Datasheet for ABIN5664982

LBP ELISA Kit

1 Image | 2 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	LBP
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	3 ng/mL - 50 ng/mL
Minimum Detection Limit:	3 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a solid phase sandwich Enzyme Linked-immunosorbent-assay (ELISA) for the quantitative measurement of natural and recombinant human LBP in serum, plasma and culture medium.
Sample Type:	Plasma, Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Specific for free LBP binding antibodies, cross reacting with : pork-, rabbit-, cattle-, dog-, horse LBP
Sensitivity:	Normal LBP range: in healthy blood donors: (5-15 ug/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.
Characteristics:	Monoclonal antibody specific for human LBP is used for coating (precoated and blocked

Product Details

	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.
Components:	1x Precoated ELISA modules, detecting antibody (POD-labelled monoclonal antibody), Human LBP-standard, Reference serum, PBS, Dilution Buffer ,Tween 20, Stopping solution, Substrate solution
Material not included:	Orbital shaker, Micro plate reader for measurement absorbance at 450 /620 nm, Precision pipettes with disposable tips, 10-1000 ul adjustable multiwell pipettes

Target Details

Target:	LBP
Alternative Name:	Lipopolysaccharide-binding Protein (LBP) (LBP Products)
Background:	Background: Natural Lipopolysccaride Binding Protein (LBP) is a 58KD glycoprotein produced in liver. It binds at lipid A of LPS with high affinity (10-9M) and reduced the cellular LPS effects at CD14+ cells (IL1ß, IL6, TNFα). It acts as opsonin for GRAM negative cells, LPS, neutrophiles and granulocytes.
Molecular Weight:	~58kDa
Gene ID:	3929
NCBI Accession:	NP_004130
UniProt:	P18428
Pathways:	TLR Signaling, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Positive Regulation of Immune Effector Process, Toll-Like Receptors Cascades, Monocarboxylic Acid Catabolic Process

Application Details

Application Notes:	**Preparation of reagents (Recommendations for 1 plate):** (A) Wash Buffer: PBS/ Tween:

Application Details

	Dissolve 1 Tablet Phosphate buffered saline (PBS, vial 5) in 200 ml distilled water - add 100 ul Tween 20 (vial 7). (Prepared wash buffer is stable for 4 weeks in 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 50ul Tween 20 from vial 7. This buffer is 1-2 weeks stable at -20oC. Attention! Use buffer for assay at room temperature. Alternatively: 250mg BSA +25ml PBS+25ul Tween 20. (D) Substrate: Vial 9 Ready for use. Mix carefully (E) Detection antibody: Vial 2 ready for use. Mix carefully. (F) Reference serum: Pipette 30ul distilled water to the vial 4 for reconstitution. For assay pipette the whole content of reconstituted vial 4 to 7970 ul dilution buffer (C) and pipette of this 100ul/well. This represents final dilution of 1:800. The reference serum contains 12.3
Sample Volume:	100 µL
Assay Time:	2.5 h
Plate:	Pre-coated
Protocol:	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:	PREPARATION OF REAGENTS A Wash Buffer: PBS/ 0.05 % Tween: Dissolve 1 Tablet phosphate buffered saline (PBS, vial 5) in 200 mL 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 50 µL Tween 20 from vial 7. This buffer is 1-2 weeks stable at -20°C. Attention! Use buffer for assay at room temperature. Alternatively: 250 mg BSA +25 mL PBS+25 µL Tween 20 D Substrate: Vial 9 Ready for use. Mix carefully E Detecting antibody: Vial 2 ready for use. Mix carefully F Reference serum: Pipette 30 µL 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 100 µL of this dilution in duplicate in reference serum wells. This represents final

	dilution of 1:800. The reference serum contains 8.3 ± 3.0 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 and secondly pipette the whole reconstituted content of vial 3 in a new vial (a) containing 3.57 mL 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.
Sample Collection:	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°C for a long-term storage.
Sample Preparation:	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 pork and rabbit LBP 1:50 to 1: 200
Assay Procedure:	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 modules (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 12-14 min in the dark at room temperature without shaking (depending from temperature in the lab). 6. Stopping Pipette 100 µL stopping solution (vial 8) to each well. Tape plate gently to mix 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 modules (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 12-14 min in the dark at room temperature without shaking (depending from temperature in the lab). 6. Stopping Pipette 100 µL stopping solution (vial 8) to each well. Tape plate gently to mix
Calculation of Results:	Remediate the optical density (OD) with blank, calculate the mean of corrected OD of standard duplicates, reference serum and the samples. 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

Application Details

	factor.
Assay Precision:	interassay vc 10%, intra assay vc 6%
Restrictions:	For Research Use only

Handling

Preservative:	Without preservative
Precaution of Use:	protect your eyes
Storage:	4 °C
Storage Comment:	Short time store at 2-8°C, Long time storage of lyophilized reference serum and standard at -20°C or -80°C, detecting monoclonal can be stored at 2-8°C

Publications

Product cited in:	Huang, Zhang, Karuna, Andrew, Juraska, Weiner, Angier, Morgan, Azzam, Swann, Edupuganti, Mgodi, Ackerman, Donnell, Gama, Anderson, Koup, Hural, Cohen, Corey, McElrath, Gilbert, Lemos: "Adults on pre-exposure prophylaxis (tenofovir-emtricitabine) have faster clearance of anti-HIV monoclonal antibody VRC01." in: **Nature communications**, Vol. 14, Issue 1, pp. 7813, (2023) (PubMed). Lê, Khorsi-Cauet, Bach, Gay-Quéheillard: "Modulation of Pseudomonas aeruginosa lipopolysaccharide-induced lung inflammation by chronic iron overload in rat." in: **FEMS immunology and medical microbiology**, Vol. 64, Issue 2, pp. 255-64, (2012) (PubMed).

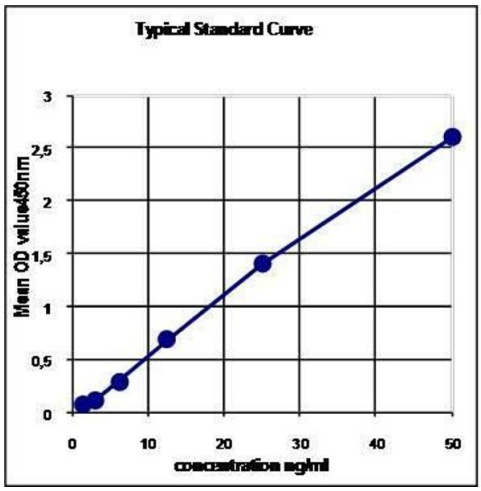
Typical Standard Curve
Mean OD value450nm
3
2,5
2
1,5
1
0,5
0
0
10
20
30
40
50
concentration ng/ml

Image 1.