

10% SERUM Kdo₂-LIPID A TREATMENT PROTOCOL

LIPID MAPS Protocol ID PP0000001001
12-7-2005

1. See Figure 1 for an overview of the time course of the 10% Serum Kdo₂-lipid A treatment.
2. Maintain sterile technique throughout the Kdo₂-lipid A treatment procedure until harvesting.
3. Plate 2×10^6 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). Make triplicate plates for each condition at each time point, starting with the 0 time point and concluding with the 24 hour time point. 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 Kdo₂-lipid A 1000x (100 µg/ml) working solution (PS0000001400) with 70% ethanol and let air dry before using.
6. Immediately after removing the plate from 37°C and before treating the cells, take an aliquot (.5 ml) of medium from each plate for the TNFα assay, place in labeled Eppendorf tubes and set aside. After beginning the Kdo₂-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 Kdo₂-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 the plate from 37°C and before harvesting the cells, take an aliquot (.5 ml) of medium from each plate for the TNFα assay, place in labeled Eppendorf tubes 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

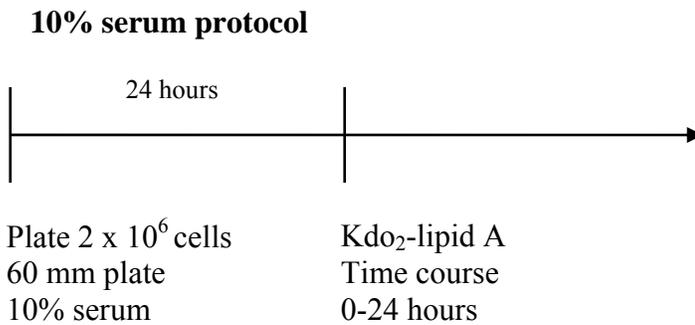
For the TNFα assay;

The TNFα aliquots from the Kdo₂-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 Kdo₂-lipid A. Send frozen labeled aliquots to ElisaTech, 12635 E. Montview Blvd., Suite 216, 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 plates on ice, aspirate the medium, and gently wash each plate 2 x 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 μ l for 20 μ l 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 Probe's manual with the exception of using 5 μ l of standards instead of 10 μ l. 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



Author: Donna Reichart
Date: 12-12-05