BioVendor Group

ENG

Instructions for Use:
HUMAN LBP ELISA

Catalogue number:
RBL016R

For research use only!

BioVendor R&D

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

CONTENT

HISTORY OF CHANGES

Previous version	Current version
	ENG.001.A
New edition	

1. INTENDED USE

Enzyme Immunoassay for the quantitative determination of Lipopolysaccharide binding protein (LBP, LPS-binding protein) in human serum and plasma.

2. STORAGE, EXPIRATION

– The kit must be stored at 2 – 8°C.
– The opened components can be stored for one week at 2 – 8°C.

3. INTRODUCTION

Lipopolysaccharide binding protein (LBP, LPS-binding protein) is a serum glycoprotein belonging to the family of lipid-binding proteins. LBP is synthesized by hepatocytes and intestinal epithelial cells. Serum concentrations of LBP range between 5 and 10 µg/ml during homeostasis, increasing up to 200 µg/ml during the acute-phase response in the course of infection [8].
LBP expression and function are strongly associated with recognition and control of bacterial infection. [4] LBP is an acute-phase reactant. As a class I acute-phase protein, LBP is induced by pro-inflammatory cytokines such as interleukins 1 and 6, tumor necrosis factor-alpha (TNF-α), and glucocorticoid hormones in liver and in non-hepatic tissues such as the gut and the lung [3].
LPS is released from CD14 in the lipid bilayer and binds to a complex of receptors including Toll-like receptor 4 (TLR-4) to initiate intracellular signaling cascades and transcription of genes mediated through NF-κB [1].
If dysregulated, these host reactions can have inadvertent outcomes such as severe sepsis, septic shock, or systemic inflammatory response syndrome (SIRS) [4].
LBP binds LPS in various pathologic states including obesity and insulin resistance.

4. TEST PRINCIPLE

The microtiter plate is coated with the antibody specifically binding the Lipopolysaccharide binding protein. The human serum or plasma is incubated in the plate with the capture antibody.
The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.
Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).
The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of LBP in the specimen. The concentration of LBP in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

5. PRECAUTIONS

- For research use only
- For professional laboratory use
- The reagents with different lot numbers should not be mixed
- To prevent cross sample contamination, use disposable labware and pipette tips
- To protect laboratory stuff, wear protective gloves and protective clothing
- The substrate solution should remain colourless, keep it protected from light
- The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

6. REAGENT SUPPLIED

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 ml
Streptavidin-HRP Conjugate	13 ml
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer 5x conc.	13 ml
Wash Buffer 15x conc.	50 ml
Substrate Solution	13 ml
STOP Solution	13 ml

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glassware and test tubes
- Microtiter plate washer
- Precision pipettes (various volumes) with tips
- Orbital shaker
- Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

8. PREPARATION OF REAGENTS

Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination. All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

8.1 Preparation of Standards

Reconstitute lyophilized Human LBP Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human LBP in Master Standard is 32 ng/ml.
Prepare set of Standard solution as follows:
Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 64 ng/ml (lyophilized)	See CoA	32 ng/ml
Std2	300 µl of Std1	300 µl	16 ng/ml
Std3	300 µl of Std2	300 µl	8 ng/ml
Std4	300 µl of Std3	300 µl	4 ng/ml
Std5	300 µl of Std4	300 µl	2 ng/ml
Std6	300 µl of Std5	300 µl	1 ng/ml
Blank	-	300 µl	0 ng/ml

8.2 Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with 100 µl deionized/distilled water. Let the QCs rehydrate for 15 min and add 900 µl of Dilution Buffer (= Dilution A). Then mix 5 µl of Dilution A + 995 µl of Dilution Buffer to get final Dilution B (i.e. 1:2000).

8.3 Preparation of Dilution Buffer 1x

Prepare a working solution of Dilution Buffer by adding 13 ml of Dilution Buffer 5x conc. to 52 ml of deionized/ distilled water (dH_2O). Mix well. Store at 4ºC for two weeks or at -20°C for long term storage.

