10% SERUM Kdo_2-LIPID A TREATMENT PROTOCOL

LIPID MAPS Protocol ID PP0000001002
01-18-06

1. See Figure 1 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 harvesting.
3. Plate 2 x 10^6 cells per 60 mm plate in 5 ml of RAW 264.7 Growth Medium 1 (PS0000000800) as recommended on the LIPID MAPS Thawing and Passage Procedure (PP0000000101). Kdo2-lipid A treated cells will be assayed at 0.5, 1, 2, 4, 8, 12 and 24 hours following addition of Kdo2-lipid A. Control cells will be assayed at time 0 (no additions) and at 0.5, 1, 2, 4, 8, 12 and 24 hours following addition of DPBS (15 conditions in triplicate = 45 plates total). Assign a barcode to each plate and enter into LIMS.
4. Incubate 24 hours at 37˚C.
5. Spray the Eppendorf containing the freshly sonicated Kdo_2-lipid A 1000x (100 µg/ml) working solution (PS0000001400) with 70% ethanol and let air dry before using.
6. Immediately after removing each plate from 37˚C and before treating the cells, take an aliquot (0.5 ml) of medium for the TNFα assay, place in a labeled Eppendorf tube and set aside. After beginning the Kdo_2–lipid A time course, spin the TNFα aliquots at 500 g for 3 min, collect an aliquot of supernatant (~0.3-0.4 ml) and place in a new labeled Eppendorf tube. Assign a barcode, enter into LIMS and freeze the aliquots at 20˚C.
7. 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.
8. Incubate for 0, .5, 1, 2, 4, 8, 12 and 24 hours at 37˚C.
9. Immediately after removing each plate from 37˚C and before harvesting the cells, take an aliquot (0.5 ml) of medium for the TNFα assay, place in a labeled Eppendorf tube and set aside. After harvesting the cells, spin the TNFα aliquots at 500 g for 3 min, collect an aliquot of supernatant (~0.3-0.4 ml) and place in a new labeled Eppendorf tube. Assign a barcode, enter into LIMS and freeze the aliquots at -20˚C

   For the TNFα assay;

   The TNFα aliquots from the Kdo_2-lipid A-treated cells must be diluted in medium at least 1:80 before assaying. ElisaTech will dilute the samples, if requested. Do not dilute aliquots from cells that were not treated with Kdo_2-lipid A. Send frozen labeled aliquots to ElisaTech, 12635 E. Montview Blvd., Suite 215, Aurora, CO 80010. For assaying in house, use the Quantikine mouse TNFα/TNFSF1A EIA kit (R&D Systems, Cat. #MTA00).
10. 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.

11. Add another 3 ml of 4˚C DPBS and scrape the cells with a scraper (see equipment list).
12. Pipette the cell suspension into an appropriate tube for either direct lipid extraction or centrifugation. Assign a barcode and enter into LIMS.
13. 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 at -20˚C for later DNA analysis in the presence of 5% v/v of 50% EtOH (HPLC grade)/ sterile water. Follow the DNA assay protocol in Molecular Probes' 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.
14. Cells can now be extracted directly or spun down for extraction of cell pellets. To centrifuge cells, spin the cell suspension at 500 g for 3 min at 4˚C.

Figure 1

10% serum protocol

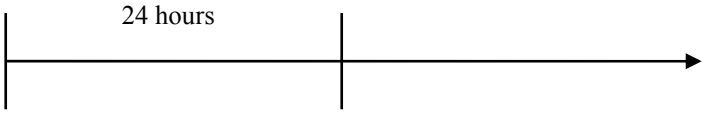

Plate 2 x 10^6 cells	Kdo_2-lipid A
60 mm plate	Time course
10% serum	0-24 hours

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Date: 1-18-06