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

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

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Abstract

Summary:

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

Materials

MATERIALS

- Mouse LBP ELISA kit **Biometec Catalog** ##43
- Shaker
- Microplate spectrophotometer
- MB grade water (diluent)

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

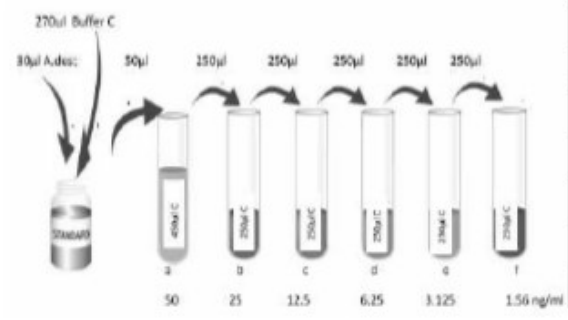
Wash Buffer (PBS/ Tween 0.05%):
Dissolve 1 Tablet Phosphate buffered saline (PBS, vial 5) in 200ml distilled water -add 100 µl Tween 20 (vial 7). (Prepared wash buffer is stable for 4 weeks at refrigerator).

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 -20ºC

Reference serum dilution:
Add 10 µl distilled water to the vial 4. This contains 12.14 ± 3.5µg/ml LBP (! new 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 -20ºC.



No	Mouse LBP µl	Dilution buffer C	Conc. ng/ml
vial a	500 µl	0	50
vial b	250 µl of vial a	250 µl	25
vial c	250 µl of vial b	250µl	12.5
vial d	250 µl of vial c	250 µl	6.25
vial e	250 µl of vial d	250 µl	3.125
vial f	250 µl of vial e	250 µl	1.56

Troubleshooting

Before start

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.