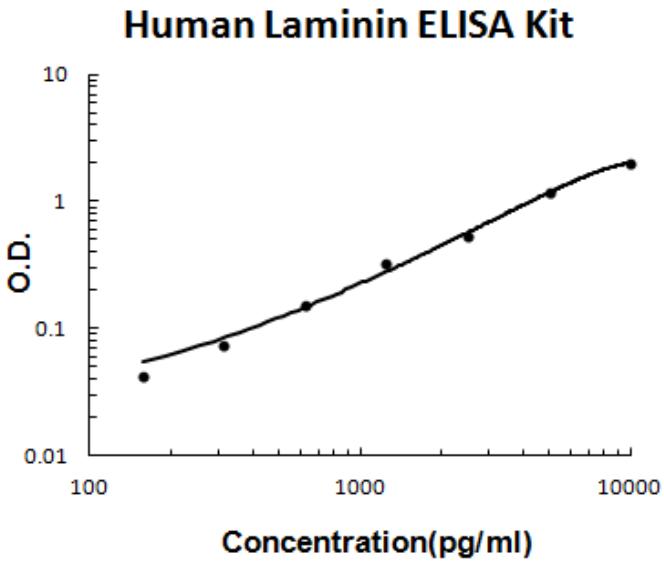
14. Calculations

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 human Laminin concentration of the samples can be interpolated from the standard curve.

Δ Note If the samples measured were diluted, make sure to account for this in your calculations.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0	0.095
156	0.137
312	0.168
625	0.247
1,250	0.425
2,500	0.624
5,000	1.284
10,000	2.110

Figure 1. Example of Laminin standard curve. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

RANGE – 156 - 10,000 pg/mL

SENSITIVITY – < 10 pg/mL

Precision –

Intra-assay precision: (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Sample	Number of measures	Mean (pg/mL)	Standard Deviation	CV%
1	16	318	23.21	7.3
2	16	1,051	47.29	4.5
3	16	4,462	348.03	7.8

Inter-assay precision: (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Number of assays	Mean (pg/mL)	Standard Deviation	CV%
1	24	295	26.25	8.9
2	24	1,015	58.87	5.8
3	24	4,289	411.74	9.6

17. Assay Specificity

No detectable cross-reactivity with other relevant proteins.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)