Core E - 0.5% SERUM Kdo_2-LIPID A TREATMENT PROTOCOL
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1. See Figure 1 of PP000000501 for an overview of the time course of the protocol.
2. Maintain sterile technique throughout the protocol until harvesting.
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.
4. In the LIMS *Treatment* application enter the cell vessel barcode (with a "B" suffix for use in an experimental procedure) that was assigned at Step 10 of the cell passage protocol 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. (A typical time course with n=1 requires 15 IDs for samples and controls.)
5. Make up Serum Deprivation Medium (DMEM, 0.5% LIPID MAPS FBS; LIPID MAPS solution protocol PS0000001000). Enter the barcode of the medium used in the comment section of the LIMS *Treatment* application.
6. 30 hours after plating, rinse the vessels one time with 37 °C DPBS.
7. Add a volume of fresh 37 °C Serum Deprivation Medium.
8. Incubate for 18 hours at 37 °C.
9. Spray the 1.5 mL microcentrifuge tube containing the freshly sonicated 1000x working solution (100 μg/mL) of Kdo_2-lipid A with 70% ethanol and let air dry before opening in the laminar flow hood.
10. Add the Kdo_2-lipid A to the medium, for a final concentration of 100 ng/mL. Add an equal amount of DPBS to controls.
11. Incubate samples and controls at 37 °C and harvest cells at specified points of the time course (see following steps for harvest procedure).
12. Immediately after removing the plate from 37 °C and before harvesting the cells, remove an aliquot (1 mL) of medium from each sample for a TNFα assay. 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.

13. Place vessels on ice, aspirate, and wash each vessel 2 times with 3 mL of 4 °C DPBS.
14. Add 1 mL of 4 °C DPBS and scrape the cells with a scraper.
15. 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.
16. Remove a 100 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 and edit 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.
17. Immediately add 2.5 mL of ice-cold methanol to each tube.
18. 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*).
19. Vortex for 10 seconds.
20. Store at 4 °C until all time points are complete.