Core E - 10% SERUM Kdo_2-LIPID A TREATMENT PROTOCOL
(Edited from Protocol ID PP0000001002, 01-18-06, by Donna Reichart)
02/10/2006
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1. See Figure 1 of PP000001002 for an overview of the time course of the 10% Serum Kdo_2-lipid A treatment.
2. Maintain sterile technique throughout the Kdo_2-lipid A treatment procedure until cell harvest.
3. Plate cells for each sample in a time course experiment from the *Core E - Thawing and Passage Procedure* step 10. Core E uses 100 mm plates: 5 x 10^6 / 10 mL media to achieve 80% confluence in approximately 30 hr. Kdo_2-lipid A treated cells are assayed at incubation (37 °C) times of 0.5, 1, 2, 4, 8, 12 and 24 hours following addition of Kdo_2-lipid A. Control cells are assayed at time 0 (no additions) and at 0.5, 1, 2, 4, 8, 12 and 24 hours following addition of DPBS (15 samples for n=1). In the LIMS *Treatment* application enter the cell vessel barcode that was assigned at Step 10 of the Core E passage procedure and generate an Experiment ID from the application. Enter the number of samples required for the experiment and label each plate with the LIMS generated sample ID.
4. Incubate for 24 hours at 37 °C.
5. Spray the 1.5 mL microcentrifuge tube containing the freshly sonicated Kdo_2-lipid A 1000x (100 μg/mL) working solution (PS0000001401) with 70% ethanol and let air dry before using.
6. Add 4.5 μL of the Kdo_2-lipid A 1000x working solution to the treatment plates for a final concentration of 100 ng/mL and 4.5 μL of DPBS to control plates.
7. Incubate for 0, 0.5, 1, 2, 4, 8, 12, and 24 hours at 37°C.
8. Immediately after removing each plate from 37 °C incubator and before harvesting the cells, remove an aliquot (0.5 mL) of medium for the TNFα assay, place in a labeled 1.5 mL microcentrifuge tube and set aside. After harvesting the cells, spin the TNFα aliquots at 500x*g* for 3 min, collect an aliquot of supernatant (~0.3-0.4 mL) and place in a new 1.5 mL microcentrifuge tube. Label the samples for TNFα assay with the barcode ID for each vessel. Print the labels from this procedure and edit the first or second line to include the notation "TNF". Freeze the aliquots at -20 °C.

   TNFα assay:

   The TNFα aliquots should not be diluted before assaying, but controls and treated samples should be labeled as such. Send frozen aliquots to ElisaTech, 12635 E. Montview Blvd., Suite 216, Aurora, CO 80010.

   For in-house analysis, use the Quantikine mouse TNFα/TNFSF1A EIA kit (R&D Systems, Cat. #MTA00).

9. After collecting the aliquot for the TNFα assay, place the plate on ice, aspirate the medium, and gently wash the plate 2x with 3 mL of 4 °C DPBS.
10. Add another 0.5 mL of 4 °C DPBS and scrape the cells with a scraper.
11. Pipette the cell suspension into a glass tube (13x100 mm) with a Teflon-lined screw cap for analysis of neutral lipids. Label each tube with the same sample barcode that was used for the corresponding plate.
12. Remove a 50 μL aliquot for DNA analysis and cell counting. Label the samples for DNA assay and cell counting with the barcode ID for each vessel. Print the labels from this procedure after editing the first or second line to include the notation "DNA". Aliquots can be frozen for later DNA analysis. Follow the DNA assay protocol in the Molecular Probes manual.
13. Immediately add 1.25 mL of ice-cold methanol to each tube.
14. To each sample and control in a glass tube with Teflon-lined screw cap, add 100 μL of the deuterium labeled internal standard solution (prepared using the protocol *Core E - Neutral Lipid Internal Standard*).
15. Vortex for 10 seconds.
16. Store at 4 °C until all time points are complete.