



## **LBP, Soluble (mouse) Detection Set [For ELISA Application]**

*Manufactured by Biometec.*

### **ALX-850-305-KI01**

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**For laboratory use only. Not for human or diagnostic use.**

### Test components:

<b>Vial 1</b>	<b>Coating antibody (monoclonal antibody to mouse LBP):</b>	<b>1 vial</b>
<b>Vial 2</b>	<b>Detecting antibody (POD-labelled monoclonal antibody to mouse LBP)</b>	<b>1 vial</b>
<b>Vial 3</b>	<b>Mouse LBP-standard</b>	<b>1 vial</b>
<b>Vial 4</b>	<b>Blocking reagent</b>	<b>1 vial</b>
<b>Vial 5</b>	<b>Substrate solution "Ready for use"</b>	<b>1 vial</b>
<b>Vial 7</b>	<b>Adsorption Buffer</b>	<b>1 Capsule</b>
<b>Vial 8</b>	<b>PBS</b>	<b>2 Tablets</b>
<b>Vial 9</b>	<b>Tween 20</b>	<b>1 vial</b>
<b>Vial 10</b>	<b>Stopping solution "Ready for use"</b>	<b>1 vial</b>
<b>Vial 11</b>	<b>Mouse reference serum mouse LBP content: 10.4± 2.2(dilution 1:800)</b>	<b>1 vial</b>

Vial 1-2 are stabilised with 0.01 % Thimerosal, vial 3 and 11 are lyophilized  
**Short time store at 2-8°C, Long time storage at -20°C or -80°C**

#### **MATERIAL REQUIRED BUT NOT PROVIDED:**

- ELISA-Plate (NUNC-Maxisorp, F-Form) or Strips
- orbital shaker
- micro plate reader for measurement absorbance at 450 nm/620
- precision pipettes with disposable tips
- 50-200 µl adjustable multiwell pipettes

#### **PREPARATION OF REAGENTS**

- A Wash Buffer:** PBS/ Tween 0.05%/Thimerosal 0.01%:  
 Dissolve 1 Tablet Phosphate buffered saline (PBS, **vial 8**) in 200ml distilled water  
 -add 0.05 % Tween 20 (100 µl, **vial 9**) and add 0.01 % Thimerosal
- B Adsorption Buffer, pH 9.4:** Dissolve content of 1 capsule (**vial 7**) in 25 ml distilled water.  
 Remove the empty capsule. **Alternatively:** 0.2M Carbonate buffer pH 9.3-9.7
- C PBS:** Dilute 1 Tablet of **vial 8** in 200 ml distilled water
- E Blocking Reagent:** Add content of the **vial 4** to 80ml PBS (Buffer **C**). Prepare just before use. Store remaining blocking reagent after reconstitution at -20°C
- F Substrate:** Ready for use
- G Coating antibody:** Add content of **vial 1** to 10 ml Adsorption Buffer (**B**) Prepare just before use.
- H Detecting antibody:** Add 10µl of **vial 2** to 12 ml blocking reagent (**E**). Prepare just before use.
- I mouse reference serum:** Add 10 µl distilled water to the **vial 11**. This contains 10.4± 2.2µg/ml LBP. For assay dilute 1: 800 and use 100µl/well.
- J mouse LBP-standard:** Add 30 µl distilled water to the **vial 3**. Then add 870µl blocking reagent (**E**) to the reconstituted standard = **vial a**. The concentration of this vial is 50ng/ml.

For standard curve prepare **vial b-f** and use **vial a –f**

No	Mouse LBP µl	Blocking reagent <b>E</b>	Concentration ng/ml
<b>vial a</b>			50
<b>vial b</b>	250 µl of <b>vial a</b>	250 µl	25
<b>vial c</b>	250 µl of <b>vial b</b>	250 µl	12.5
<b>vial d</b>	250 µl of <b>vial c</b>	250 µl	6.25
<b>vial e</b>	250 µl of <b>vial d</b>	250 µl	3.125
<b>vial f</b>	250 µl of <b>vial e</b>	250 µl	1.56

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

#### **PRINCIPLE OF TEST**

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

The mouse LBP kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). Monoclonal antibody specific for mouse LBP is used for coating (1. incubation). After blocking of free binding sites the plate will be incubated with the antigen (standard or sample). During this incubation, mouse 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 mouse LBP (third incubation). Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of sulphuric 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. The mouse 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 mouse LBP containing solutions as well as recombinant LBP solutions are suitable for use in the test. Samples containing a visible precipitate must be 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 be diluted with blocking buffer.

For normal serum samples a dilution of 1:800 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% till 13.6% depending of concentration

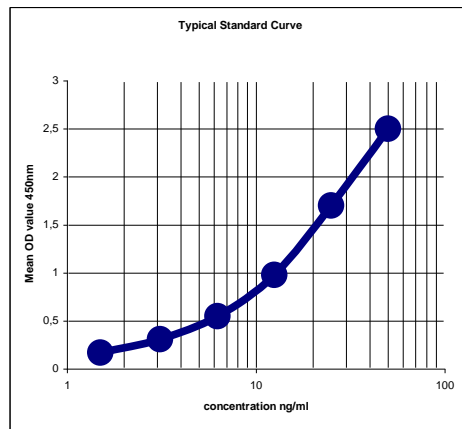
**Intraassay** variation coefficient: 2.4%, n=50 plasma samples

**Effective range:** 5 -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. Coating

Pipette 100 µl coating antibody (G) to each well and incubate over night at 4°C.  
Cover the plate.

2. 1 x washing with Wash Buffer (A). Remove the Wash Buffer carefully after each wash.

### 3. Blocking

Add 200 µl Blocking reagent (E) to each well and incubate at room temperature for 30 minutes at orbital shaker (300 U/min)

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

### 5. Samples

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

6. 3 x washing with Wash Buffer (A).

### 7. Detecting antibody

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

8. 3 x washing with Wash Buffer (A).

### 9. Substrate

Add 100 µl Substrate solutions (F) to each well. Incubate 5-7 min in the dark at room temperature without shaking.

### 10. Stopping

Add 100 µl stopping solution (vial 10) to each well. Tape gently to mix plate

11. Read absorbance at 450 nm (reference wave length 620)

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 2001, 167:1624-1628

