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Human Lipopolysaccharide Binding Protein (LBP) ELISA Kit Multispecies Reactive

Catalog No: CKH113 **Lot Number:** TBD **Size:** 1 Plate (96 tests) **Expiration Date:** TBD

NOTE: this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at tech@cellsciences.com

INTRODUCTION:

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-Immuno-Sorbent Assay (ELISA). Monoclonal antibody specific for human LBP is used for coating (pre-coated and blocked modules). In the first step, the plate is 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. The plate is then incubated with a POD-labelled antibody specific for human LBP (second incubation). Detection step includes TMB as chromogen. The enzyme reaction is stopped by the addition of Stop 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.

KIT COMPONENTS:

Item	Description	Quantity
CKH113-1	Pre-coated ELISA 96 well plate	1
CKH113-2	Detection antibody (POD-labelled monoclonal antibody to human LBP, 14 ng/mL) “ready-to-use”	1 vial
CKH113-3	Human LBP-standard (6 µg/mL)	1 vial
CKH113-4	Reference serum (9.5 µg/mL)	1 vial
CKH113-5	PBS	2 tablets
CKH113-6	Dilution buffer	1 vial
CKH113-7	Tween 20	1 vial
CKH113-8	Stop solution-“ready-to-use”	1 vial
CKH113-9	Substrate solution-“ready-to-use”	1 vial

Note: Vials CKH113-3 and CKH113-4 are lyophilized.

STORAGE:

Store the kit at 2-8 °C, for short term storage.
Store detection monoclonal antibody (CKH113-2) at 2-8 °C.
Long term storage of Human LBP standard and the reference serum (vials CKH113-3 & 4), store at -20 °C to -80 °C.
For storage of prepared regents, see “Preparation of Reagents” section.

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MATERIAL REQUIRED BUT NOT PROVIDED:

- orbital shaker
- microplate reader for measurement of absorbance at 450 nm/620 nm
- precision pipettes with disposable tips
- 10-1000 µL adjustable multi-well pipettes

PREPARATION OF REAGENTS (RECOMMENDATIONS FOR 1 PLATE):

A. Wash Buffer (PBS/Tween 0.05%):
Dissolve 1 tablet phosphate buffered saline (PBS, CKH113-5) in 200 mL distilled water.
Add 100 µL Tween 20 (CKH113-7).
Prepared Wash Buffer is stable for 4 weeks at 2-8 °C.

B. PBS:
Dilute 1 tablet of CKH113-5 in 200 mL distilled water.

C. Dilution Buffer:
Dissolve content of CKH113-6 with 50 mL PBS (Buffer B) and add 50 µL Tween 20 from CKH113-7.
Alternatively: 250 mg BSA + 25 mL PBS + 25 µL Tween 20
This buffer is stable at -20 °C for 1-2 weeks.
Note: Use buffer for assay at room temperature.

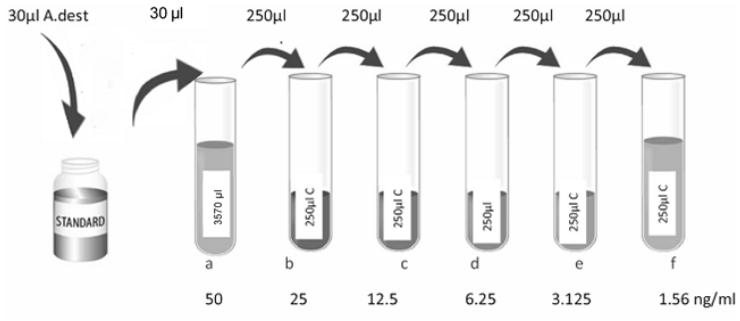
D. Substrate:
CKH113-9 - Ready to use. Mix gently.

E. Detection Antibody:
CKH113-2 - Ready to use. Mix gently.

F. Reference Serum:
Pipette 30 µL distilled water into CKH113-4 for reconstitution. For assay, pipette the entire contents of reconstituted CKH113-4 into 7970 µL dilution buffer (C) and gently mix. Pipette 100 µL of this dilution in duplicate into reference serum wells. This represents a final dilution of 1:800. The reference serum contains 8.3 ± 3.0 µg/mL LBP.
Reconstituted reference serum is stable for one week at 2-8 °C.

G. Human LBP Standard: (prepare just before use)
Pipette 30 µL distilled water to CKH113-3 for reconstitution. Then, pipette the entire contents of reconstituted vial 3 into a new vial (vial a), containing 3.57 mL dilution buffer (C), for a total volume of 3.60 mL and mix gently. For the standard curve, prepare vials b-f and use vials a–f.

ID	Human LBP	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



Reconstituted standard stable for a maximum of one week at 2-8 °C.

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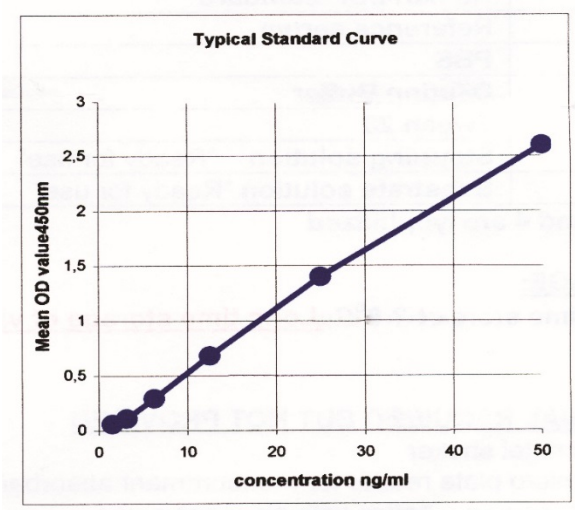
PREPARATION OF SAMPLES

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. Use of lipemic and hemolyzed samples is not possible. *Samples should be frozen at -20 °C for long term storage.*

Depending on the concentration of LBP in the samples, these have to be diluted with dilution buffer (C).
For normal human serum samples, a dilution of 1:800 is recommended. For animal sera: goat and sheep have recommended dilutions of 1:2, 1:4 to 1:20; bovine LBP 1:10 to 1:100; porcine and rabbit LBP 1:50 to 1:200.

ASSAY CHARACTERISTICS

Normal LBP range (with human LBP standard):
Human LBP in healthy blood donors: 5-15 µg/mL
Bovine LBP range: 0.05 - 2.5 µg/mL
Sheep - Goat LBP: 10 - 30 ng/mL
Porcine - Rabbit LBP: 4 - 10 µg/mL
Inter-Assay CV: 9.8-17.8% depending on concentration
Intra-Assay CV: 6.1%
Effective range: 5-50 ng/mL, linear until 25 ng/mL
Cross-reactivity: Porcine, Rabbit, Bovine, Canine, Equine LBP
Specificity: specific for free LBP



ASSAY PROCEDURE

Note: 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 the pre-coated modules.
Incubate for 1 hour at room temperature using shaker.

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

3. Detection antibody
Add 100 µL detection antibody (E, CKH113-2) to each well, and incubate at room temperature for 1 hour using shaker.

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

5. Substrate
Pipette 100 µL substrate solution (D, CKH113-9) to each well.
Incubate for **12-14 minutes** in the dark at room temperature without shaking.

6. Stopping
Pipette 100 µL stop solution (CKH113-8) to each well.
Tap plate gently to mix.

7. Read absorbance of wells at 450 nm (reference wave length 620).

8. Calculate LBP concentration
Calculate the mean optical density (OD) of standard duplicates, reference serum, and the samples. Design a standard curve by plotting the mean OD of the 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 by the dilution factor.

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