8.4 Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 ml of Wash Buffer 15x conc. to 700 ml of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

9. PREPARATION OF SAMPLES

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70ºC. Lipemic or haemolytic samples may cause false results.
Recommended dilution of **serum and plasma** is 1:2000. It is recommended to use the two-step dilution.
Dilution A (20x) for both singlets and duplicates: 5 µl of samples + 95 µl of Dilution Buffer.
Dilution B (100x): 5 µl of Dilution A + 495 µl of Dilution Buffer, for both singlets and for duplicates.

Do not store the diluted samples.

10. ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µl of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µl/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µl of Biotin-labelled Antibody into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm. Wash the wells as described in point 3.
5. Pipette 100 µl of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
6. Wash the wells as described in point 3.
7. Pipette 100 µl Substrate solution, incubate for **10 min**, at 25°C ±2°C. Avoid exposure to the light during this step.
8. Pipette 100 µl of STOP solution.
9. Read the signal at 450 or 450/630 nm within 15 min

11. PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:2000 as recommended and assayed. The results are multiplied by the dilution factor.

11.1 Sensitivity

The limit of detection, defined as a concentration of human LBP giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.125 ng/ml of sample.

11.2 Precision

11.2.1 Intra-assay

Sample	Mean (µg/ml)	SD	CV (%)
1	19.0	0.5	1.4
2	4.6	0.2	2.3

11.2.2 Inter-assay (Run – to – run)

Sample	Mean (µg/ml)	SD	CV (%)
1	8.5	677	8.0
2	2.8	58	2.0

11.3 Accuracy

11.3.1 Dilution linearity

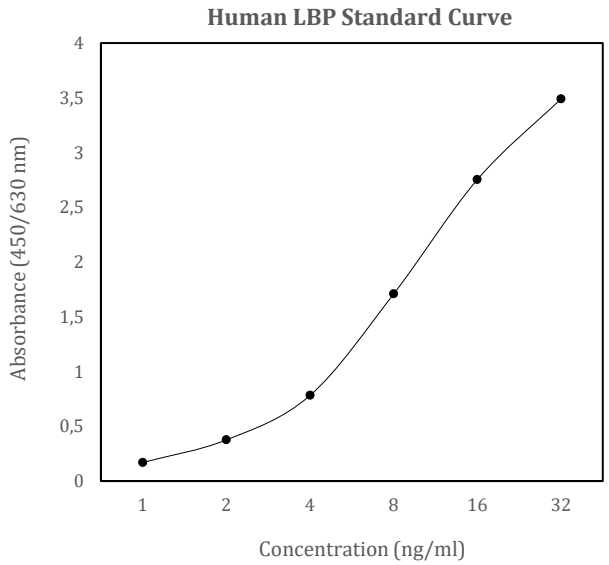
Sample	Dilution	Measured concentration (µg/ml)	Expected concentration (µg/ml)	Yield (%)
1	-	61.2	-	-
	2x	29.0	31	95
	4x	14.3	15	93
	8x	9.2	8	120
2	-	70.8	-	-
	2x	31.4	35	89
	4x	16.6	18	94
	8x	10.5	9	118

11.3.2 Spiking Recovery

Sample	Spike (µg/ml)	Measured concentration (µg/ml)	Expected concentration (µg/ml)	Yield (%)
1	-	9.4	-	-
	32	40.8	41.4	99
	16	25.1	25.4	99
	8	17.1	17.4	99

