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Cat.:044 a	**Multispecies Enzyme Immunoassay for Quantification of free human LBP** Useful for bovine-, pork-, rabbit- dog LBP

Recommendations for Use

Test components

1	Precoated ELISA modules	1
Vial 2	detecting antibody (POD-labelled monoclonal antibody to human LBP, 10ng/ml) "Ready for use"	1 vial
Vial 3	Human LBP-standard (8µg/ml)	1 vial
Vial 4	Reference serum (9.5µg/ml)	1 vial
Vial 5	PBS	2 tabl.
Vial 6	Dilution Buffer	1 vial
Vial 7	Tween 20	1 vial
Vial 8	Stopping solution "Ready for use"	1 vial
Vial 9	Substrate solution "Ready for use"	1 vial

vial 3 and 4 are lyophilized

STORAGE:

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

MATERIAL REQUIRED BUT NOT PROVIDED

- orbital shaker
- micro plate reader for measurement absorbance at 450 /620 nm
- precision pipettes with disposable tips
- 10-1000 µl adjustable multiwell pipettes

PREPARATION OF REAGENTS

A **Wash Buffer**: *PBS/ 0.05%Tween:* Dissolve 1 Tablet phosphate buffered saline (PBS, **vial 5**) in 200 ml distilled water, add 100µl Tween 20 (**vial 7**). (Prepared wash buffer is stable for 4 weeks at refrigerator).

B **PBS**: Dilute 1 Tablet of **vial 5** in 200 ml distilled water

C **Dilution buffer:** Dissolve content of **vial 6** with 50 ml PBS (Buffer **B**) and add 50µl Tween 20 from **vial 7**. This buffer is 1-2 weeks stable at -20°C. Attention! Use buffer for assay at **room temperature.** **Alternatively:** 250mg BSA +25ml PBS+25µl Tween 20

D **Substrate:** **Vial 9** Ready for use**.** Mix carefully

E **Detecting antibody**: **Vial 2** ready for use. Mix carefully

F **Reference serum:** Pipette 30µl distilled water to the **vial 4** for reconstitution. For assay pipette the whole content of reconstituted **vial 4** to 7970 µl dilution buffer (**C**), gently mix and pipette 100µl of this dilution in duplicate in reference serum wells. This represents final dilution of 1:800. The reference serum contains **9.5 ± 3.5** µg/ ml LBP. Reconstituted reference serum is stable for 1 week at refrigerator.

G **human LBP-standard:** Firstly pipette 30 µl distilled water to the **vial 3** for reconstitution and secondly pipette the whole reconstituted content of **vial 3** in a new vial (a) containing 1570µl Dilution Buffer (C) and mix carefully. This represents = vial a. For standard curve prepare vial b-f and use a-f.

No	human LBP µl	Dilution buffer **C**	Concentration ng/ml
vial a			50
vial b	250 µl of vial a	250 µl	25
vial c	250 µl of vial b	250 µl	12.5
vial d	250 µl of vial c	250 µl	6.25
vial e	250 µl of vial d	250 µl	3.125
vial f	250 µl of vial e	250 µl	1.5

Prepare just before use. **Store the standard at -20°C.**

Reconstituted standard is stable at refrigerator at maximum 1 week.

PRINCIPLE OF TEST

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

The human LBP 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.

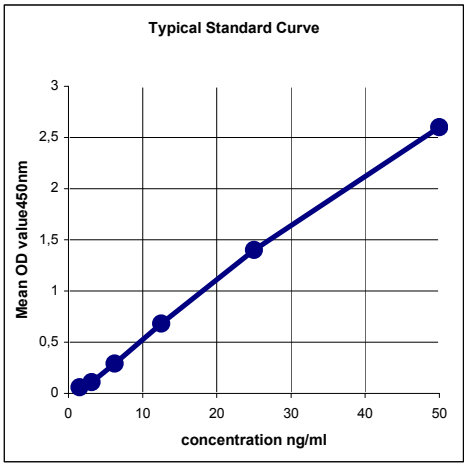
PREPARATION OF SAMPLES

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 CHARACTERISTIC

Normal LBP range (with human LBP standard) :
human LBP in healthy blood donors: (5-15 µg/ml)
cattle LBP range 0.05-2.5µg/ml
sheep - goat LBP: 10- 30ng/ml
pork- rabbit LBP: 4 -10µg/ml
Interassay variation coefficient: 9.8 till 17.8 depending of concentration
Intraassay variation coefficient: 6.1%,
Effective range: 5 -50ng/ml, linear till 25ng/ml
Cross reaction: pork-, rabbit-, cattle-, dog-, horse LBP
Specificity: specific for free LBP



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 (A).

3. **Detecting antibody**
Pipette 100 µl detecting antibody (E, **vial 2**) to each well and incubate at room temperature for 1 hour at shaker.

4. 3 x washing with Wash Buffer (A).

5. **Substrate**
Pipette 100 µl substrate solution (D, **vial 9**) to each well. Incubate **10 min** in the dark at room temperature without shaking (depending from temperature in the lab).

6. **Stopping**
Pipette 100µl stopping solution (**vial 8**) to each well. Tape plate gently to mix

7. **Read absorbance** of wells at 450 nm (reference wave length 620)

8. **Calculate the LBP concentration**
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 (b-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.

References
Schroedl, W. et al. A novel acute phase marker in cattle: Lipopolysaccharide binding protein (LBP) J. of Endocrine Research 7, 2001, p. 49-52
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