Core J Procedure Protocol
Compactin, Kdo2 Lipid A Treatment

Overview

1. One large experiment will be completed to provide treated cells to each of five cores: 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. Each core will receive triplicate samples of each time and treatment, for a total of 27 samples. Included will be medium for TNFα assay and cells for DNA assay.

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)
4. Spray the reagent tubes with 70% ethanol and let air dry before using.

Treatment

1. Remove 12 dishes from the incubator and label 3 through 15 (12 hour dishes).
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. Repeat this treatment procedure with dishes 16 through 27 (24 hour dishes). For 0 hours, do not treat with anything, not even mevalonate. Label three dishes 1, 2, and 3, 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. Gently wash each plate twice with 3 ml of cold PBS.
4. Add 3 mL of PBS to each dish and scrape the cells with a scraper.
5. Transfer the cells to a 15 mL conical polypropylene tube and pipette 10x with a p1000 to suspend the cells. *(core I samples – use kimax glass tube)*
6. Remove 400 µL of the cell suspension to an eppendorf on ice for DNA assay.
7. Pellet the remaining cells by centrifuging at 2000 rpm for 5 minutes.
8. Aspirate the supernatant and snap freeze the pellets in liquid nitrogen. *(core I samples – do not snap freeze, place directly at -80˚C)*
9. 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 each core as outlined in 10% Serum Kdo2-Lipid A Treatment Protocol (PP0000001004).

Cell Processing for DNA Assay

1. Add 20 µL of 50% etOH in H_2O to each sample for DNA analysis. Store at -80˚C.
2. DNA is to be assayed by each core according to the LIPID MAPS DNA Assay (PP0000002700).

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

Table 2 outlines which sample number corresponds to which treatment

Figure 1

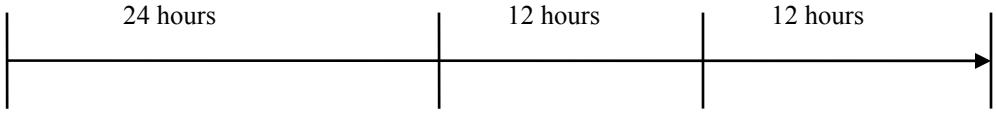

Plate 2x10^6 RAW cells
60 mm plate

Treatment
Harvest 0 hr

Harvest 12 hr

Harvest 24 hr

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

Author: Bonne Thompson
Date: 7/18/06