ELISA KIT FOR HUMAN NEUTROPHIL DEFENSINS (ALPHA DEFENSINS, HNP1-3)

Catalog nr: HK317 (2 x 96 determinations)

Description: Human neutrophil defensins (alpha-defensins, HNP1-3) 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 percent of the total defensin content of these cells. Measured amount of defensins is 3-5 mg per million human neutrophils. Activation of neutrophils leads to rapid release of defensins. Thus, only one cell type, neutrophils, may be the source of HNP1-3 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 HNP 1-3 ELISA shows cross reactivity with Rhesus monkey HNP 1-3.

Application: The human HNP1-3 ELISA has been developed for the quantitative measurement of natural and recombinant human HNP1-3 in cell culture medium, plasma and other biological fluids. In plasma samples human HNP1-3 can be measured accurately if plasma samples are diluted at least 2000 times for human and at least 4x for Rhesus monkey. Most reliable results are obtained if EDTA plasma is used. HNP1-3 are highly absorbing to Ig and other proteins therefore samples can only be measured accurately if diluted with supplied dilution buffer. Please be aware that human HNP1-3 is released from neutrophils into serum in the process of blood coagulation. This will lead to false positive and difficult to interpret results of serum samples. Therefore it is advised to use 'careful plasma'.

Features:
- Minimum concentration which can be measured is 50 pg/ml human HNP1-3.
- Measurable concentration range of 41-10,000 ng/ml.
- Working volume of 100 µl/well.

Typical standard curve:

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Principle:
- The human HNP1-3 ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
- The efficient format of 2 plates with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are captured by a solid bound specific antibody.
- Biotinylated tracer antibody will bind to captured human HNP1-3.
- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
- Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of citric acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human HNP1-3 standards (log).
- The human HNP1-3 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.
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Website: [www.hbt.nl](http://www.hbt.nl)  All Hbt products are subject to strict quality control procedures. Version: 07-2007
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NEUTROPHIL DEFENSINS (ALPHA DEFENSINS, HNP1-3)

Storage and stability
Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least six months.
After reconstitution the reagents are stable for 1 month if stored at 2-8°C.

Cross Reactivity
Potential cross reacting proteins detected in the human HNP1-3 ELISA.

<table>
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<tr>
<th>Cross reactant</th>
<th>Reactivity</th>
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<tr>
<td>Rhesus monkey</td>
<td>strong</td>
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Precautions
For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and Federal rules in the use of this product. Hbt is not responsible for any patent infringements that might result with the use of or derivation of this product.

References
5. Baroncelli, S et al; Characterization of α-defensins plasma levels in Macaca fascicularis and correlations with virological parameters during SHIV89.6PFvill experimental infection. AIDS Res Hum Retroviruses 2007, 23: 287

Also available
HK314 Human BPI ELISA kit, 2 x 96 determinations
HK315 Human LBP ELISA kit, 2 x 96 determinations
HK321 Human LL-37 ELISA kit, 2 x 96 determinations
HK324 Human MPO ELISA kit, 2 x 96 determinations
HK325 Human Calprotectin ELISA kit, 2 x 96 determinations