



Product Manual

Kallikrein-6 (human) ELISA Kit

Catalog #: ADI-900-217



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Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Enzo Life Sciences Technical Support if necessary.

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INTENDED USE

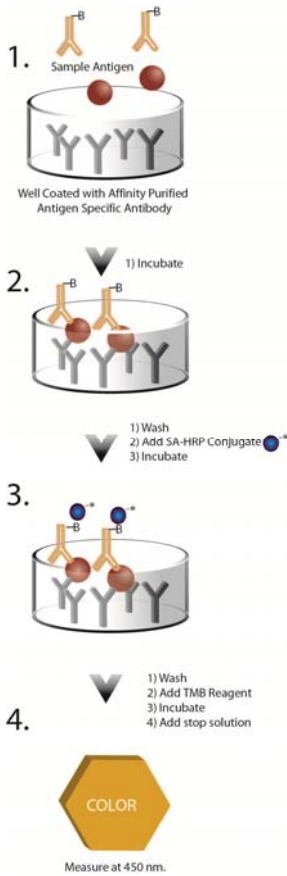
The Kallikrein-6 (human) ELISA Kit is intended for the quantitative determination of the Cancer Antigen Kallikrein-6 concentration in human serum.

SUMMARY AND EXPLANATION

Kallikreins are a subgroup of serine proteases having diverse physiological functions. Growing evidence suggests that many kallikreins, including kallikrein-6 (KLK6), are implicated in carcinogenesis and some have potential as novel cancer and other disease biomarkers. The KLK6 gene is one of the fifteen kallikrein subfamily members located in a cluster on chromosome 19. The encoded KLK6 enzyme is regulated by steroid hormones. In tissue culture, KLK6 has been found to generate amyloidogenic fragments from the amyloid precursor protein, suggesting a potential for involvement in Alzheimer's disease.

PRINCIPLE OF THE TEST

The Kallikrein-6 (human) 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 KLK6 molecule for solid phase immobilization (on the microtiter wells). Standards, calibrators, and patient samples are incubated with a biotin conjugated anti-KLK6 monoclonal antibody and the solid phase antibody on the plate simultaneously. Wells are then washed and incubated with Streptavidin conjugated to HRP which is used 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 KLK6 is present. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of KLK6 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.



MATERIALS SUPPLIED

1. **Microtiter plate coated with Monoclonal anti-KLK6**
2. **KLK6 reference standards:** 6 vials (ready to use), 0.35ml
3. **Calibrators 1 and 2:** 1 vial each (ready to use), 0.35ml
4. **Biotin-labeled monoclonal anti-KLK6 antibody,** 6ml
5. **10X Streptavidin-HRP** (add 10ml diH₂O for 1X), 1.1ml
6. **TMB Reagent (One-Step),** 11ml
7. **Stop Solution,** 11ml
8. **10X Wash Concentrate,** 30ml

STORAGE

1. Store the 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 to heat, sun, or strong light.

OTHER MATERIALS NEEDED

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 or immunoassay 4P data analysis software

WARNINGS AND PRECAUTIONS

1. This test kit is designed for RESEARCH USE ONLY.
2. 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.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. Reagents contain Thimerosal as a preservative.
5. 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.
6. 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 10X Wash bottle to 270ml of distilled or deionized water. Store at room temperature (18-26°C).
2. Prepare 1X Streptavidin-HRP by adding 10ml distilled or deionized water to contents of bottle and mix gently.
3. All serum samples should be diluted a minimum of 1:5 in 1X PBS before being added to the plate. An example for a sample being run in triplicate would be 35ul of serum into 140ul of 1X PBS for 175ul total. Then apply 50ul of diluted serum into each well for the assay.

ASSAY PROCEDURE

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

1. Dispense 50µl of KLK6 standards, calibrators, and diluted specimens into appropriate wells.
2. Dispense 50µl of Biotin-labeled Antibody into each well and incubate at room temperature for 2 hours with gentle agitation.
3. Remove samples by emptying the plate contents into a waste container.
4. 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.
5. Strike the microtiter plate sharply onto absorbance paper or paper towels to remove all residual liquid droplets.
6. Dispense 100µl Strept-HRP into each well and incubate at room temperature for 30 minutes with gentle agitation.
7. Repeat steps 4 and 5.
8. Dispense 100µl of TMB Reagent into each well and incubate at room temperature in the dark for 30 minutes with gentle agitation.
9. Stop the reaction by adding 100µl of Stop Solution into each well.
10. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of KLK6 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program (such as AssayBlaster™, ENZO catalog number ADI-28-0002). Such software is often supplied by plate reader manufacturers. If data reduction software is not readily available, the concentration of KLK6 can be calculated as follows:

1. Calculate the average Net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

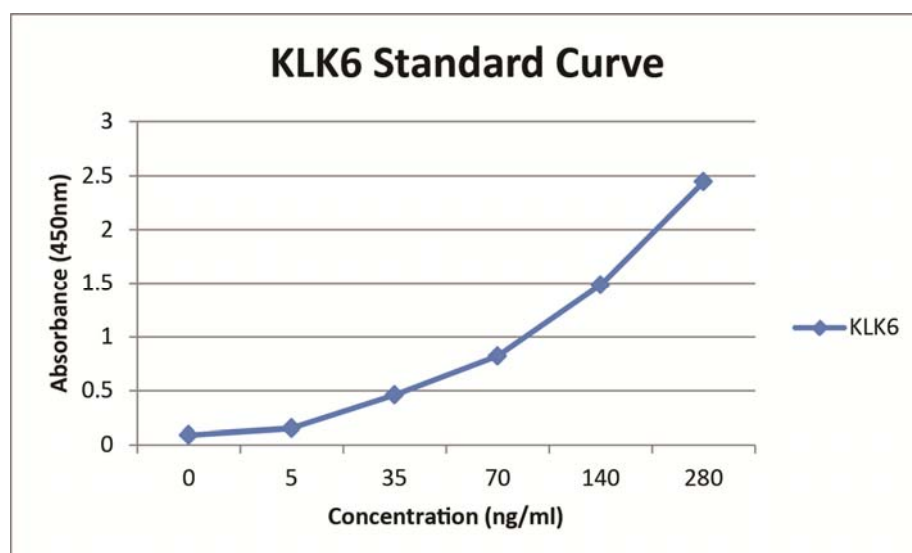
3. Approximate a straight line through the points. The concentration of KLK6 in the unknowns can be determined by interpolation.

TYPICAL RESULTS

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against KLK6 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.

Standards	KLK6 values (ng/ml)	Absorbance (450nm)
1	0	0.0920
2	5	0.1540
3	35	0.4615
4	70	0.8225
5	140	1.4865
6	280	2.4470

TYPICAL STANDARD CURVE



CALIBRATORS

Calibrators consist of protein standard diluted in 1X PBS, 3% BSA. Calibrators should read within the given range in this kit insert for assay run to be considered valid. Calibrators are stable at 4°C for 6 months. Avoid repeated freeze/thaw cycles. Do not mix calibrators from different kit lots.

Calibrator 1 range: 15-35ng/ml

Calibrator 2 range: 80-120ng/ml

EXPECTED VALUES AND SENSITIVITY

The expected KLK6 assay values for healthy women are yet to be determined in this assay. The minimum detectable concentration of KLK6 in this assay is estimated to be 1ng/ml.

No cross-reactivity with other serine proteases was detected for this assay. However, due to limited resources, it is impossible for us to test all similar proteins. Therefore, some cross reactivity may still exist.

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.



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NOTES



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