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

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SOP for LBP ELISA after DSS-induced injury

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ABSTRACT

SOP for LBP ELISA after DSS-induced injury

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Protocol

1 Concentrations of plasma LPS-binding protein (LBP) were examined via Mouse LBP SimpleStep ELISA according to the manufacturer's protocol (Abcam, #ab269542).

2 Plasma samples were prepared at a 1:16,000 dilution.

3 Standards, antibody cocktail containing detector and capture antibodies, and 1x wash buffer were prepared according to the manufacturer's protocol.

4 In the plate, 50µL of standard or sample was added to appropriate wells in duplicate.

5 50µL of antibody cocktail was added to all wells.

6 The plate was then incubated at RT for 1 hour on a plate shaker at 400 rpm.

7 Wells were decanted and washed three times with 150µL 1x wash buffer.

8 100µL of TMB Development Solution was added to each well and allowed to incubate in the dark for 12 minutes with shaking at 400rpm

9 100µL of Stop Solution was added to each well and placed on the shaker for 1 minute at 400 rpm to mix.

10 Absorbance of 450nm light was recorded with a FLUOstar Omega microplate reader (BMG Labtech).

11 The standard curve was created by plotting the blank-subtracted average LBP standard absorbance against the standard LBP concentrations.

12 Sample LBP concentrations were calculated by interpolating the blank-subtracted average absorbance values against the standard curve, multiplied by the dilution factor.