Core J Procedure Protocol
Compactin, Kdo2 Lipid A Treatment Modified

Overview

1. One large experiment will be completed to provide treated cells to each of six cores: G, E, H, I, J, and K.
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.
4. Six replicates will be made for each time and treatment. Four aliquots will be generated from each replicate. Every core will receive an aliquot from three of the six replicates for a total of 27 samples per core.
5. Data will be normalized to DNA content (pmol lipid per μg DNA). DNA and TNFa data will be generated by core J and sent to the other cores.

Setup

1. Plate 2 x 10^7 cells per 150 mm plate in 20 mL of Dye-Free RAW Growth Medium (PS0000002400) 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 7 through 30 (12 hour dishes).
2. Treat each dish with the appropriate reagents:

Group 1: -C-K	20 μL mevalonate 50 mM 100 μL PBS 20 μL PBS
Group 2: +C-K	20 μL mevalonate 50 mM 100μL compactin 10 mM 20 μL PBS
Group 3: -C+K	20 μL mevalonate 50 mM 100 μL PBS 20 μL Kdo2 Lipid A Working Solution
Group 4: +C+K	20 μL mevalonate 50 mM 100 μL compactin 10 mM 20 μL Kdo2 Lipid A Working Solution

3. Note the time and return the dishes to the incubator.
4. Repeat this treatment procedure with dishes 31 through 54 (24 hour dishes). For 0 hours, do not treat with anything, not even mevalonate. Label six 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. Remove 5 mL medium to a snap-cap tube and store at -20˚C for core G analysis.
4. Remove remaining medium to 15 mL conical tube and store at -20˚C.
5. Gently wash each plate twice with 5 ml of cold PBS.
6. Add 4 mL of PBS to each dish and scrape the cells with a scraper.
7. Transfer the cells to a 15 mL conical polypropylene tube and suspend cells well.
8. Remove 100 µL of the cell suspension to an eppendorf on ice for DNA assay.
9. Divide the 4 mL cells into aliquots of 1 mL each, placing into appropriate tubes.
10. Pellet the cells by centrifuging at 2000 rpm for 5 minutes.
11. Aspirate the supernatant and snap freeze the pellets in liquid nitrogen.
12. Store frozen pellets 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. TNFα is to be assayed by Jay Westcott at ELISA Tech (Aurora, CO).

Cell Processing for DNA Assay

1. Store at -80˚C.
2. DNA is to be assayed by core J according to the LIPID MAPS DNA Assay (PP0000002700).

All samples will be shipped to their cores on dry ice via Fed-Ex

Table 2 outlines which sample number corresponds to which treatment

Figure 1

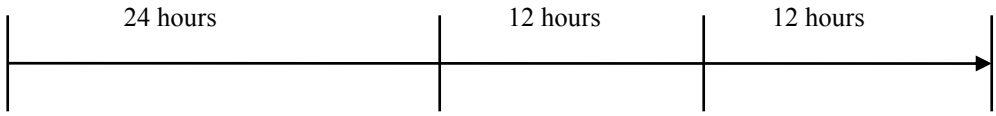

Table 1

	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	rep	#
0	-C-K	A	1
0	-C-K	B	2
0	-C-K	C	3
0	-C-K	D	4
0	-C-K	E	5
0	-C-K	F	6

hour	Rx	rep	#
12	-C-K	A	7
12	-C-K	B	8
12	-C-K	C	9
12	-C-K	D	10
12	-C-K	E	11
12	-C-K	F	12
12	+C-K	A	13
12	+C-K	B	14
12	+C-K	C	15
12	+C-K	D	16
12	+C-K	E	17
12	+C-K	F	18
12	-C+K	A	19
12	-C+K	B	20
12	-C+K	C	21
12	-C+K	D	22
12	-C+K	E	23
12	-C+K	F	24
12	+C+K	A	25
12	+C+K	B	26
12	+C+K	C	27
12	+C+K	D	28
12	+C+K	E	29
12	+C+K	F	30

hour	Rx	rep	#
24	-C-K	A	31
24	-C-K	B	32
24	-C-K	C	33
24	-C-K	D	34
24	-C-K	E	35
24	-C-K	F	36
24	+C-K	A	37
24	+C-K	B	38
24	+C-K	C	39
24	+C-K	D	40
24	+C-K	E	41
24	+C-K	F	42
24	-C+K	A	43
24	-C+K	B	44
24	-C+K	C	45
24	-C+K	D	46
24	-C+K	E	47
24	-C+K	F	48
24	+C+K	A	49
24	+C+K	B	50
24	+C+K	C	51
24	+C+K	D	52
24	+C+K	E	53
24	+C+K	F	54

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