

HBT ELISA TEST KITS FOR HNP 1-3 (HUMAN NEUTROPHIL DEFENSINS 1-3)

Human neutrophil defensins (alpha-defensins) belong to the family of cationic trisulfide-containing microbicidal peptides. Besides microbicidal, the peptides exert chemotactic, immunomodulating and cytotoxic activity and participate in host defense and inflammation.

Azurophilic granules of neutrophils contain Human Neutrophil Peptide (HNP) 1-4 which are highly homologous. The three principal human defensins, HNP 1-3, are unique to neutrophils and account for about 99 of the total defensin content of these cells. Measured amount of defensins is 3 -5 µg per million human neutrophils.

Activation of neutrophils leads to rapid release of defensins. Thus, only one cell type, neutrophils, may be the source of HNP 1-3 measured in plasma and other body fluids during infection and inflammation. In normal plasma low levels of HNP are present ranging from undetectable level to 50-100 ng/ml, while in septic conditions the levels of HNP might be elevated to 10 mg/ml and even more. Activation of neutrophils in blood as occurs during clotting, as well as long storage of anticoagulated blood leads to a release of HNP, thus careful plasma sampling is important for possible detection of HNP. Defensins are relatively resistant to proteolysis, low pH and boiling, but have a tendency to bind to a variety of materials, including plastic and proteins. The HBT HNP sandwich assay is appropriate for detection of HNP 1-3 in culture media, plasma and other biological fluids. The HBT HNP sandwich assay shows cross reactivity with Rhesus monkey and cynomolgous macaques HNP1-3.

PRINCIPLE OF THE TEST

The Hbt HNP 1-3 ELISA test kit is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. Samples and standards are incubated in microtiter wells coated with antibodies recognizing Human HNP 1-3. During this incubation Human HNP 1-3 is captured by solid bound antibody. Unbound material present in the sample is removed by washing. Next biotinylated second antibody (tracer) to Human HNP 1-3 is added to the wells. If HNP 1-3 was present in the sample, the tracer antibodies will bind to the captured HNP 1-3. The excess tracer is removed by washing. Next a streptavidin-peroxidase conjugate is applied to the wells, this conjugate reacts specifically with the biotinylated tracer antibody bound onto the detected HNP 1-3. The excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine (TMB) is added to the wells. Colour develops proportionally to the amount of human HNP 1-3 present in the sample. The enzyme reaction is stopped by the addition of citric acid and the absorption at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The human HNP 1-3 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

SPECIAL FEATURES OF THE KIT

- Ready-to-use (i.e. pre-coated microwells).
- High specificity.
- High reproducibility
- High sensitivity. The minimum concentration which can be measured is 50 pg/ml of HNP 1-3.
- Large measurable concentration range. Standard curve from 41-10,000 pg/ml.
- Efficient format. Two plates with each twelve 8-well strips allow free choice of batch size for the assay.
- Simple, rapid procedure. Four pipetting steps are required to complete the assay. Working time 3½ hours.

AVAILABILITY

The Hbt Human HNP 1-3 test is available in a kit for 2 x 96 determinations.

PRODUCT NUMBER: HK317

Hbt HNP 1-3 ELISA Kit

For research purposes only.

Caution: Not for use in humans.