



## **LBP, Soluble (mouse) ELISA Kit**

*Manufactured by Biometec.*

### **ALX-850-305/1-KI01**

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#### **NORTH AMERICA**

AXXORA, LLC  
6181 Cornerstone Court East  
Suite 103  
San Diego, CA 92121-4727  
Phone: (858) 550-8828  
Fax: (858) 550-8825  
E-mail: [axxora-usa@axxora.com](mailto:axxora-usa@axxora.com)

#### **SWITZERLAND/REST OF THE WORLD**

ALEXIS CORPORATION  
Industriestrasse 17, Postfach  
CH-4415 Lausen / Switzerland  
Phone: +41 61 926 89 89  
Fax: +41 61 926 89 79  
E-mail: [alexis-ch@alexis-corp.com](mailto:alexis-ch@alexis-corp.com)

#### **GERMANY**

AXXORA DEUTSCHLAND GmbH  
Marie-Curie-Strasse 8  
DE-79539 Lörrach  
Phone: (07621) 5500 522  
Fax: (07621) 5500 523  
E-mail: [axxora-de@axxora.com](mailto:axxora-de@axxora.com)

#### **UK & IRELAND**

AXXORA (UK) LTD.  
P.O. Box 6757  
Bingham, Nottingham NG13 8LS  
Phone: +44 1949 836111  
Fax: +44 1949 836222  
E-mail: [axxora-uk@axxora.com](mailto:axxora-uk@axxora.com)

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

### Test components:

<b>1</b>	<b>Precoated ELISA modules</b>	<b>1 plate</b>
<b>Vial 2</b>	<b>Detecting antibody (POD-labelled monoclonal antibody to mouse LBP) "Ready for use"</b>	<b>1 vial</b>
<b>Vial 3</b>	<b>Mouse LBP-standard</b>	<b>1 vial</b>
<b>Vial 4</b>	<b>Mouse reference serum mouse LBP content: 10.4± 2.2(dilution 1:800)</b>	<b>1 vial</b>
<b>Vial 5</b>	<b>PBS</b>	<b>2 tabl.</b>
<b>Vial 6</b>	<b>Dilution Buffer</b>	<b>1 vial</b>
<b>Vial 7</b>	<b>Tween 20</b>	<b>1 vial</b>
<b>Vial 8</b>	<b>Stopping solution "Ready for use"</b>	<b>1 vial</b>
<b>Vial 9</b>	<b>Substrate solution "Ready for use"</b>	<b>1 vial</b>

Vial 2 is stabilised with stabilisation solution and 0.01 % Thimerosal, 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 nm/620
- precision pipettes with disposable tips
- 10-1000 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 5**) in 200ml distilled water -add 0.05 % Tween 20 (100 µl, **vial 7**) and add 0.01 % Thimerosal
- 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 mouse reference serum:** Add 10 µl distilled water to the **vial 4**. This contains 10.4± 2.2µg/ml LBP. For assay dilute 1: 800 and use 100µl/well.
- G mouse LBP-standard:** Firstly pipette 30 µl distilled water to the **vial 3** for reconstitution and secondly pipette 500µl dilution buffer (**C**) in this vial and mix carefully, thirdly pipette the whole reconstituted content of **vial 3** (530µl) in a new vial (**a**) containing 370µl dilution buffer (**C**) and mix carefully. This represents 50ng/ml= **vial a**. For standard curve prepare **vial b-f** and use **vial a –f**

No	Mouse LBP µl	Dilution buffer <b>C</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 precoated modules. In the first step the precoated modules 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 will be removed by washing. Now the plate will be incubated with a POD-labelled antibody specific for mouse 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 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:** 1 -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-f) or diluted samples in duplicate into the corresponding wells of the precoated modules and incubate for one hour at room temperature and shaking.

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 12-15 min in the dark at room temperature without shaking.

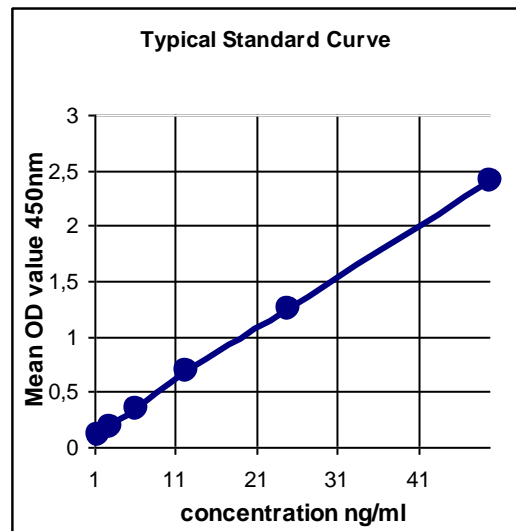
### 6. Stopping

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 2001, 167:1624-1628