



# LBP (Human) ELISA Kit

Catalog Number KA0448

96 assays

Version: 18

Intended for research use only

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## **Introduction**

### **Intended Use**

The LBP (Human) ELISA Kit has been developed for the quantitative measurement of natural and recombinant human LBP in serum, plasma and culture medium.

### **Principle of the Assay**

The LBP (Human) ELISA Kit is a solid phase sandwich Enzyme Linked-Immunosorbent Assay (ELISA). Monoclonal antibody specific for human LBP is used for coating (precoated and blocked modules). In the first step, the plate will be incubated with the antigen (standard or sample). During this incubation, human LBP is captured by solid bound antibody. Unbound material present in the sample is removed by washing. Now the plate will be incubated with a POD-labelled antibody specific for human LBP (second incubation). Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of stopping solution 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 LBP concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

## General Information

### Materials Supplied

List of component

Component	Amount	Vial number
Precoated ELISA modules	96 wells	1
Detecting antibody (POD-labelled monoclonal antibody to human LBP, 14 ng/mL) "Ready for use"	1 vial	Vial 2
Human LBP-standard (6 µg/mL)	1 vial	Vial 3
Reference serum (9.5 µg/mL)	1 vial	Vial 4
PBS	2 tablets	Vial 5
Dilution Buffer	1 vial	Vial 6
Tween 20	1 vial	Vial 7
Stopping solution "Ready for use"	1 vial	Vial 8
Substrate solution "Ready for use"	1 vial	Vial 9

Vial 3 and 4 are lyophilized.

### Storage Instruction

Short time store at 2-8°C, Long time storage of vial 3 and 4 at -20°C or -80°C, Detecting monoclonal can be stored at 2-8°C.

### Materials Required but Not Supplied

- ✓ Orbital shaker
- ✓ Micro plate reader for measurement absorbance at 450 /620 nm
- ✓ Precision pipettes with disposable tips
- ✓ 10-1000 µL adjustable multiwell pipettes

## Assay Protocol

### Reagent Preparation

- ✓ Wash Buffer  
PBS/ 0.05% Tween: Dissolve 1 Tablet phosphate buffered saline (PBS, vial 5) in 200 mL distilled water; add 100  $\mu$ L Tween 20 (vial 7). (Prepared wash buffer is stable for 4 weeks at refrigerator).
- ✓ PBS  
Dilute 1 Tablet of vial 5 in 200 mL distilled water.
- ✓ Dilution buffer  
Dissolve content of vial 6 with 50 mL PBS and add 50  $\mu$ L Tween 20 from vial 7. This buffer is 1-2 weeks stable at -20°C. Attention! Use buffer for assay at room temperature. Alternatively: 250 mg BSA +25 mL PBS+25  $\mu$ L Tween 20.
- ✓ Substrate  
Vial 9 ready for use. Mix carefully
- ✓ Detecting antibody  
Vial 2 ready for use. Mix carefully
- ✓ Reference serum  
Pipette 30  $\mu$ L distilled water to the vial 4 for reconstitution. For assay pipette the whole content of reconstituted vial 4 to 7970  $\mu$ L dilution buffer, gently mix and pipette 100  $\mu$ L of this dilution in duplicate in reference serum wells. This represents final dilution of 1:800. The reference serum contains  $8.3 \pm 3.0$   $\mu$ g/mL LBP. Reconstituted reference serum is stable for 1 week at refrigerator.
- ✓ Human LBP-standard  
Firstly pipette 30  $\mu$ L distilled water to the vial 3 for reconstitution and secondly pipette the whole reconstituted content of vial 3 in a new vial (a) containing 3.57 mL Dilution Buffer and mix carefully. This represents = vial a. For standard curve prepare vial b-f and use a-f. Prepare just before use. Store the standard at -20°C.

No	human LBP $\mu$ L	Dilution buffer	Concentration ng/mL
vial a			50
vial b	250 $\mu$ L of vial a	250 $\mu$ L	25
vial c	250 $\mu$ L of vial b	250 $\mu$ L	12.5
vial d	250 $\mu$ L of vial c	250 $\mu$ L	6.25
vial e	250 $\mu$ L of vial d	250 $\mu$ L	3.125
Vial f	250 $\mu$ L of vial e	250 $\mu$ L	1.5

Reconstituted standard is stable at refrigerator at maximum 1 week.

