Core D Procedure Protocol
25OHC Treatment
3-22-10

Overview

1. One large experiment will be completed to provide samples to all cores.
2. Untreated cells will be harvested at 0 hours. Treated cells will be harvested at 30 m, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr. See Figure 1 for an overview of the treatment time course.
3. Table 1 for an overview of the treatment.
4. Only one replicate will be made for each time and treatment. 10 aliquots will be generated from each replicate. Cores E, G, J and K will receive one aliquot, cores H and I will receive two aliquots and core G will receive 10-20 mL of medium from each treatment.
5. Data will be normalized to DNA content (pmol lipid per μg DNA). DNA and TNFa data will be generated by core D. DNA and TNFa assay data will be emailed to each core.

Setup

1. Seed 2 - 100 mm TC plates with 1 x 10^7 cells in 10 mL Bone Marrow-derived Macrophage Growth Medium 2 (PS0000003201) or BMDMGM 3 (Low lipid serum plus Mevalonate FC 50 uM and Compactin FC 50 uM) (PS0000004500).
2. Incubate 16 hours at 37°C.

Reagent Preparation

1. Mevalonate 50 mM – (PS0000003800)
2. Compactin 50mM – (PS0000003900)
3. 25OHC 5mM – (PS0000004600)

Treatment

1. Treat each plate with the appropriate reagents:

BMDMGM2 - 25OHC	10 uL DMSO
BMDMGM2 + 25OHC	10 uL 25OHC
BMDMGM3 – 25OHC	10 uL DMSO
BMDMGM3 + 25OHC	10 uL 25OHC

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

Harvest

1. At the appropriate time, remove the plates from the incubator.
2. Immediately remove medium from each plate and place in labeled 50 mL polypropylene tubes for Core G.
3. Remove .5 mL of medium from each treatment and place in labeled Eppendorf tubes for the TNFα assay.
4. Place the plates on ice and wash twice with 5 mL of cold DPBS.
5. Add 5 mL of cold DPBS to the first plate and 5.5 mL to the second plate of each treatment and scrape the cells with a scraper.
6. Transfer the cells to a labeled 50 mL conical tube on ice.
7. Gently suspend cells by swirling or lightly vortexing for 10 secs.
8. Remove 2 x 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.
10. Freeze and store cells at -80°C.

Medium Processing for TNFα Assay

1. Centrifuge the TNFα aliquots at top speed in refrigerated 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.

All samples will be shipped to their cores on dry ice.

Figure 1

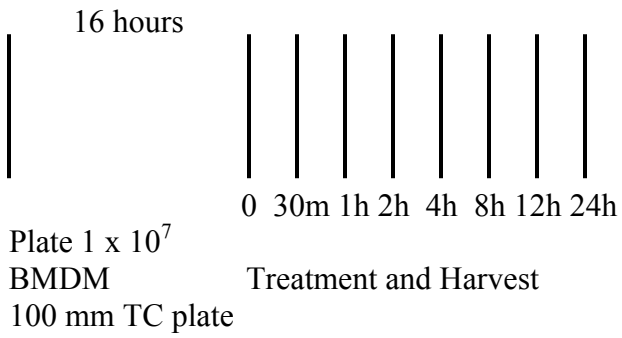

Table 1: Number labeling of samples, time and treatment

Sample #	Tmt Hour	Rx	Media
1	0	-25OHC	BMDMGM2
2	0	-25OHC	BMDMGM3
3	30 m	- 25OHC	BMDMGM2
4	30 m	+25OHC	BMDMGM2
5	30 m	- 25OHC	BMDMGM3
6	30 m	+25OHC	BMDMGM3
7	1	- 25OHC	BMDMGM2
8	1	+25OHC	BMDMGM2
9	1	- 25OHC	BMDMGM3
10	1	+25OHC	BMDMGM3
11	2	- 25OHC	BMDMGM2
12	2	+25OHC	BMDMGM2
13	2	- 25OHC	BMDMGM3
14	2	+25OHC	BMDMGM3
15	4	- 25OHC	BMDMGM2
16	4	+25OHC	BMDMGM2
17	4	- 25OHC	BMDMGM3
18	4	+25OHC	BMDMGM3
19	8	- 25OHC	BMDMGM2
20	8	+25OHC	BMDMGM2
21	8	- 25OHC	BMDMGM3
22	8	+25OHC	BMDMGM3
23	12	- 25OHC	BMDMGM2
24	12	+25OHC	BMDMGM2
25	12	- 25OHC	BMDMGM3
26	12	+25OHC	BMDMGM3
27	24	- 25OHC	BMDMGM2
28	24	+25OHC	BMDMGM2
29	24	- 25OHC	BMDMGM3
30	24	+25OHC	BMDMGM3

Author: Donna Reichart
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