KAMIYA BIOMEDICAL COMPANY

MULTISPECIES LBP Quantification ELISA

For the quantitative determination of natural and recombinant human, bovine, pig, rabbit, dog LBP in serum, plasma and culture medium

Cat. No. KT-1005

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

Product Information

Multispecies Enzyme Immunoassay for quantification of free human LBP
Useful for bovine, pig, rabbit, dog LBP
Cat. No. KT-1005

INTENDED USE

The human LBP kit has been developed for the quantitative measurement of natural and recombinant human LBP 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 (POD-labelled monoclonal antibody to human LBP) "Ready for use"	1 vial
Vial 3	Human LBP-standard (lyophilized)	1 vial
Vial 4	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 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.

REAGENT PREPARATION

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:** 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. **Alternatively:** 250mg BSA +25ml PBS+25µl Tween 20.
Attention! Use buffer for assay at **room temperature.**

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

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

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 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℃ 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 Pig 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
Pig- rabbit LBP: 4 -10µg/ml

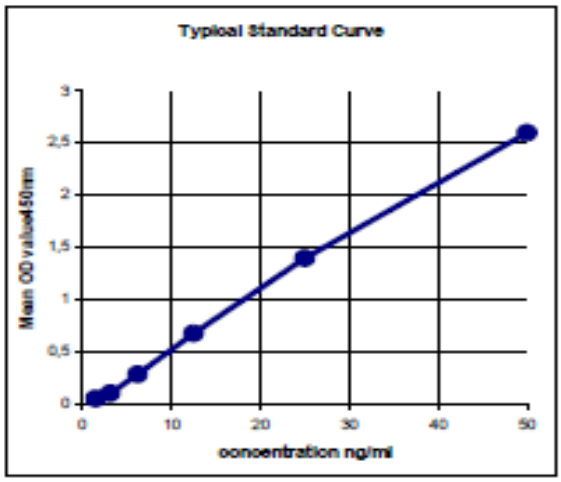
Interassay variation coefficient: 9.8 till 17.8 depending of concentration

Intraassay variation coefficient: 6.1%,

Effective range: 5 -50ng/ml, linear till 25ng/ml

Cross reaction: Pig-, 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 module (**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.

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. 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.

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