KAMIYA BIOMEDICAL COMPANY

MOUSE LBP quantification ELISA

For the quantitative determination of natural and recombinant mouse LBP in serum, plasma and culture medium

Cat. No. KT-1002

For Research Use Only. Not for use in diagnostic procedures.

Product Information

Enzyme Immunoassay for Determination of mouse LBP
(useful also for rat and goat LBP)
Cat. No. KT-1002

INTENDED USE

The kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of natural and recombinant mouse LBP (both free and LPS-bound) in serum, plasma and culture medium. For research use only. Not for use in diagnostic procedures.

COMPONENTS

No.	Reagents	Quantity
1	Precoated modules, 96-well strip plate	1 plate
Vial 2	Detecting antibody (HRP-labelled monoclonal antibodies to mouse LBP) "Ready for use"	1 vial
Vial 3	Mouse LBP-standard (5µg/ml, lyophilized)	1 vial
Vial 4	Mouse reference serum (lyophilized)	1 vial
Vial 5	PBS	2 tab.
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

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Orbital shaker.
2. Micro plate reader for measurement absorbance at 450/620nm.
3. Precision pipettes with disposable tips.
4. 10-1000 µl adjustable multiwell pipette.

STORAGE

All the reagents should be kept according to the labels on vials.
Short time store at 2-8℃, Long time storage of **vials 3** and **vial 4** at -20℃ or -80℃**.** Detecting monoclonal can be stored at 2-8℃.

PRINCIPLE

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 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.

REAGENT PREPARATION

⚠ **Note: Use all reagents for assay at room temperature.**

A Wash buffer: PBS/ Tween 0.05%:
Dissolve one 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 Mouse reference serum: Add 10µl distilled water to **vial 4**. This contains 10.4 ± 3.5µg/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, ***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 and mix carefully. ***Finally*** this last vial contains 500µl standard dilution containing 50ng/ml LBP = vial a. For standard curve prepare vial **b**-f and use vial **a** –f.
Prepare just before use. ***Store the standard at -20°C.***

No.	Mouse 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	1.56

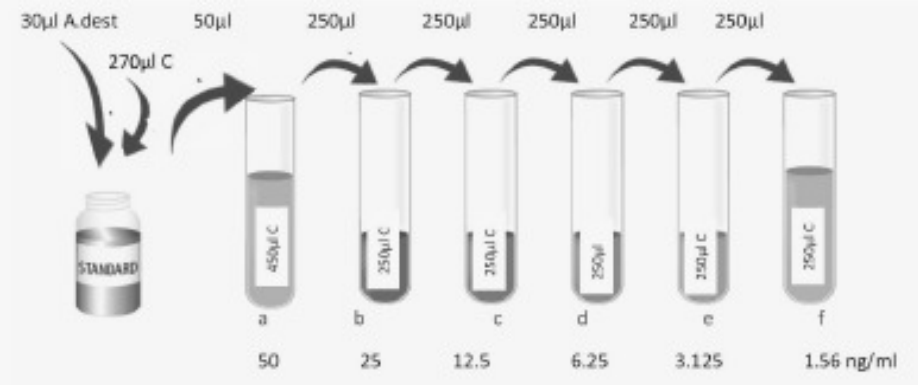
SAMPLE PREPARATION

Kamiya Biomedical Company is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

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 haemolysed probes are not possible.

Samples should be frozen at -20℃ for a long term storage.

Depending on the concentration of mouse LBP in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:800 is recommended, for rat serum 1:50 to 1:200.



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.

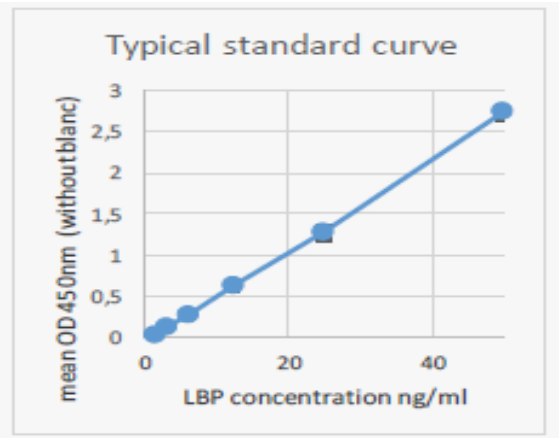
Intraassay variation coefficient: 2.4%, n=50 plasma samples.

Effective range: 1.56-50 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, 1.56ng/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 (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 solution (**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 plate gently to mix plate.

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

CALCULATION LBP CONCENTRATION

Calculate the mean of optical density (OD) of standard duplicates, reference serum and the samples (reduced for blanc). 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.

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