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UC Davis - Lipoprotein Binding Protein (LBP)-Endotoxemia Assay

Trina Knotts¹

¹University of California, Davis

Mouse Metabolic Phenotyping Centers

Tech. support email: info@mmpc.org

Lili Liang

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Protocol status: Working
We use this protocol and it's working

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21001

ABSTRACT

Summary:

Plasma samples will be assayed for lipopolysaccharide binding protein (LBP) as surrogate for bacterial LPS/measure of endotoxemia via ELISA.

MATERIALS

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⊗ Mouse LBP ELISA kit Biometec Catalog
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⊗ Shaker Contributed by users

⊗ Microplate spectrophotometer Contributed by users

⊗ MB grade water (diluent) Contributed by users

Reagent Preparation: *(According to the manufacturer.)*

Wash Buffer (PBS/ Tween 0.05%):

Dissolve 1 Tablet Phosphate buffered saline (PBS, vial 5) in 200ml distilled water - add 100 µl Tween 20 (vial 7). (Prepared wash buffer is stable for 4 weeks at refrigerator).

Phosphate Buffered Saline (PBS):

Dilute 1 Tablet of vial 5 in 200 ml distilled water

Dilution Buffer:

Add content of the vial 6 to 50ml PBS (Buffer C). Prepare just before use. Store remaining dilution buffer after reconstitution at -20°C

Reference serum dilution:

Add 10 µl distilled water to the vial 4. This contains 12.14 ± 3.5µg/ml LBP (! new

Keywords:
lipopolysaccharide binding
protein (LBP)

Reference for Lot #141016). For assay dilute 1:800 (10µl serum +7990µl dilution buffer and use 100µl/well.

LBP standards :

Firstly, pipette 30 µl distilled water to the vial 3 for reconstitution and secondly add 270µl dilution buffer (C) to this vial and mix carefully, thirdly pipette 50µl from this vial to a new vial containing 450µl dilution buffer (C) and mix carefully. Finally this last vial contains 500µl standard dilution and containing 50ng/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.

BEFORE START INSTRUCTIONS

IMPORTANT: Check kit datasheet for lot-specific instructions that may modify general protocol.

- 1 Prepare kit reagents.
- 2 Dilute mouse plasma or serum samples 1:800.
- 3 Add 100 µl of standards (50, 25, 12.5, 6.25, 3.12, 1.56 ng/ml) or diluted samples in duplicate into the corresponding wells of the precoated modules and incubate for one hour at room temperature and shaking (300rpm).

- 4 Wash 3X with Wash Buffer.
- 5 Add 100 µl detecting antibody to each well and incubate at room temperature for 1 hour at shaker.
- 6 Wash 3X with Wash Buffer.
- 7 Add 100 µl Substrate solution to each well. Incubate 12-14 min **in the dark** at room temperature **without** shaking. Cover with foil during incubation or place in drawer.
- 8 Add 100 µl stopping solution to each well. Tap gently to mix.
- 9 Read absorbance at 450 nm (reference wave length 620 nm)
- 10 Calculate the LBP concentration by first plotting the OD means of standards (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.