### **Sample Preparation**

- ✓ Serum, plasma and other human LBP containing solutions are suitable for use in the test. Samples containing a visible precipitate must be clarified prior to use in the assay. Lipemic and haemolysed probes are not possible.
- ✓ Samples should be frozen at -20°C for a long term storage.
- ✓ Depending on the concentration of LBP in the samples, these have to be diluted with dilution buffer.
- ✓ For normal human serum samples a dilution of 1:800 is recommended. For animal sera (goat, sheep we recommended dilutions of 1:2, 1:4 to 1:20), for cattle LBP 1:10 to 1:100, for pork and rabbit LBP 1:50 to 1:200.

### **Assay Procedure**

Let all reagents reach room temperature and mix thoroughly

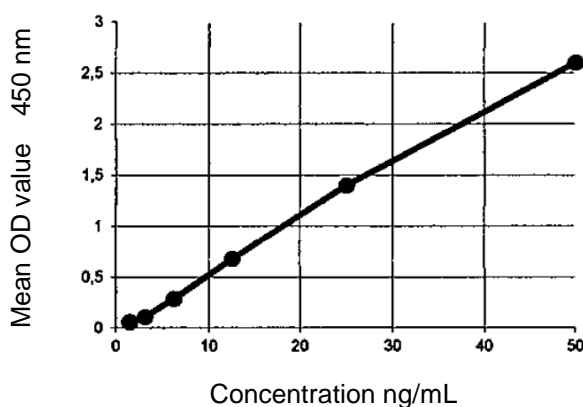
1. Samples: Pipette 100 µL of standards (50, 25, 12.5, 6.25, 3.12, 1.5 ng/mL= vial a-f), reference serum or diluted samples in duplicate into the corresponding wells of precoated modules (1) and incubate for one hour at room temperature and shaking.
2. 3 x washing with Wash Buffer.
3. Detecting antibody: Pipette 100 µL detecting antibody (vial 2) to each well and incubate at room temperature for 1 hour at shaker.
4. 3 x washing with Wash Buffer.
5. Substrate: Pipette 100 µL substrate solution (vial 9) to each well. Incubate 12-14 min in the dark at room temperature without shaking (depending from temperature in the lab).
6. Stopping: Pipette 100 µL stopping solution (vial 8) to each well. Tape plate gently to mix.
7. Read absorbance of wells at 450 nm (reference wave length 620).

## Data Analysis

### Calculation of Results

Calculate the mean of optical density (OD) of standard duplicates, reference serum and the samples. Design a standard curve by plotting the OD means of standards (a-f) (y-axis) and the LBP concentration (x-axis). Calculate the LBP concentration from the mean OD of samples from the standard curve and multiply with dilution factor.

#### ✓ Typical Standard Curve



### Performance Characteristics

- ✓ Normal LBP range (with human LBP standard):  
 Human LBP in healthy blood donors: (5-15  $\mu\text{g/mL}$ )  
 Cattle LBP range 0.05-2.5  $\mu\text{g/mL}$   
 Sheep-Goat LBP: 10-30 ng/mL  
 Pork-Rabbit LBP: 4-10  $\mu\text{g/mL}$
- ✓ Inter-assay variation coefficient: 9.8 till 17.8 depending of concentration
- ✓ Intra-assay variation coefficient: 6.1%,
- ✓ Effective range: 5 -50 ng/mL, linear till 25 ng/mL
- ✓ Cross reaction: pork-, rabbit-, cattle-, dog-, horse LBP
- ✓ Specificity: specific for free LBP

## Resources

### References

1. Schroedl, W. et al. A novel acute phase marker in cattle: Lipopolysaccharide binding protein (LBP) J. of Endocrine Research 7, 2001, p. 49-52
2. Berner, R. et al.: Elevated levels of Lipopolysaccharide –binding protein and soluble CD14 in plasma in neonatal early-onset sepsis, Clinical and diagnostic laboratory immunology 2002, Vol 9, p. 440-445



**Plate Layout**

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