Core D 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 one or two aliquots, depending on their requirements.
5. Data will be normalized to DNA content (pmol lipid per µg DNA). DNA and TNFa data will be generated by core D. 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 Primary Macrophage Growth Medium 2 (PS0000003600).
2. Incubate 24 hours at 37°C.

Reagent Preparation

1. Mevalonate 50 mM – (RD0000000955)
2. Compactin 50 mM – (RD000000954)
3. Kdo2 Lipid A working solution – prepare from Kdo2 Lipid A stock solution (PS0000001401)

Treatment

1. Before treatment, refresh medium and label plates per time and group number.
2. Treat each plate with the appropriate reagents:

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

3. Note the time and return the plates to the incubator.
4. For 0 hours, do not treat with anything, not even mevalonate. Proceed with harvest.

Harvest

1. At the appropriate time, remove the plates from the incubator.
2. Immediately remove .5 ml of medium from each plate and place in labeled Eppendorf tubes for the TNFα assay.
3. Remove 5 mL of medium from each plate and place in labeled 15 ml polypropylene tubes for Core G.
4. Place the plates on ice, aspirate remaining medium and wash twice with 10 ml of cold DPBS.
5. Add 5 mL of cold DPBS to each plate and scrape the cells with a scraper.
6. Transfer the cells to a labeled 50 mL conical tube on ice.
7. Add another 5 ml of cold DPBS to each plate, scrape and transfer to corresponding tube. Gently suspend cells by swirling or lightly vortexing for 10 secs.
8. Remove 200 µL of the cell suspension to an Eppendorf on ice for the DNA assay.
9. Divide the 10 mL cells into aliquots of **1 mL/2 x 10^6 cells each**, placing into 13x100mm glass tubes with Teflon-lined screw caps or 15 ml polypropylene tubes.
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 (~0.4 ml) and place in a new labeled Eppendorf tube. Freeze the aliquots at -20°C.
2. TNFα is to be assayed by Jay Westcott at ELISA Tech (Aurora, CO).

Cell Processing for DNA Assay

1. Store at -20°C.
2. DNA is to be assayed by core D according to the LIPID MAPS DNA Assay (PP0000002700).

All samples will be shipped to their cores on dry ice

Figure 1

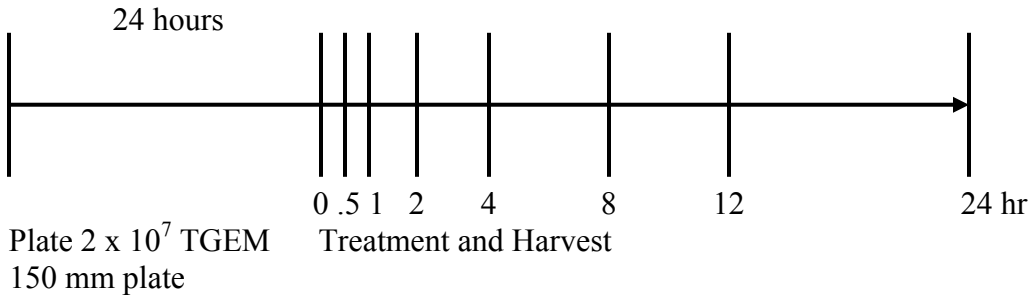

Table 1

Final Concentrations	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: **Time and group number labeling of samples**

hour	Group #	Rx
0		-C-K
0.5	Grp 1	-C-K
0.5	Grp 2	+C-K
0.5	Grp 3	-C+K
0.5	Grp 4	+C+K
1	Grp 1	-C-K
1	Grp2	+C-K
1	Grp3	-C+K
1	Grp4	+C+K
2	Grp1	-C-K
2	Grp2	+C-K
2	Grp3	-C+K
2	Grp4	+C+K
4	Grp 1	-C-K
4	Grp 2	+C-K
4	Grp 3	-C+K
4	Grp 4	+C+K
8	Grp 1	-C-K
8	Grp 2	+C-K
8	Grp 3	-C+K
8	Grp 4	+C+K
12	Grp 1	-C-K
12	Grp 2	+C-K
12	Grp 3	-C+K
12	Grp 4	+C+K
24	Grp 1	-C-K
24	Grp 2	+C-K
24	Grp 3	-C+K
24	Grp 4	+C+K

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