12. CALCULATION

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

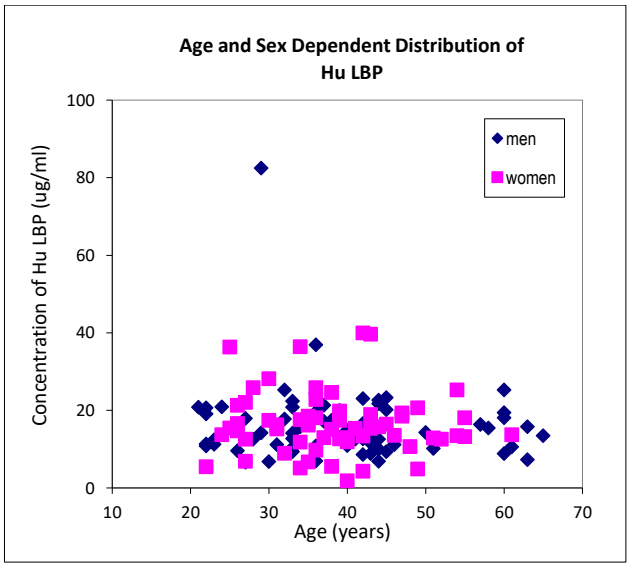


13. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 135 unselected donors (74 men + 61 women) 21-65 years old were assayed with the Human LBP ELISA in our laboratory.

13.1 Age dependent distribution of LBP

Sex	Age (years)	n	LBP (ug/ml)				
			Mean	Median	SD	Min	Max
Men	21-29	14	19.35	13.42	18.07	6.67	82.47
	30-39	21	16.49	16.26	6.78	6.78	36.94
	40-49	27	14.29	12.59	4.57	6.85	23.31
	50-65	12	17.03	16.39	4.36	10.16	25.30
Women	22-29	11	17.39	15.47	8.37	5.46	36.40
	30-39	25	16.80	17.01	7.08	5.18	36.45
	40-49	18	16.33	15.40	9.74	1.82	39.95
	50-61	7	15.92	13.35	4.57	12.59	25.26



Human LBP concentration plotted against donor age and sex.

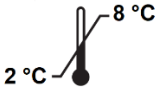
13.2 Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological references ranges for LBP levels with the assay.

