



ELISA PRODUCT INFORMATION & MANUAL

Human Kallikrein 8/Neuropsin ELISA Kit (Colorimetric)

NBP2-62176

Enzyme-linked Immunosorbent Assay for
quantitative detection of Human Kallikrein 8/
Neuropsin.

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

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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

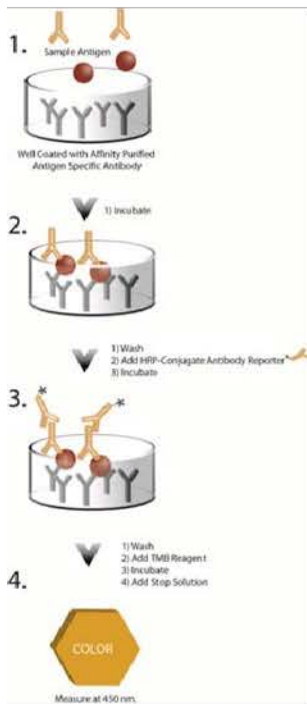
The Kallikrein 8/Neurotrophin (human) ELISA Kit is intended for the quantitative determination of the Cancer Antigen Kallikrein 8/Neurotrophin (KLK8) concentration in human serum.

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 8/Neurotrophin (KLK8) is one of the fifteen kallikrein subfamily member. The KALLIKREIN 8/NEUTROPSIN enzyme exhibits distinct patterns of expression that suggest roles in brain plasticity and ovarian cancer.

PRINCIPLE OF THE TEST

The Kallikrein 8/Neurotrophin (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 Kallikrein 8/Neurotrophin molecule for solid phase immobilization (on the microtiter wells). Standards, calibrators, and patient samples are incubated with an anti-Kallikrein 8/Neurotrophin polyclonal antibody and the solid phase antibody on the plate simultaneously. Wells are then washed and incubated with a Goat anti-Rabbit antibody conjugated to HRP which is used as a reporting agent. Excess Goat anti-Rabbit -HRP is then washed off and a solution of TMB Reagent is added and incubated resulting in the development of a blue color if Kallikrein 8/Neurotrophin is present. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of Kallikrein 8/Neurotrophin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.



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MATERIALS SUPPLIED

1. **Microtiter plate coated with Monoclonal anti-Kallikrein 8/Neuropsin**
2. **Kallikrein 8/Neuropsin reference standards:** 6 vials (ready to use), .35ml
3. **Calibrators 1 and 2:** 1 vial each (ready to use), 0.35ml
4. **Polyclonal anti-Kallikrein 8/Neuropsin antibody,** 6ml
5. **10X HRP-Conj. Antibody Reporter** (add 12ml diH₂O for 1X), 1.33ml
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

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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. Samples can be run NEAT in this assay. No dilution is necessary.

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 Kallikrein 8/Neurotrophin Reporter by adding 12ml distilled or deionized water to contents of bottle and mix gently.

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ASSAY PROCEDURE

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

1. Dispense 50µl of Kallikrein 8/Neuropsin standards, calibrators, and specimens into appropriate wells.
2. Dispense 50µl of Polyclonal 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 HRP-Conj. Antibody Reporter 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.
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 Kallikrein 8/Neurotrophin 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 Kallikrein 8/Neurotrophin 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:

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

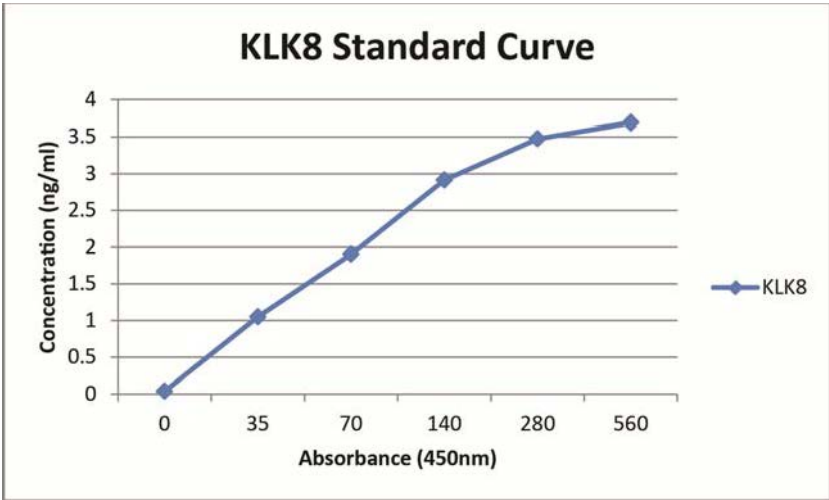
3. Approximate a straight line through the points. The concentration of Kallikrein 8/Neurotrophin in the unknowns can be determined by interpolation.

TYPICAL RESULTS

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against Kallikrein 8/Neuropsin 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	Kallikrein 8/Neuropsin values (ng/ml)	Absorbance (450nm)
1	0	0.0370
2	35	1.0480
3	70	1.9025
4	140	2.9125
5	280	3.4725
6	560	3.6875

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: 5-25ng/ml

Calibrator 2 range: 40-70ng/ml

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EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have Kallikrein 8/Neuropsin assay values below 5ng/ml according to a limited set of non-cancerous post- menopausal serum samples. The minimum detectable concentration of Kallikrein 8/Neuropsin in this assay is estimated to be 0.1ng/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.

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

US Patent # 7,014,993

US Patent # 7,537,901

US Patent # 7,759,103

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