Core J Procedure Protocol
Compactin, Kdo2 Lipid A Treatment 2008

Overview

1. One large experiment will be completed to provide samples to each of seven cores: D, G, E, H, I, J, and K.
2. Untreated cells will be harvested at 0 hours. Treated cells will be harvested as a full time course at 0.5, 1, 2, 4, 8, 12, and 24 hours. 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. Only one replicate will be made for each time and treatment. Ten aliquots will be generated from each replicate. Each core will receive will receive one or two aliquots, depending on their requirements and the growth of the cells.
5. Data will be normalized to DNA content (pmol lipid per µg DNA). DNA and TNFa data will be generated by core J. DNA assay data will be made available through the LIMS Shipment module. TNFa data will be emailed to each core.

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 12 dishes from incubator and label 18 through 29 (8, 12 & 24 hr 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 6 through 17 (1, 2, & 4 hr dishes), then with dishes 2 through 5 (0.5 hr dishes). For 0 hours, do not treat with anything, not even mevalonate. Proceed with harvest.

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 about 12 mL medium to a conical tube and store at -20˚C for core G.
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 10 mL of cold PBS to each dish and scrape the cells with a scraper.
7. Transfer the cells to a 15 mL conical tube on ice and suspend cells well.
8. Remove 100 µL of the cell suspension to an eppendorf on ice for DNA assay.
9. Divide the 10 mL cells into aliquots of 1 mL each, placing into 13x100mm glass tubes with Teflon-lined screw caps.
10. Freeze and store 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. 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

Table 2 outlines which sample number corresponds to which treatment

Figure 1

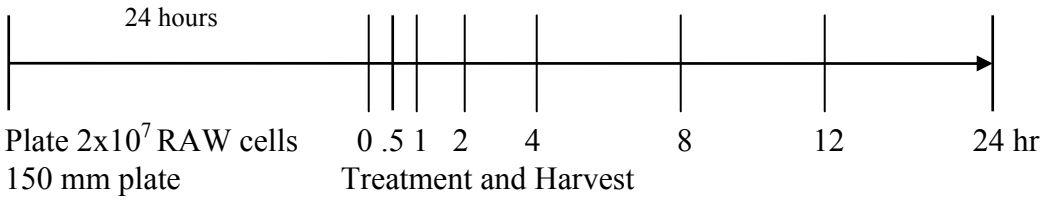

Table 1

	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	Sample #
0	-C-K	1
0.5	-C-K	2
0.5	+C-K	3
0.5	-C+K	4
0.5	+C+K	5
1	-C-K	6
1	+C-K	7
1	-C+K	8
1	+C+K	9
2	-C-K	10
2	+C-K	11
2	-C+K	12
2	+C+K	13

hour	Rx	Sample #
4	-C-K	14
4	+C-K	15
4	-C+K	16
4	+C+K	17
8	-C-K	18
8	+C-K	19
8	-C+K	20
8	+C+K	21
12	-C-K	22
12	+C-K	23
12	-C+K	24
12	+C+K	25
24	-C-K	26
24	+C-K	27
24	-C+K	28
24	+C+K	29

Author: Bonne Thompson
Date: 2/6/08