

HBT ELISA TEST KIT FOR NITROTYROSINE

Nitrotyrosine has been identified as a marker of inflammation and NO production. Nitrotyrosine is formed in presence of the active metabolite NO. Various pathways including the formation of peroxynitrite lead to nitrotyrosine production. Since nitrotyrosine is a stable endproduct of peroxynitrite oxidation, assesment of its plasma concentration may be useful as a marker of NO-dependent damage in vivo. Since NO_x is only an indicator for enhanced NO production, protein associated nitrotyrosine might be a more suitable marker for damage induced by reactive nitrogen intermediates derived from NO. Furthermore, most proteins have a longer half life in the circulation than NO_x levels. The presence of nitrotyrosine has been detected in various inflammatory processes including atherosclerotic plaques, celiac disease, rheumatoid arthritis, chronic renal failure and septic shock.

PRINCIPLE OF THE TEST

The Hbt Nitrotyrosine 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 nitrotyrosine. During this incubation nitrotyrosine is captured by the solid bound antibody. Unbound material present in the sample is removed by washing. Biotinylated second antibody (tracer) to nitrotyrosine is added to the wells. If nitrotyrosine was present in the sample the tracer antibodies will bind to the nitrotyrosine. 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 nitrotyrosine. Excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine (TMB) is added to the wells. Colour develops proportionally to the amount of nitrotyrosine 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 nitrotyrosine standards.

The nitrotyrosine 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).
- Specific for nitrotyrosine, no cross reaction with tyrosine, 3-chlorotyrosine or phenylalanine.
- The minimum concentration which can be measured is 2 nM nitrotyrosine.
- Large measurable concentration range. Standard curve from 2-1,500 nM.
- Efficient format. 2 plates with twelve disposable 8-well strips allow free choice of batch size for the assay.
- High reproducibility.
- Simple, rapid procedure. Four pipetting steps are required to complete the assay. Working time 3½ hours.

LITERATURE

1. ter Steege, J et al., *Free Radic Biol Med.*, 1998, **25**(8): 953-963.

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

PRODUCT NUMBER: HK501

Hbt Nitrotyrosine test kit

For research purposes only.

Caution: Not for use in humans.