



ELISA PRODUCT INFORMATION & MANUAL

Kallikrein 7 NBP2-62157

Enzyme-linked Immunosorbent Assay for quantitative
detection of Human Kallikrein 7.

For research use only.

Not for diagnostic or therapeutic procedures.

Product Manual

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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 Novus Biologicals Technical Support if necessary.

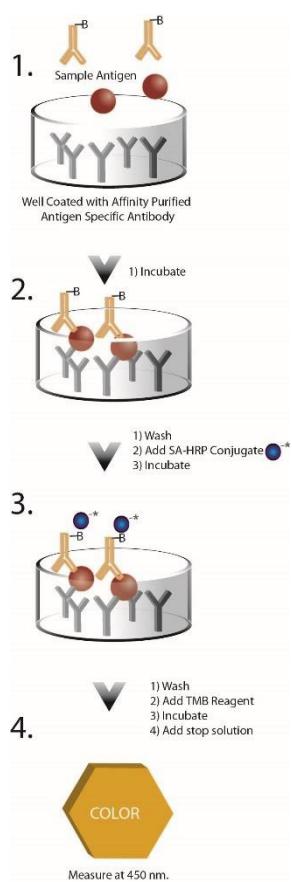
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INTENDED USE

The Kallikrein 7 (human) ELISA Kit is intended for the quantitative determination of the Cancer Antigen KLK7 concentration in human serum.



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. Kallikrein-7 (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 Kallikrein 7 (KLK7) 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 a biotin conjugated anti-KLK7 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 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 450 nm.

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MATERIALS SUPPLIED

1. **Microtiter plate coated with Monoclonal anti-KLK7**
2. **KLK7 reference standards:** 6 vials (ready to use), 0.35 mL
3. **Calibrators 1 and 2:** 1 vial each (ready to use), 0.35 mL
4. **Biotin-labeled monoclonal anti-KLK7 antibody,** 6 mL
5. **10X Streptavidin-HRP** (add 10ml diH₂O for 1X), 1.1 mL
6. **TMB Reagent** (One-Step), 11 mL
7. **Stop Solution,** 11 mL
8. **10X Wash,** 30 mL

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 450 nm
6. Absorbance paper or paper towel
7. Graph paper or immunoassay 4P data analysis software

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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 270 mL of distilled or deionized water. Store at room temperature (18-26°C).
2. Prepare 1X Streptavidin-HRP by adding 10 mL 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 35 uL of serum into 140 uL of 1X PBS for 175 uL total. Then apply 50 uL of diluted serum into each well for the assay.

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ASSAY PROCEDURE

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

1. Dispense 50 µL of KLK7 standards, calibrators, and diluted specimens into appropriate wells.
2. Dispense 50 µL 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 300 µL 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 100 µL Strept-HRP into each well and incubate at room temperature for 30 minutes with gentle agitation.
7. Repeat steps 4 and 5.
8. Dispense 100 µL 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 100 µL 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 450 nm with a microtiter plate reader within 15 minutes.

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CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of KLK7 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program. Such software is often supplied by plate reader manufacturers. If data reduction software is not readily available, the concentration of KLK7 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 KLK7 in the unknowns can be determined by interpolation.

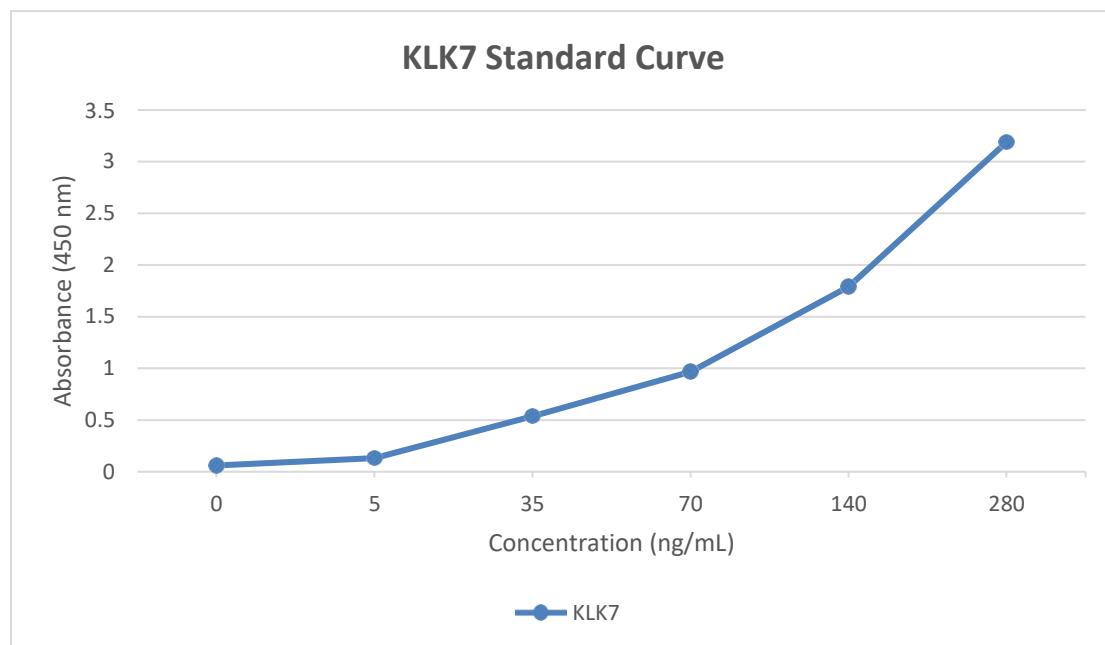
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TYPICAL RESULTS

Results of a typical standard run with optical density readings at 450 nm 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.

Standards	KLK7 values (ng/mL)	Absorbance (450 nm)
1	0	0.0610
2	5	0.1305
3	35	0.5355
4	70	0.9675
5	140	1.7890
6	280	3.1885

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: 35-65 ng/mL

Calibrator 2 range: 180-220 ng/mL

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EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have KLK7 assay values below 8 ng/mL according to a limited set of non-cancerous post-menopausal serum samples. The minimum detectable concentration of KLK7 in this assay is estimated to be 0.5 ng/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.

This assay and its constituent parts are protected by the following patents:

US Patent # 6,294,344

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