D6002- Lipopolysaccharide Binding Protein (LBP)- Endotoxemia Assay

Version: 1.1
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Summary: Plasma samples will be assayed for lipopolysaccharide binding protein (LBP) as surrogate for bacterial LPS/measure of endotoxemia via ELISA.

Reagents and Materials: *(prepared according to manufacturer)*

Reagent/Material	Vendor	Stock Number
Mouse LBP ELISA kit	Biometec	#043
Shaker		
Microplate spectrophotometer		
MB grade water (diluent)		

Protocol: *(performed according to manufacturer's 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.

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:

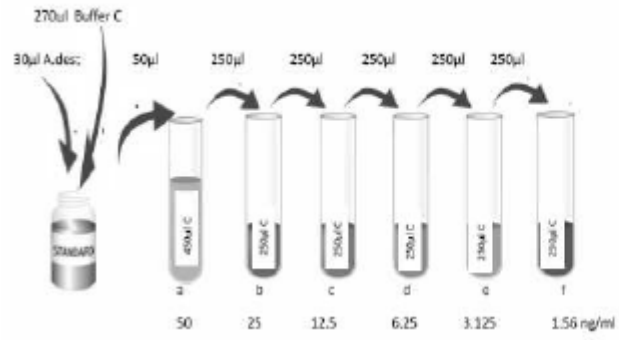
Add content of the vial 6 to 50ml PBS (Buffer C). Prepare just before use. Store remaining dilution buffer after reconstitution at -20^{o}C

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**