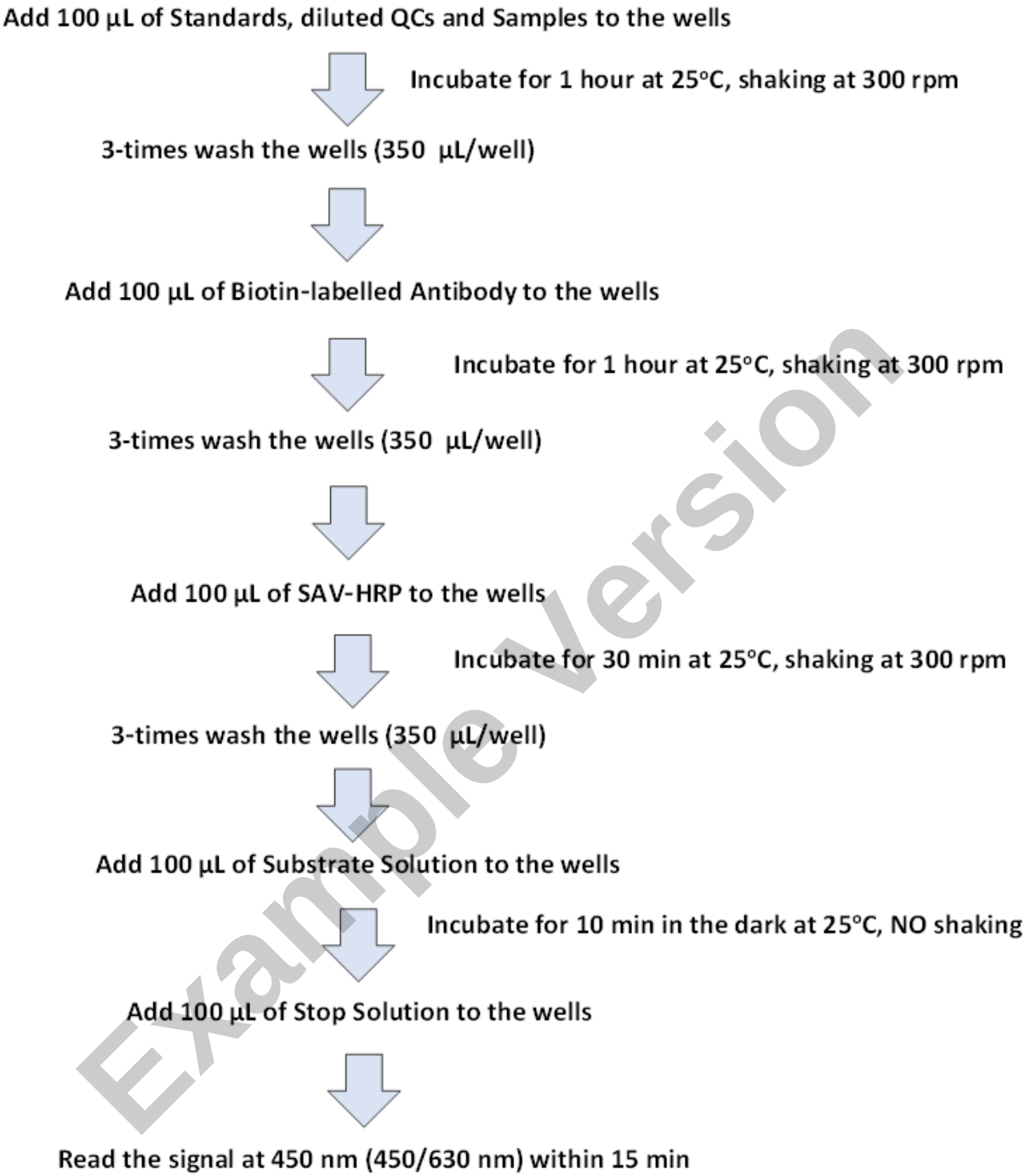
14. REFERENCES

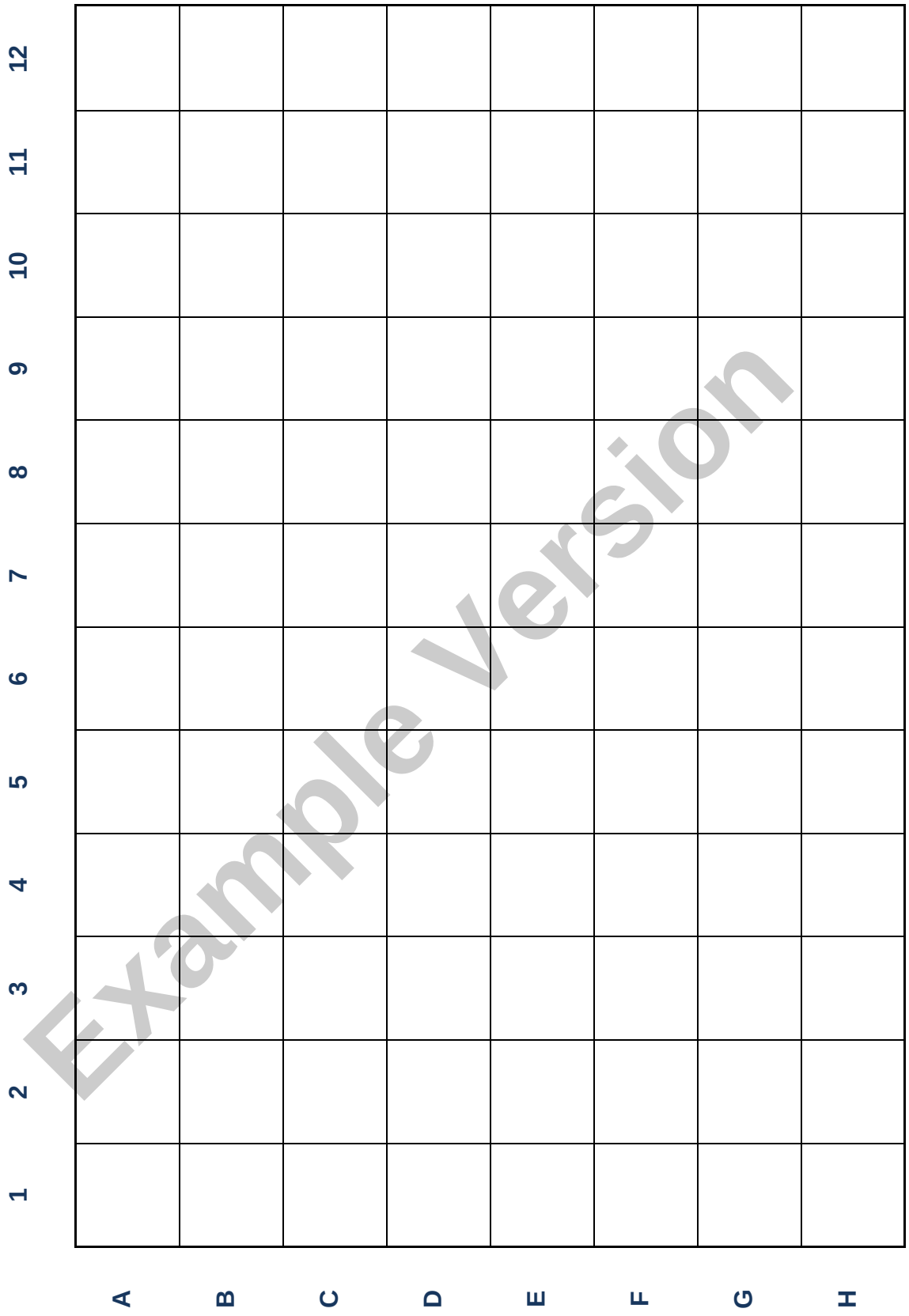
1. Aldag J, Persson T, Hartmann RK: 2´-Fluoro-Pyrimide-Modiefied RNA Aptamers Specific for Lipopolysaccharide Binding Protein (LBP). J of Molecular Science. 19 (2018)
2. Trojanova I, Kozarova M, Petrasova D, Malachovska Z, Paranicova I, Joppa P, Tkacova R: Circulating Lipopolysaccharide-Binding Protein and Carotid Intima-Media Thickness in Obstructive Sleep Apnea. J of Physiological Research. 67: 69-78 (2018)
3. Kong Y, Li Z, Tang T, Wu H, Liu J, Gu L, Zhao T, Huang Q: The level of lipopolysaccharide-binding protein is elevated in adult patients with obstructive sleep apnea. J of Pulmonary Medicine. 18 (2018)
4. Kopp F, Kupsch S, Schromm AB: Lipopolysaccharide-binding protein is bound and internalized by host cells and colonizes with LPS in the cytoplasm: Implications for a role of LBP in intercellular LPS-signaling. J of Biochimica et Biophysica Acta. 1863: 660-672 (2016)
5. Pal GD, Shaikh M, Forsyth CB, Ouyang B, Keshavarzian A, Shannon KM: Abnormal lipopolysaccharide binding protein as marker of gastrointestinal inflammation in Parkinson disease. J of Neuroscience. 9 (2015)
6. Krasity BC, Troll JV, Weiss JP, McFall-Ngai MJ: LBP/BPI proteins and their relatives: Conservation over evolution and roles in mutualism. J of Biochem Soc Trans. 39 (4): 1039-1044 (2011)
7. Prucha M, Herold I, Zazula R, Dubska L, Dostal M, Hildebrand T, Hyanek J: Significance of lipopolysaccharide-binding protein (an acute phase protein) in monitoring critically ill patients. J of Critical Care. 7 (6): 154-159 (2003)
8. Gutsmann T, Müller M, Carroli SF, MacKenzie RC, Weise A, Seydel U: Dual Role of Lipopolysaccharide (LPS)-Binding Protein in Neutralization of LPS and Enhancement of LPS-Induced Activation of Mononuclear Cells. J of Infection and Immunity. 69 (11): 6942-6950: (2001)
9. Vesy CJ, Kitchens RL, Wolfbauer G, Albers JJ, Munford RS: Lipopolysaccharide-Binding Protein and Phospholipid Transfer Protein release Lipopolysaccharides from Gram-Negative Bacterial Membranes. J of Infection and Immunity. 68 (5): 2410-2417 (2000)

15. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

16. ASSAY PROCEDURE - SUMMARY





BioVendor R&D®

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com