



Kallikrein-Related Peptidase 7 (KLK7) ELISA

Catalog Number: KL731-K01

1 x 96 Wells

For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.

v. 1.0

EAGLE BIOSCIENCES, INC.

20A Northwest Blvd., Suite 112, Nashua, NH 03063

Phone: 866-419-2019 Fax: 617-419-1110

www.EagleBio.com



INTENDED USE

The Eagle Biosciences Kallikrein-Related Peptidase 7 (KLK7) ELISA Kit is intended for the quantitative determination of the Kallikrein-Related Peptidase 7 (KLK7) concentration in human serum. The Kallikrein-Related Peptidase 7 (KLK7) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

SUMMARY AND EXPLANATION

Kallikreins are a subgroup of serine proteases having diverse physiological functions. Growing evidence suggests that many kallikreins are implicated in carcinogenesis and some have potential as novel cancer and other disease biomarkers. KLK7 is one of the fifteen kallikrein subfamily members. The KLK7 enzyme is thought to be involved in the proteolysis of intercellular cohesive structures preceding desquamation, which is the shedding of the outermost layer of the epidermis.

PRINCIPLE OF THE TEST

The KLK7 (SCCE) ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact KLK7 molecule for solid phase immobilization (on the microtiter wells). Standards, calibrators, and patient samples are incubated with the solid phase antibody on the plate. Wells are then washed and incubated with a biotin conjugated anti-KLK7 monoclonal antibody. After a second wash Streptavidin conjugated to HRP is added as a reporting agent. Excess streptavidin-HRP is then washed off and a solution of TMB Reagent is added and incubated resulting in the development of a blue color if KLK7 is present. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of KLK7 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

MATERIALS PROVIDED

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|--|--------|
| 1. Microtiter plate coated with Monoclonal anti-KLK7 | |
| 2. KLK7 reference standards: 6 vials (ready to use) | 0.45ml |
| 3. Calibrators 1 and 2: 1 vial each (ready to use) | 0.22ml |
| 4. Biotin-labeled monoclonal anti-KLK7 antibody | 14ml |
| 5. 10X Streptavidin-HRP (add 12ml H ₂ O for 1X) | 1.33ml |
| 6. TMB Reagent (One-Step) | 11ml |
| 7. Stop Solution | 11ml |
| 8. Wash Concentrate 10X: 2 bottles | 15ml |

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. 1X PBS
3. Precision pipettes
4. Disposable pipette tips
5. ELISA reader capable of reading absorbance at 450nm
6. Absorbance paper or paper towel
7. Graph paper



STORAGE AND STABILITY

1. Store the Kallikrein-Related Peptidase 7 (KLK7) ELISA assay kit at 2 – 8°C.
2. Keep microplate sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents of the Kallikrein-Related Peptidase 7 (KLK7) ELISA assay kit to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. The calibrators contain human source components, which have been tested and found to have no evidence of infection with HIV, HCV, or HBV. Since no method can completely rule out the presence of blood borne diseases, these reagents should be handled as if they were potentially infectious.
2. This Kallikrein-Related Peptidase 7 (KLK7) ELISA assay kit is designed for RESEARCH USE ONLY.
3. Please refer to the U.S. Department of Health and Human Services (Bethesda, MD, USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.
4. The components in this Kallikrein-Related Peptidase 7 (KLK7) ELISA assay kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Reagents contain Thimerosal as a preservative.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting as well as following the exact time and temperature requirements prescribed is essential. Any deviation from this may yield invalid data.
7. Follow local guidelines for disposal of all waste material.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Bring frozen samples to room temperature and mix thoroughly before analysis.

REAGENT PREPARATION

1. Prepare 1X Wash buffer by adding contents of the bottles (15ml each, 10X) to 270ml of distilled or deionized water. Store at room temperature (18-26°C).
2. Prepare 1X Streptavidin-HRP by adding 12ml distilled or deionized water to contents of bottle and mix gently.
3. Dilute samples 1:2 in 1X PBS and mix gently.



ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (18-26°C) and gently mix.

1. Wash wells one time with 300µl/well 1X PBS, remove liquid from wells, and pat dry on absorbance paper or paper towel.
2. Dispense 100µl of KLK7 standards, calibrators, and diluted specimens into appropriate wells.
3. Incubate at room temperature (18-26°C) for 60 minutes with gentle agitation.
4. Remove samples by emptying the plate contents into a waste container.
5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel after each wash.
6. Strike the microtiter plate sharply onto absorbance paper or paper towels to remove all residual liquid droplets.
7. Dispense 100µl of Biotin-labeled Antibody into each well and incubate at room temperature for 60 minutes with gentle agitation.
8. Repeat steps 5 and 6.
9. Dispense 100µl Strept-HRP into each well and incubate at room temperature for 30 minutes with gentle agitation.
10. Repeat steps 5 and 6.
11. Dispense 100µl of TMB Reagent into each well and incubate at room temperature in the dark for 30 minutes.
12. Stop the reaction by adding 100µl of Stop Solution into each well.
13. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
14. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of standards, calibrators, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of KLK7 in ng/ml from the standard curve and multiply by the dilution factor.



Example of Standard Curve

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against KLK7 concentrations shown in the X axis. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

KLK7 values (ng/ml)	Absorbance (450nm)
0	0.0890
2	0.0975
8	0.1185
32	0.2070
128	0.5385
512	1.9580

CALIBRATORS

Calibrator 1 range: 30-50ng/ml

Calibrator 2 range: 130-150ng/ml

EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have KLK7 assay values below 65ng/ml according to a limited set of post-menopausal serum samples. The minimum detectable concentration of KLK7 in this assay is estimated to be 2ng/ml.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.