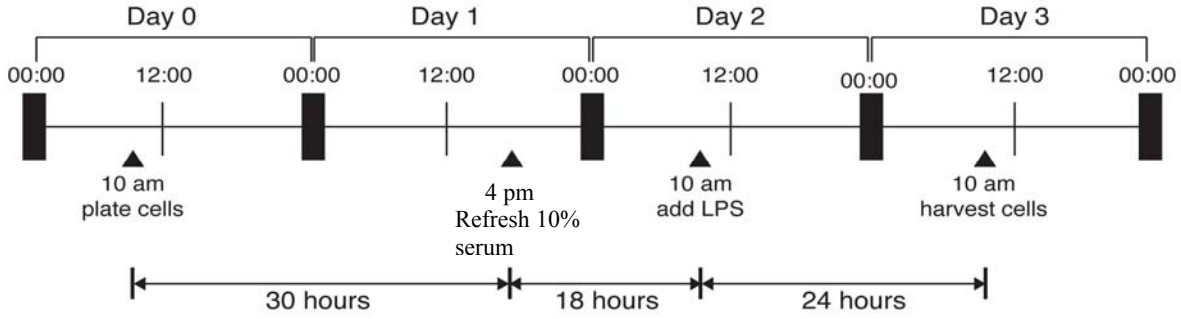
10% SERUM Kdo_2 LIPID A TREATMENT PROTOCOL
Version 3, 8-26-05

1. See Figure 1 for an overview of the time course of the Kdo_2 induction protocol.
2. Maintain sterile technique throughout the Kdo_2 induction protocol until harvesting.
3. Plate cells as recommended on the LIPID MAPS Thawing and Passage Procedure in growth medium to achieve 80% confluence at 30 hours following plating.
4. Assign a barcode to each plate/vessel and enter into LIMS.
5. Thirty hours after plating, rinse the vessels 1 X with 37˚ C DPBS.
6. Add a volume of fresh 37˚ C growth medium.
7. Incubate for 18 hours at 37˚ C.
8. Immediately after removing the plate from 37˚ C and before treating the cells, take an aliquot (1 ml) of medium from each condition for the TNFα assay. Assign a barcode, enter into LIMS and freeze the aliquots at -20˚ C.
9. Spray the Eppendorf containing the freshly sonicated 1000x working solution (100 μg/ml) of Kdo_2 lipid A with 70% ethanol and let air dry before using.
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 for 24 hours at 37˚ C.
12. Immediately after removing the plate from 37˚ C and before harvesting the cells, take an aliquot (1 ml) of medium from each condition for the TNFα assay. Assign a barcode, enter into LIMS and freeze the aliquots at -20˚ C.
    For the TNFα assay;
    The TNFα aliquots from the Kdo_2 induced cells must be diluted in medium 1:40 and 1:80 before assaying. Do not dilute aliquots from cells that were not treated with Kdo_2 lipid A. 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 X with an appropriate volume of 4˚ C DPBS.
14. Add another fresh volume of 4˚ C DPBS and scrape the cells with a scraper (see equipment list).
15. Pipet the cell suspension into an appropriate tube for either direct lipid extraction or centrifugation. Assign a barcode and enter into LIMS.
16. Suspend the cells and take an aliquot, e.g., 200 μl for 20 μl duplicates, for DNA analysis. Assign a barcode and enter into LIMS. Aliquots can be frozen for later DNA analysis. Follow the DNA assay protocol in Molecular Probe's manual with the exception of using 5 μl of standards instead of 10 μl. If you anticipate having a lower DNA concentration, use less standard for your DNA curve.
17. Cells can now be extracted directly or spun down for extraction of cell pellets. To centrifuge cells, spin the cell suspension at 2000 rpm for 5-10 minutes at 4˚ C.

Figure 1


Day 0
Day 1
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Day 3
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10 am
plate cells
4 pm
Refresh 10%
serum
10 am
add LPS
10 am
harvest cells
30 hours
18 hours
24 hours