

HBT ENDOBLOCK TEST: A SCREENING ASSAY FOR INHIBITORS OF LPS-LBP BINDING

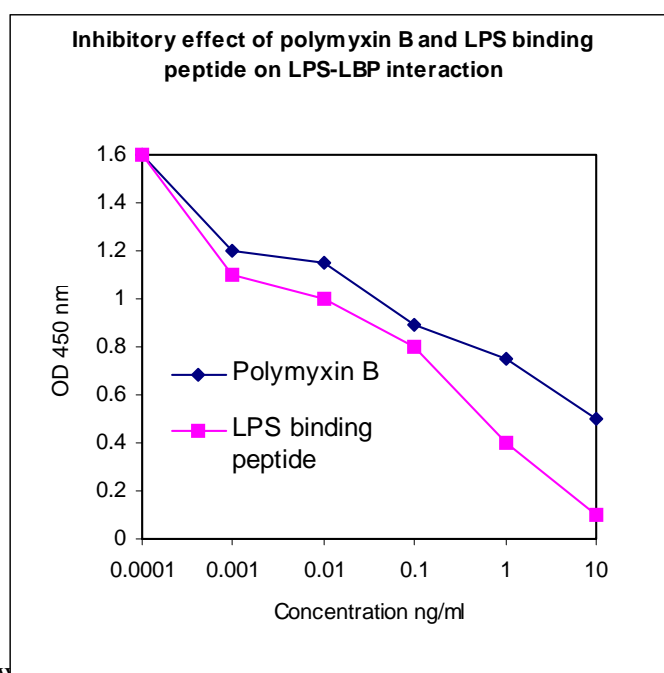
The Hbt ENDOBLOCK test has been developed for the detection of compounds that inhibit binding of endotoxin to LBP. Binding to lipopolysaccharide (LPS) binding protein (LBP) is an important step in the biological response to endotoxins. Inhibition of endotoxin binding to LBP is found to be the major mechanism of inhibition of endotoxin toxicity by natural or synthetic compounds. A wide range of compounds ranging from Polymyxin B to endotoxin toxicity reducing peptides have been shown to share the common property that they prevent binding of endotoxins to LBP.

The Hbt ENDOBLOCK test permits the study and selection of compounds that prevent endotoxins from binding to LBP. The assay is based upon the inhibition principle: addition of a compound that competes with LBP for binding to LPS, which leads to reduction of binding of biotin labeled LPS detected by a HRP labeled conjugate.

PRINCIPLE OF THE TEST

The Hbt ENDOBLOCK test consists of a microtiter plate that is coated with an antibody to LBP that is reactive with LBP of a wide variety of animals. This antibody interacts with LBP in such configuration that LBP is still highly reactive with endotoxin. The binding to LPS is however abrogated very efficiently by endotoxin inhibitors such as LBP-, LAL- and BPI like peptides, Polymyxin B and many other inhibitors.

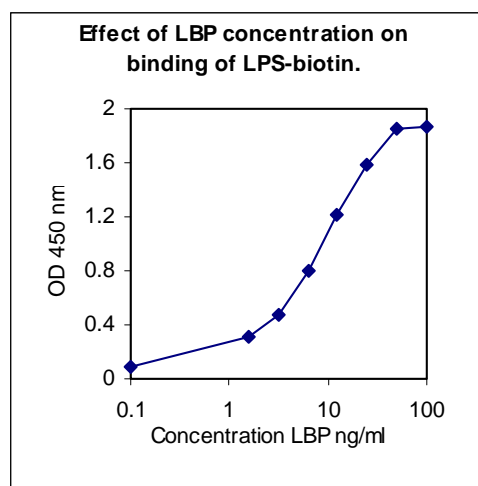
LBP is incubated in the microtiter wells, during this incubation LBP is captured by the solid bound antibody. Unbound material present in the solution is removed by washing. For testing of the endotoxin toxicity inhibitory capacity of the samples, the samples or Polymyxin B, as a standard, are preincubated with biotinylated LPS. After preincubation the mixtures are added to the wells. If an endotoxin inhibitor is present in the sample, the inhibitor competes with LBP for binding to LPS. The excess biotinylated LPS and inhibitor is removed by washing. Next a streptavidin-peroxidase conjugate is applied to the wells, this conjugate reacts specifically with the biotinylated LPS bound onto LBP. The excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine (TMB), is added to the wells. Colour develops inverse proportionally to the endotoxin toxicity inhibitory capacity of 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 of the Polymyxin B inhibitor. The endotoxin toxicity inhibitory capacity of samples can be qualitative determined from the standard curve of the inhibitor Polymyxin B.



ADDITIONAL PRODUCT INFORMATION:

The assay can be used to investigate LPS-LBP interactions also in other ways. First it can be used as described above for detection of compounds that interact with LPS and inhibit the binding of LPS on LBP. The test compound can be incubated with LPS for a given period of time (for many peptides 30 min at 37°C will be sufficient; it is imperative to use a control test compound that lacks inhibitory properties).The user might also be interested to test other procedures. For some peptides, inhibitors, the assay works also very efficiently in case the test compound is added to the plate simultaneously with or just before addition of biotinylated LPS.

Second, the assay can be used as an alternative to an ELISA for LBP of man and various animal species since it permits quantification of LBP. In this test system LBP of man, sheep, pig, goat, rabbit, dog, rat, bovine and cynomolgous monkey (not murine LBP) can be determined.



Third, the assay allows detection of compounds that react with LBP and inhibit LBP to interact with LPS. To this end LBP is added to the plate, incubated and excess LBP is washed away. Next the compound to be tested should be added to the plate. The compound is left to interact with LBP for a defined period of time (eg 30 min at 37°C) and subsequently excess compound is washed away. Hereafter biotinylated LPS is added to the plate, incubated, excess washed away. Next a streptavidin-peroxidase conjugate is applied to the wells, this conjugate reacts specifically with the biotinylated LPS bound onto the LBP. The excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine (TMB), is added to the wells. Colour develops inverse proportionally to the LBP blocking capacity of the compound to be tested. The enzyme reaction is stopped by the addition of citric acid and the absorption at 450 nm is measured with a spectrophotometer.

SPECIAL FEATURES OF THE KIT

- Ready-to-use (i.e. pre-coated microwells).
- High specificity.
- High reproducibility.
- Large measurable concentration range. Standard curve of endotoxin toxicity inhibition capacity of 10 – 10,000 ng/ml Polymyxin B.
- Efficient format. One plate with 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.

LITERATURE

1. Scott, M et al., *J. Immunol.*, 2000, 164: 549-553.

AVAILABILITY

The Hbt ENDOBLOCK test is available in kits for 1 x 96 determinations.

PRODUCT NUMBER: HIT301

Hbt ENDOBLOCK test

OPTIONAL AVAILABLE

1 liter of endotoxin free de-ionized water

PRODUCT NUMBER: HE003

Hbt endotoxin free water

For research purposes only.

Caution: Not for use in humans