

## **HBT ELISA TEST KIT FOR HUMAN SOLUBLE CD14**

The Hbt human sCD14 test kit has been developed for the quantitative measurement of natural and recombinant human sCD14 in serum, plasma and culture medium.

CD14, the 55-kDa glycoprotein known to function as a receptor for LPS is expressed mainly on the surface of monocytes/macrophages, and PMN, the cells responsible for scavenging of LPS and bacteria. Although monocytes and PMN are the main CD14 expressing cells few reports have described CD14 expression on B-cells, mesangial cells and basophils. The plasma protein LBP plays an important role in the LPS-CD14 mediated cell activation. In addition to the function as receptor for LPS, several other functions have been ascribed to CD14; next to the recognition of micro-organisms by the innate immune system CD14 also plays a role in cell-cell interactions.

Besides the membrane bound form of CD14 also the soluble form of CD14 (sCD14), which lacks the GPI anchor, is involved in LPS-induced cell activation. Two forms of sCD14 are known to exist. An approximately 48 kDa form, derived from monocytes membrane CD14, and a 56 kDa form, speculated to be directly released in plasma or supernatant after processing are known to exist.

sCD14 affects LPS actions via several pathways. sCD14 is an intermediate in the transfer of LPS to lipoproteins, resulting into neutralization of LPS. Besides, sCD14 facilitates LPS activation of CD14-membrane negative cells like endo- and epithelium. Furthermore, high concentrations of sCD14 were shown to block LPS-induced activation of monocytes. sCD14 thus both enhances and reduces cellular responses to LPS.

sCD14 was demonstrated to be present in plasma in levels from 2 - 4 µg/ml and to be enhanced in infectious diseases.

### **PRINCIPLE OF THE TEST**

The Hbt human sCD14 ELISA 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 sCD14. During this incubation sCD14 is captured by the solid bound antibody. Unbound material present in the sample is removed by washing. Biotinylated second antibody (tracer) to sCD14 is added to the wells. If sCD14 was present in the sample, the tracer antibodies will bind to the captured sCD14. Excess tracer is removed by washing. Streptavidin-peroxidase conjugate is applied to the wells, this conjugate reacts specifically with the biotinylated tracer antibody bound onto the detected sCD14. Excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine (TMB) is added to the wells. Colour develops proportionally to the amount of sCD14 present in the sample. The enzyme reaction is stopped by the addition of citric acid and the absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance versus the corresponding concentrations of the standards.

The sCD14 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 (ie. pre-coated microwells).
- High specificity for sCD14 due to the use of two monoclonal antibodies directed against different epitopes on the molecule.
- The minimum concentration which can be measured is 2 ng/ml .
- Large measurable concentration range. Standard curve from 2-100 ng/ml.
- Efficient format: 2 plates with twelve disposable 8-well strips allow free choice of batch size for the assay.
- Standardization. The calibration standards have been standardized by Hbt sCD14 .
- High reproducibility.
- Simple, rapid procedure. Four pipetting steps are required to complete the assay. Working time 3½ hours.

**AVAILABILITY:** The Hbt sCD14 test is available in a kit for 2x96 determinations.

**PRODUCT NUMBER: HK320**

Hbt human sCD14 test kit

For research purposes only.

Caution: Not for use in humans.