Core J Procedure Protocol
Compactin, Kdo2 Lipid A Treatment for RNA Harvest

Overview

1. Three experiments will be done for a total of three biological replicates.
2. Untreated cells will be harvested at 0 hours. Treated cells will be harvested at 12 and 24 hours post-treatment. See Figure 1 for an overview of the treatment time course.
3. There will be four different treatments consisting of all the permutations with and without compactin and Kdo2 Lipid A. All four treatments will be supplemented with mevalonate. See Table 1 for an overview of the four treatment groups.

Setup

1. Plate 2 x 10^6 cells per 60 mm plate in 5 mL of RAW Growth Medium (PS0000000901) as recommended in the LIPID MAPS Thawing and Passage Procedure (PP0000000101).
2. Incubate 24 hours at 37˚C.

Reagent Preparation

1. Mevalonate 50 mM – prepare from 0.2 M mevalonate (PS0000002800)
2. Compactin 10 mM – thaw from -80˚C
3. Kdo2 Lipid A working solution – prepare from Kdo2 Lipid A stock solution (PS0000001401)

Treatment

1. Remove 24 dishes from the incubator and label 4 through 27.
2. Treat each dish with the appropriate reagents:

Group 1: -C-K	5 µL mevalonate 50 mM 25 µL PBS 5 µL PBS
Group 2: +C-K	5 µL mevalonate 50 mM 25 µL compactin 10 mM 5 µL PBS
Group 3: -C+K	5 µL mevalonate 50 mM 25 µL PBS 5 µL Kdo2 Lipid A Working Solution
Group 4: +C+K	5 µL mevalonate 50 mM 25 µL compactin 10 mM 5 µL Kdo2 Lipid A Working Solution

3. Note the time and return the dishes to the incubator.
4. For 0 hours, do not treat with anything, not even mevalonate. Label dishes 1 through 6 and proceed with harvesting.

Harvest

1. At the appropriate time, remove the dishes from the incubator and place on ice.
2. Remove 1 mL of medium from each dish for the TNFα assay, place in labeled Eppendorf tubes, and place at 4˚C until processing.
3. Aspirate remaining medium.
4. Gently wash each plate twice with 3 ml of cold PBS.
5. Add 1.5 mL of Trizol to each dish and allow cells to dissolve, 2 to 5 minutes.
6. Transfer the cells in Trizol to a 2 mL Eppendorf tube.
7. Store 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.

Figure 1

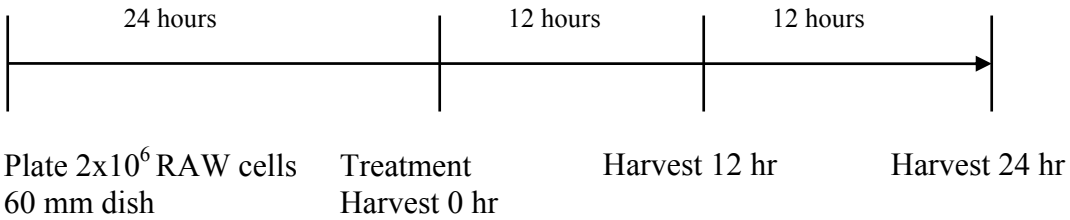

Table 1

	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	-	-	+	+

Table 2

hour	Rx	sample
0	-C-K	1
0	-C-K	2
0	-C-K	3

hour	Rx	sample
12	-C-K	4
12	-C-K	5
12	-C-K	6
12	+C-K	7
12	+C-K	8
12	+C-K	9
12	-C+K	10
12	-C+K	11
12	-C+K	12
12	+C+K	13
12	+C+K	14
12	+C+K	15

hour	Rx	sample
24	-C-K	16
24	-C-K	17
24	-C-K	18
24	+C-K	19
24	+C-K	20
24	+C-K	21
24	-C+K	22
24	-C+K	23
24	-C+K	24
24	+C+K	25
24	+C+K	26
24	+C+K	27

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Date: 5/21/07