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SOP for LBP ELISA after DSS-induced injury

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Protocol status: Working

We use this protocol and it's working

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Abstract

SOP for LBP ELISA after DSS-induced injury

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.