PROTOCOL FOR Kdo_2-LIPID A TREATMENT OF THIOGLYCOLLATE AND BONE MARROW DERIVED MACROPHAGES

LIPID MAPS Protocol ID PP0000001801
06-07-07

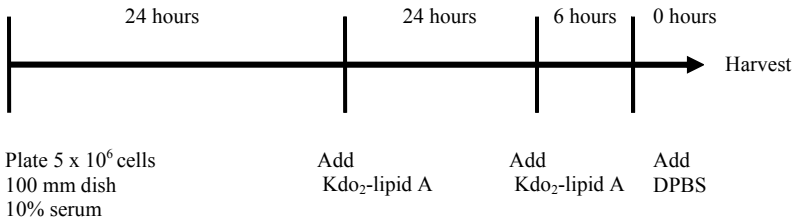
This protocol is an updated version of Protocol PP0000001800, titled "Kdo_2 Treatment of Primary Macrophages" and thus maybe considered as "version 2".

1. See Figure 1 for an overview of the Kdo_2-lipid A treatment protocol.
2. Maintain sterile technique throughout the Kdo_2-lipid A treatment procedure until harvesting.
3. Plate 5 x 10^6 cells per 100 mm dish in 7 mL 37˚C Primary Macrophage Growth Medium 1 (PS0000001700) or 7 mL 37˚C Bone Marrow Derived Growth Medium 1 (PS0000002900).
4. Incubate 24 hours at 37˚C.
5. Before Kdo_2 treatment, aspirate the medium from each plate and add fresh 37˚C medium.
6. Spray the Eppendorf tube containing freshly-sonicated Kdo_2-lipid A 1000x (100 μg/ml) working solution (PS0000001400) with 70% ethanol and let air dry.
7. Treat the cells with 7 μl Kdo_2-lipid A 1000x working solution for a final concentration of 100 ng/ml starting at the 24 hour time point. 18 hours later, treat the 6 hour time point. At 0 hour, add 7 μl DPBS to the 0 hour time point and harvest all. Assign a barcode to each plate and enter into LIMS.
8. 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).
9. After collecting the aliquot for the TNFα assay, place the plate on ice, aspirate the medium, add 0.5 mL 4˚C methanol or DPBS for scraping the cells and 0.5 ml methanol or DPBS for rinsing the cells.
10. Pipette the cell suspension into an appropriate tube for direct lipid extraction. Assign a barcode and enter into LIMS.

Figure 1



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