cellsciences.com

Mouse Lipopolysaccharide Binding Protein (LBP) ELISA Kit

Catalog No.: CKM043 **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 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. This kit is also useful for detection of rat LBP.

The mouse LBP Kit is a solid phase sandwich Enzyme Linked-Immunosorbent Assay (ELISA). Monoclonal antibody specific for mouse LBP is used on well of the pre-coated plate. In the first step, the pre-coated wells will be incubated with the antigen (standard or sample). During this incubation, mouse LBP is captured by solid bound antibody. Unbound material present in the sample is removed by washing. Next the plate is incubated with a POD-labelled antibody specific for mouse 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 spectro-photometer. A standard curve is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The mouse LBP concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

KIT COMPONENTS:

1	Pre-coated ELISA 96 well plate	1
Vial 2	Detection antibody (HRP-labelled monoclonal antibody to mouse LBP) “Ready to use”	1 vial
Vial 3	Mouse LBP-standard (TBD µg/mL)	1 vial
Vial 4	Mouse reference serum (TBD ± µg/mL)	1 vial
Vial 5	PBS	2 tablets
Vial 6	Dilution buffer	1 vial
Vial 7	Tween 20	1 vial
Vial 8	Stop solution “Ready to use”	1 vial
Vial 9	Substrate solution “Ready to use”	1 vial

Note: Vials 3 and 4 are lyophilized.

STORAGE:

Store the kit at 2-8°C until the expiration date.
Store detection monoclonal antibody (vial 2) at 2-8°C.
For long-term storage of the Mouse LBP standard and the reference serum (vials 3 and 4), store at -20°C or -80°C.
For storage of prepared regents, see “Preparation of Reagents” section.

MATERIAL REQUIRED BUT NOT PROVIDED:

- orbital shaker
- microplate reader for measurement of absorbance at 450 nm/620 nm
- precision pipettes with disposable tips

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

- 10-1000 µL adjustable multi-well pipettes

PREPARATION OF REAGENTS:

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 2-8 °C.

B. PBS:

Dilute 1 tablet of Vial 5 in 200 mL distilled water.

C. Dilution Buffer: prepare just before use

Dissolve content of Vial 6 with 50 mL PBS (Buffer B).
Prepared Dilution Buffer is stable at -20 °C for 1-2 weeks.
Note: Use buffer for assay at room temperature.

D. Substrate:

Vial 9 - Ready to use. Mix gently.

E. Detection Antibody:

Vial 2 - Ready to use. Mix gently.

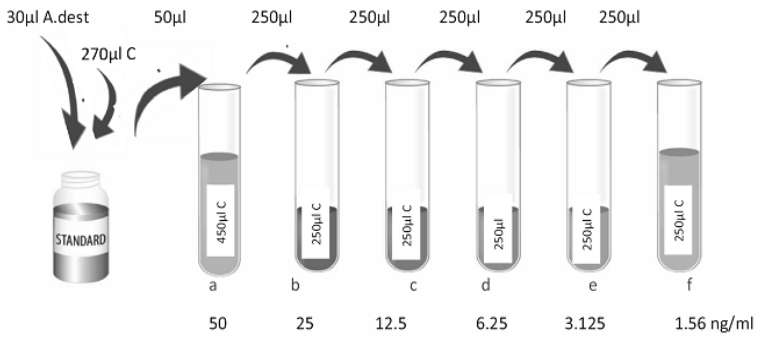
F. Reference Serum:

Add 10 µL distilled water to Vial 4.
The reference serum contains 12.14 ± 3.5 µg/mL LBP.
For assay, dilute 1:800 (10 µL serum + 7990 µL dilution buffer) and use 100 µL/well.
Reconstituted reference serum is stable for one week at 2-8 °C.

G. Mouse LBP Standard: prepare just before use

Pipette 30 µL distilled water to Vial 3 for reconstitution. Then, add 270 µL dilution buffer (C), for a total volume of 300 µL. Mix gently. *Store the standard at -20 °C.*
Transfer 50 µL of this reconstituted LBP standard to a new vial and add 450 µL dilution buffer (C). This is "vial a" of the standard dilutions, containing 500 µL at 50 ng/mL LBP.
For the standard curve, prepare vials b-f.

Vial	Mouse LBP	Dilution buffer C	Concentration (ng/mL)
a	500 µL	0	50
b	250 µL of vial a	250 µL	25
c	250 µL of vial b	250 µL	12.5
d	250 µL of vial c	250 µL	6.25
e	250 µL of vial d	250 µL	3.125
f	250 µL of vial e	250 µL	1.56



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

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

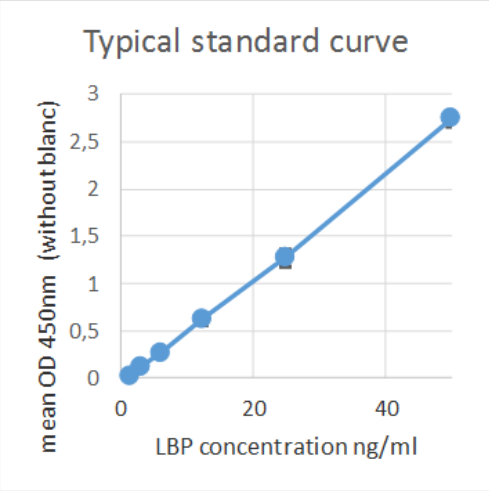
PREPARATION OF SAMPLES

Serum, plasma and other mouse LBP containing solutions, as well as recombinant LBP solutions are suitable for use in the test. Samples containing a visible precipitate must be clarified prior to use in the assay. Lipemic and hemolytic probes are not possible. Samples should be frozen at -20 °C for long term storage.

Depending on the concentration of mouse LBP in the samples, these have to be diluted with dilution buffer. For normal mouse serum samples, a dilution of 1:800 is recommended. For rat serum, use 1:50 to 1:200.

ASSAY CHARACTERISTICS

Normal LBP range in untreated mice: 2-15 µg/mL. (Acute phase sera may contain a factor of 10 to 100 fold more LBP.)
Interassay CV: 7% to 13.6% depending on the concentration
Intrassay CV: 2.4%, n = 50 plasma samples
Effective range: 1 - 50 ng/mL
Cross-reactivity: rat LBP
Specificity: detects free and bound LBP
Recovery of recombinant LBP in LBP-depleted sera is 100%.



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, 1.56 ng/mL = vials a-f), reference serum or diluted samples in duplicate into the corresponding wells of the pre-coated plate. Incubate for 1 hour at room temperature using shaker (300 rpm).

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

3. Detection antibody
Add 100 µL detection antibody (E, vial 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, vial 9) to each well. Incubate for **12-14 minutes** in the dark at room temperature without shaking.

6. Stopping
Pipette 100 µL stop solution (Vial 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 (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 by the dilution factor.

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com