ELISA PRODUCT INFORMATION & MANUAL

Hepsin

NBP2-62147

Enzyme-linked Immunosorbent Assay for quantitative detection of Human Hepsin.

For research use only.

Not for diagnostic or therapeutic procedures.

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Product Manual

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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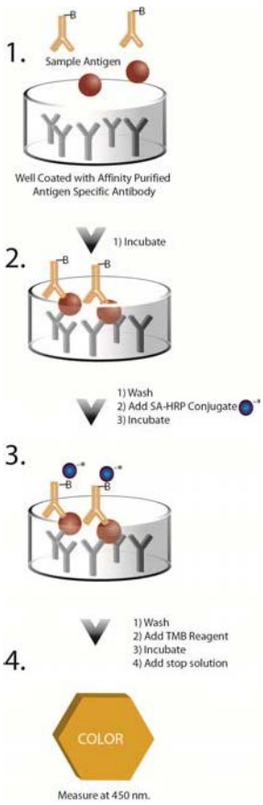


INTENDED USE

The Hepsin (human) ELISA Kit is intended for the quantitative determination of the Cancer Antigen Hepsin concentration in human plasma or serum.

SUMMARY AND EXPLANATION

The Hepsin gene encodes a type II transmembrane serine protease. The encoded protein has an extracellular region that consists of two domains, a catalytic serine protease domain and a non-catalytic scavenger receptor cysteine-rich domain. This protein may be involved in diverse cellular functions including blood coagulation, maintenance of cell morphology and the growth and progression of certain cancers, particularly prostate cancer.

PRINCIPLE OF THE TEST

The Hepsin (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 Hepsin molecule for solid phase immobilization (on the microtiter wells). Standards, calibrators, and samples are incubated with the solid phase antibody. The wells are then washed and incubated with a biotin conjugated anti-Hepsin monoclonal antibody. The wells are then washed again 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 Hepsin is present. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of Hepsin 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-Hepsin**
2. **Hepsin reference standards:** 6 vials (ready to use), 0.35ml
3. **Calibrator:** 2 vials (ready to use), 0.35ml
4. **Biotin-labeled monoclonal anti-Hepsin** antibody, 11ml
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 or plasma should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives or plasma samples collected in Heprin tubes 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 or plasma samples should be diluted a minimum of 1:2 in 1X PBS before being added to the plate. An example for a sample being run in triplicate would be 175ul of serum into 175ul of 1X PBS for 350ul total. Then apply 100ul 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 100µl of Hepsin standards, calibrators, and diluted specimens into appropriate wells and incubate at room temperature for 1.5 hours with gentle agitation.

2. Remove samples by emptying the plate contents into a waste container.

3. 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.

4. Tap the microtiter plate gently onto absorbance paper or paper towels to remove all residual liquid droplets.

5. Dispense 100µl of Biotin-labeled Antibody into each well and incubate at room temperature for 1 hour with gentle agitation.

6. Repeat steps 3 and 4.

7. Dispense 100µl Streptavidin-HRP into each well and incubate at room temperature for 30 minutes with gentle agitation.

8. Repeat steps 3 and 4.

9. Dispense 100µl of TMB Reagent into each well and incubate at room temperature in the dark for 30 minutes with gentle agitation.

10. Stop the reaction by adding 100µl of Stop Solution to each well.

11. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

12. 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 hepsin 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 hepsin 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:

   Average Net OD = Average Bound OD - 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:

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

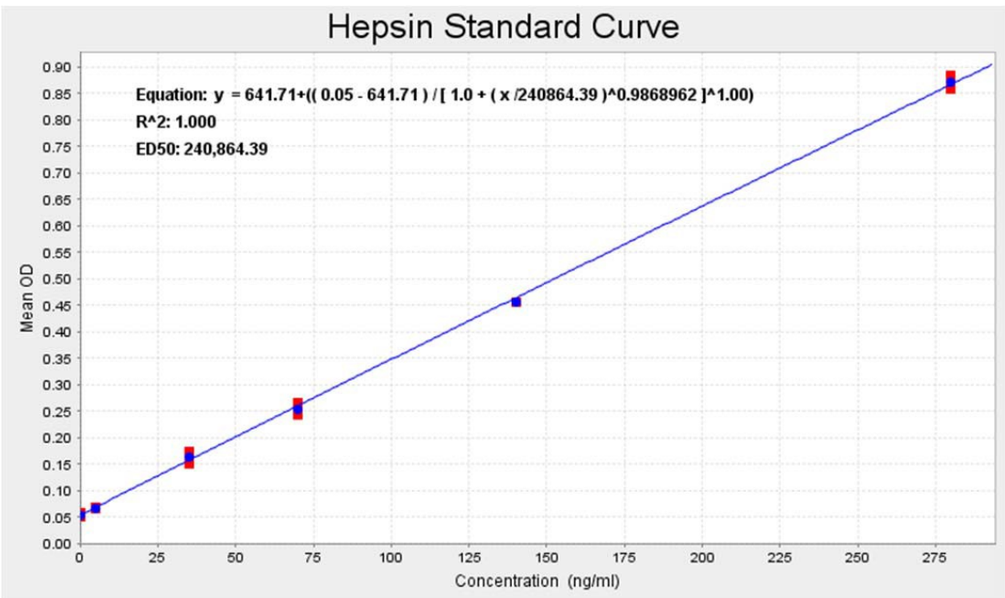
3. Approximate a straight line through the points. The concentration of hepsin in the unknowns can be determined by interpolation.

TYPICAL RESULTS

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against Hepsin 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	Hepsin values (ng/ml)	Absorbance (450nm)
1	0	0.0540
2	5	0.0675
3	35	0.1635
4	70	0.2545
5	140	0.4560
6	280	0.8710

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 4ºC for 6 months. Avoid repeated freeze/thaw cycles. Do not mix calibrators from different kit lots.

Calibrator 1 range: 60-90 ng/ml

Calibrator 2 range: 150-210 ng/ml

EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have Hepsin assay values below 15 ng/ml according to a limited set of non-cancerous post-menopausal serum samples. The minimum detectable concentration of Hepsin in this assay is estimated to be 1.0 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 # 6,268,165

US Patent # 6,303,318

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