

## Whole blood assay - LPS

**Aim:**

To induce sufficient levels of IL8 and prostanoids from patient (SLE, IIM) whole blood to be able to see a suppressive effect of added SGC probes, chemicals and blocking antibodies.

Reagent	Final concentration in cell culture	Stock konc	Vendor/provider
LPS	10 ug/ml	1mg/ml	Sigma cat#L6529
IL8 ELISA kit			MabTech cat#3560-1H

**Procedure:**

1. Dilute probes (typically 1 or 0,1uM final dilution) and DMSO (0,01 and 0,001% final dilution) in PBS and dispense 25ul/well into 96 well U-bottomed cell culture plates, triplicate wells
2. Pipette 225ul whole heparinized blood/well
3. Preincubate the plate 30 min at 37C 5% CO<sub>2</sub>
4. Dilute LPS in PBS and add 10ul/well, final concentration 10ug/ml
5. Incubate the plate 24hr at 37C 5% CO<sub>2</sub>
6. Centrifuge the plate 3000g collect supernatants.
7. Freeze the supernatants at -20C until analysis of IL8 concentration using ELISA, and for prostanoid concentration using mass spectrometry