Datasheet for ABIN5664983

LBP ELISA Kit

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Overview

Quantity:	96 tests
Target:	LBP
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	1.5 ng/mL - 50 ng/mL
Minimum Detection Limit:	1.5 ng/mL
Application:	ELISA

Product Details

Purpose:	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.
Sample Type:	Plasma, Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	For free and bound LBP binding antibodies
Sensitivity:	Normal LBP range in untreated mice: (2-15ug/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
Characteristics:	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

Product Details

	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.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. 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
Components:	1x Precoated ELISA modules, detecting antibody (POD-labelled monoclonal antibody), Mouse 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:	16803
UniProt:	Q61805
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:** (A) Wash Buffer: PBS/ Tween 0.05%: Dissolve 1 Tablet Phosphate buffered saline (PBS, vial 5) in 200ml distilled water -add 100 ul 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 -20oC. D Substrate: Vial 9 Ready for use, mix carefully. E Detection antibody: Vial 2 Ready for use, mix carefully. F mouse reference serum: Add 10 ul distilled water to the vial 4. This contains 10.4
Sample Volume:	100 µL
Assay Time:	2.5 h
Plate:	Pre-coated
Protocol:	The mouse LBP kit is a solid phase sandwich Enzyme-Linked-Immunosorbent Assay (ELISA). Monoclonal antibody specific for mouse LBP used for precoated modules. In the first step, the precoated modules incubated with the antigen (standard or sample). During this incubation, mouse LBP captured by solid bound antibody. Unbound material present in the sample removed by washing. Now the plate incubated with a POD-labelled antibody specific for mouse LBP (second incubation). Revelation step includes TMB as chromogen. The enzyme reaction stopped by the addition of stopping solution and the absorption at 450 nm measured with a spectrophotometer. A standard curve obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The mouse LBP concentration of samples with unknown concentrations, which run concurrently with the standards, can be determined from the standard curve.
Reagent Preparation:	A Wash Buffer: PBS/ Tween 0.05 % : 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: Add content of the vial 6 to 50 mL 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 Reference serum: Add 10 µL distilled water to the vial 4. This contains 12.14 ± 3.5 µg/mL LBP (! new reference). For assay dilute 1:800 (10 µL serum +7990 µL dilution buffer and use 100 µL/well. G LBP-standard: Firstly, pipette 30 µL distilled water to the vial 3 for reconstitution and secondly add 270 µL dilution buffer (C) to this vial and mix carefully, thirdly pipette 50 µL from this vial to a new vial containing 450 µL dilution buffer (C) and mix carefully. Finally this last vial contains 500 µL standard dilution and containing 50 ng/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.

Application Details

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 mouse LBP in the samples, these have to dilute with dilution buffer. For mouse serum 1:800 and for normal rat serum samples dilution 1:50 to 1:200, is recommended,
Assay Procedure:	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, 1.56 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 (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 solutions (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 gently to mix plate 7. Read absorbance at 450 nm (reference wave length 620)
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 factor.
Assay Precision:	interassay vc 10%, intra assay vc 5%
Restrictions:	For Research Use only

Handling

Preservative:	Without preservative
Precaution of Use:	protect your eyes
Storage:	-20 °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:	Depke, Steil, Domanska, Völker, Schütt, Kiank: "Altered hepatic mRNA expression of immune response and apoptosis-associated genes after acute and chronic psychological stress in mice." in: **Molecular immunology**, Vol. 46, Issue 15, pp. 3018-28, (2009) (PubMed).

Images

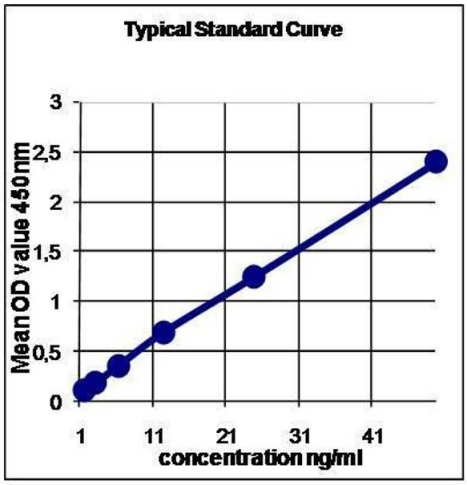

Image 1.