Core J Procedure Protocol
Compactin, Kdo2 Lipid A Treatment
in Dye-Free Growth Medium 2008

Setup

1. See Figure 1 for an overview of the treatment time course.
2. See Table 1 for an overview of the four treatment groups.
3. Maintain sterile technique throughout the treatment procedure until harvesting.
4. Plate 2 x 10^6 cells per 60 mm plate in 5 ml of Dye-Free Growth Medium (PS0000002400) as recommended in the LIPID MAPS Thawing and Passage Procedure (PP0000000101). Make triplicate plates for each condition at each time point. Assign a barcode to each plate and enter into LIMS.
5. Incubate 24 hours at 37˚C.

Reagent Preparation

1. Mevalonate 20 mM – prepare from 0.2 M mevalonate (PS0000002200)
2. Compactin 10 mM – thaw from -80˚C
3. Kdo2 Lipid A working solution – prepare from Kdo2 Lipid A stock solution (PS0000001401)
4. Spray the reagent tubes with 70% ethanol and let air dry before using.

Treatment

1. Remove 12 dishes from the incubator and label 24A through 24L.
2. Treat each dish with the appropriate reagents:

Group 1: -C-K	Dishes A, B, C	11.25 µL mevalonate 22.5 µL PBS 4.5 µL PBS
Group 2: +C-K	Dishes D, E, F	11.25 µL mevalonate 22.5 µL compactin 4.5 PBS
Group 3: -C+K	Dishes G, H, I	11.25 µL mevalonate 22.5 µL PBS 4.5 µL Kdo2 Lipid A
Group 4: +C+K	Dishes J, K, L	11.25 µL mevalonate 22.5 µL compactin 4.5 µL Kdo2 Lipid A

3. Note the time and return the dishes to the incubator.
4. Repeat this treatment procedure for every time point, working backwards from 24 hours to 12, 8, 4, 2, 1, and 0.5.
5. For 0 hours, do not treat with anything, not even mevalonate. Label three dishes A, B, and C, take the medium for TNFα, and proceed with harvesting.

Harvest

1. At the appropriate time, remove the dishes from the incubator and place on ice.
2. Before harvesting the cells, remove 1 mL of medium from each plate for the TNFα assay, place in labeled Eppendorf tubes, and place at 4˚C until processing.
3. Aspirate the remaining medium from the dish.
4. Gently wash each plate twice with 2 ml of cold PBS.
5. Add 2 mL of PBS to each dish and scrape the cells with a scraper.
6. Transfer the cells to a 13x100mm glass tube and pipette 5x with a serological pipette to suspend the cells.
7. Remove 100 µL cell suspension to an eppendorf for DNA assay. Store at -80˚C.
8. Centrifuge the cells 200 rpm (1000g) for 5 minutes to pellet.
9. Aspirate medium and freeze cells at -80˚C.

Medium Processing for TNFα Assay

1. Centrifuge the TNFα aliquots at top speed in coldroom microfuge for 2 min, collect an aliquot of supernatant (~0.8 ml) and place in a new labeled Eppendorf tube. Freeze the aliquots at -80˚C.
2. 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).

DNA Assay

1. Follow LIMS protocol PP0000002700.

Figure 1

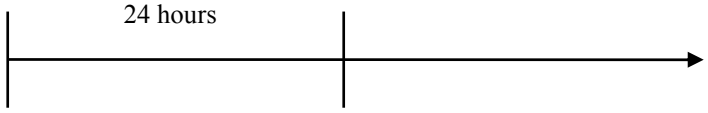

Plate 2 x 10^6 cells
60 mm plate
Dye-Free GMed

Compactin/Kdo2 LipidA
Time course
0-24 hours

Table 1

	Group 1: -C-K	Group 2: +C-K	Group 3: -C+K	Group 4: +C+K
Mevalonate 50 µM	+	+	+	+
Compactin 50 µM	-	+	-	+
Kdo2 LipidA 100 ng/mL	-	-	+	+

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