LPS SOLUBILIZATION PROTOCOL
Version 2, 4-22-05

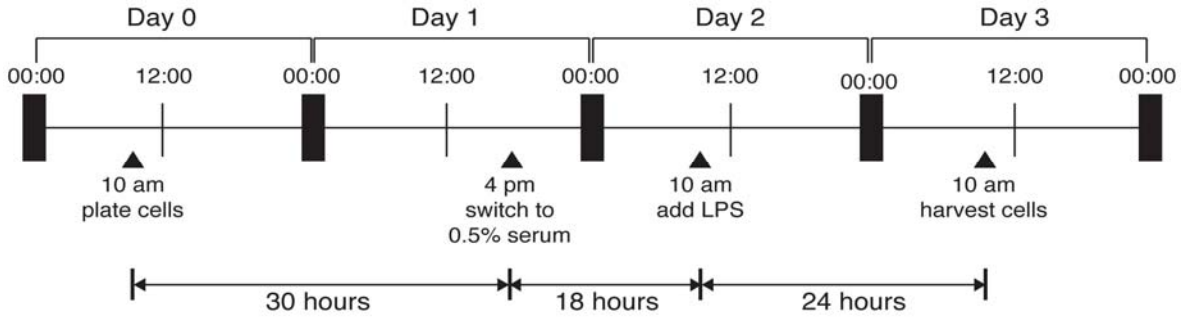
1. Dissolve the KDO Lipid A in sterile DPBS to a final concentration of 1mg/ml.
2. Sonicate in a bath sonicator (NOT with tip sonicator) for 5 minutes. Solution will appear uniformly opalescent.
3. Transfer to a 1.5 ml Eppendorf tube. This is the stock KDO Lipid A solution. Assign a barcode and enter into LIMS. This 1 mg/ml stock solution can be stored at -20^{o} C. This 1 mg/ml stock solution must be sonicated as in step 2 prior to making a working 1000x solution.
4. Make up a working 1000x solution in a 1.5 ml Eppendorf tube. Dilute one part of the KDO Lipid A 1 mg/ml stock solution with 9 parts DPBS (final concentration 100 μg/ml). Assign a barcode and enter into the LIMS. This working solution can be stored at -20^{o} C. This 100 μg/ml working solution must be sonicated as in step 2 above prior to addition to cells.

LPS INDUCTION PROTOCOL

1. See Figure 1 for an overview of the time course of the LPS induction protocol.
2. Maintain sterile technique throughout the LPS 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. Make up Serum Deprivation Media (DMEM, 0.5% LIPID MAPS FBS, see table below). Assign a barcode to each bottle and enter into LIMS
6. Thirty hours after plating, rinse the vessels 1 X with 37˚ C DPBS.
7. Add a volume of fresh 37˚ C Serum Deprivation medium.
8. Incubate for 18 hours at 37˚ C.
9. 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.
10. Spray the Eppendorf containing the freshly sonicated 1000x working solution (100 ug/ml) of KDO lipid A with 70% ethanol and let air dry before using.
11. Add the KDO Lipid A to the medium, for a final concentration of 100ng/ml. Add an equal amount of DPBS to controls.
12. Incubate for 24 hours at 37˚ C.
13. 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 LPS 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 lipid A. Send frozen aliquots to ElisaTech, 12635 E. Montview Blvd., Suite 216, Aurora, CO 80010.

14. Place vessels on ice, aspirate, and wash each vessel 2 X with an appropriate volume of 4˚ C DPBS.
15. Add another fresh volume of 4˚ C DPBS and scrape the cells with a scraper (see equipment list).
16. Pipet the cell suspension into an appropriate tube for either direct lipid extraction or centrifugation. Assign a barcode and enter into LIMS.
17. 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 ul of standards instead of 10 ul. If you anticipate having a lower DNA concentration, use less standard for your DNA curve.
18. 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



RAW 264.7 Tissue Culture Reagents – Serum Deprivation Media

Reagent	Source	Catalog No.	F.W. or Stock Conc.	Quantity	Final Conc.
Dulbecco's Modified Eagle's Medium (DMEM)	Cellgro	10-013	1X DMEM with 4.5g/l Glucose and 4mM L-Glutamine	497.5 ml	
Heat-inactivated fetal calf serum (FCS)	Hyclone	SH30071.03 ANG19242	100%	2.5 ml	0.5%

To heat-inactivate the serum:
1. Thaw at 4° C.
2. Heat at 56° C for 30 minutes.
3. Aliquot serum in 50 ml tubes and store at 4° C until use.

CELLGRO 800-235-5476
HYCLONE 800-492-5663