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Cat. No 043 lot 130318	Enzyme Immunoassay for Determination of mouse LBP (useful also for rat LBP)

Recommendations for Use

Test components:

1	Precoated ELISA modules	1 plate
2	Detecting antibody (HRP-labelled monoclonal antibody to mouse LBP) "Ready for use"	1 vial
3	LBP-standard (15µg/ml)	1 vial
4	Mouse reference serum	1 vial
5	PBS	2 tab.
6	Dilution Buffer	1 vial
7	Tween 20	1 vial
8	Stopping solution "Ready for use"	1 vial
9	Substrate solution "Ready for use"	1 vial

Vials 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 /620nm
- precision pipettes with disposable tips
- 10-1000 µl adjustable multiwell pipettes

PREPARATION OF REAGENTS:

A Wash Buffer: PBS/ Tween 0.05%:
Dissolve 1 Tablet Phosphate buffered saline (PBS, **vial 5**) in 200ml distilled water -add 100 µl 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

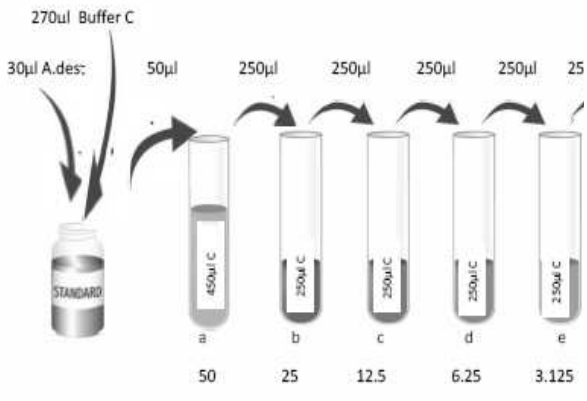
C Dilution buffer: Add content of the **vial 6** to 50ml PBS (Buffer **C**). Prepare just before use. Store remaining dilution buffer after reconstitution at -20°C

D Substrate: **Vial 9** Ready for use, mix carefully.

E Detecting antibody: **Vial 2** Ready for use, mix carefully

F Reference serum: Add 10 µl distilled water to the **vial 4**. This contains 12.14 ± 3.5µg/ml LBP. For assay dilute 1:800 (10µl serum +7990µl dilution buffer and use 100µl/well.

G LBP-standard: Firstly, pipette 30 µl distilled water to the **vial 3** for reconstitution and secondly add 270µl dilution buffer (**C**) to this vial and mix carefully, thirdly pipette 50µl from this vial to a new vial containing 450µl dilution buffer (**C**) and mix carefully. Finally this last vial contains 500µl standard dilution and containing 50ng/ml LBP = vial a. For standard curve prepare vial b-e and use vial a –ePrepare just before use. **Store the standard at -20°C.**



No	Mouse LBP µl	Dilution buffer C	Conc. ng/ml
vial a	500 µl	0	**50**
vial b	250 µl of vial a	250 µl	**25**
vial c	250 µl of vial b	250µl	**12.5**
vial d	250 µl of vial c	250 µl	**6.25**
vial e	250 µl of vial d	250 µl	**3.125**

PRINCIPLE OF TEST

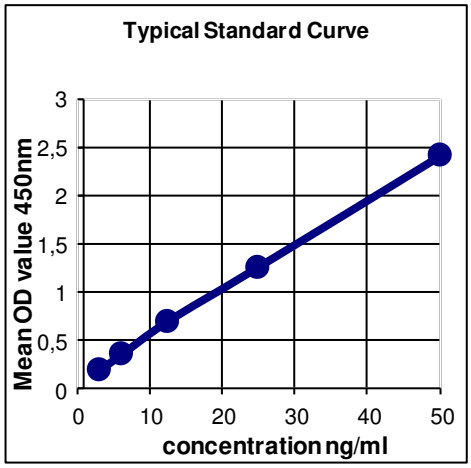
The mouse LBP kit has been developed for the quantitative measurement of natural and recombinant mouse LBP (both free and LPS-bound) in serum, plasma and culture medium.
The mouse LBP kit is a solid phase sandwich Enzyme-Linked-Immunosorbent Assay (ELISA). Monoclonal antibody specific for mouse LBP used for precoated modules. In the first step, the precoated modules incubated with the antigen (standard or sample). During this incubation, mouse LBP captured by solid bound antibody. Unbound material present in the sample removed by washing. Now the plate incubated with a POD-labelled antibody specific for mouse LBP (second incubation). Revelation step includes TMB as chromogen. The enzyme reaction stopped by the addition of stopping solution and the absorption at 450 nm measured with a spectrophotometer. A standard curve obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The mouse LBP concentration of samples with unknown concentrations, which run concurrently with the standards, can be determined from the standard curve.

PREPARATION OF SAMPLES

Serum, plasma and other mouse LBP containing solutions as well as recombinant LBP solutions are suitable for use in the test. Samples containing a visible precipitate clarified prior to use in the assay. Lipemic and haemolytic probes are not possible.
Samples should be frozen at -20°C for long-term storage.
Depending on the concentration of mouse LBP in the samples, these have to dilute with dilution buffer. For mouse serum 1:800 and for normal **rat serum samples dilution 1:50 to 1:200**, is recommended,

ASSAY CHARACTERISTIC

Normal LBP range in untreated mice: (2-15µg/ml). Acute phase sera containing factor 10 to 100 more LBP
Interassay variation coefficient: 7% until 13.6% depending of concentration
Intraassay variation coefficient : 2.4%, n=50 plasma samples
Effective range: 3 -50 ng/ml
Cross-reaction: rat LBP
Specificity: detected free as well as bound LBP
Recovery of recombinant LBP in LBP depleted sera is 100%



ASSAY PROCEDURE

Let all reagents reach room temperature and mix thoroughly

1. **Samples**
Add 100 µl of standards (50, 25, 12.5, 6.25, 3.12 ng/ml= vial a-e) or diluted samples in duplicate into the corresponding wells of the precoated modules and incubate for one hour at room temperature and shaking (300rpm).

2. 3 x washing with Wash Buffer (A).

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

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

5. **Substrate**
Add 100 µl Substrate solutions (D, vial 9) to each well. Incubate **12**-14 min **in the dark** at room temperature without shaking.

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

7. **Read absorbance** 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.

Ref.
Heinrich,J.-M. Bernheiden, M. Minigo,G. Schütt, C. *et al.*: The Essential Role of Lipopolysaccharide-Binding Protein in Protection of Mice Against a Peritoneal Salmonella Infection Involves the rapid Induction of an Inflammatory Response, J. of Immunology,167, 2001:1624-1628

Myot, G. et al.: Prognostic values of α_2-macroglobulin, fibrinogen and albumin in regards to mortality and fraility in old rats, experimental Gerontology 42, 6(2007) 498

Takano K. et al. Successful treatment of acute lung injury with Pitavastatin in septic mice: Potential role of glucocorticoid receptor expression in alveolar macrophages. J. Pharmacology and exp. Therapeutics 336, 2011, 2381-2390