Protocol
ATP/POVPC-peptide Costimulation Time course
(Ishita Shah 01/17/12)

Overview

1. One large experiment will be completed at Core G to provide samples to all the lipidomic cores.
2. RAW 264.7 (Mouse leukemic monocyte macrophage cell line) will be pre-treated for 4hrs with POVPC-peptide (40 µM) followed by subsequent stimulation with ATP(2 mM) for 15min, 30min, 1hr, 2hr, 4hr, 8hr and 20hr. As controls, cells treated with either POVPC-peptide or ATP as well as untreated cells will be harvested at every time point.
3. One technical replicate will be made for each time and treatment; three subsequent experiments will be performed. 12 aliquots will be generated from each replicate. Cores I, G, and J will receive one aliquot, cores H, E and K will receive two aliquots and core G will receive 15 mL of medium from each treatment.
4. Data will be normalized to cell counts. Cell count data will be generated by core G using Invitrogen Countess.

Setup

1. Seed T-75 flasks for each treatment with 1.2 x 10^7 cells in 15 ml dye-free DMEM (Dulbecco's Modified Eagle's Medium)-(SG1201170571)
2. Incubate for 16-18 hours at 37^0C

Reagent Preparation

1. ATP (200mM solution) – (SG1201170569)
2. POVPC-peptide (10mM solution)- (SG1201170570)

Treatment

1. Treat each flask with appropriate reagents:
    a. Pretreatment (priming): 4hr POVPC-peptide (40µM final concentration , 60 µl of 10mM POVPC-peptide in 15ml media). In control experiments, cells will receive 60 µl of H_2O.
    b. Stimulation with ATP: after 4hr of priming with POVPC-peptide, ATP is added (2mM final concentration, 150 µl of 200mM ATP in 15ml media). In control experiments, cells will receive 150 µl of PBS (RG0000001143).
2. The flasks are incubated at 37^0C

Harvest

1. At appropriate time points remove the flasks from the incubator.
2. Collect medium in 50ml conical polypropylene tubes for Core G.
3. Wash the cells twice with 10ml of PBS.
4. Add 12 ml of PBS and scrape the cells with cell scraper.

5. Transfer the cells to a 50ml conical tube. Gently suspend cells by swirling or lightly vortexing for 10 secs.
6. Remove 100ul of cells suspension for counting the cells.
7. Make 12 aliquots from the remaining cell suspension.
8. Dispense 1ml into 13 x 100mm glass tubes (Kimble Chase Part #45066A-13100) with Teflon-lined screw caps.
9. Freeze and store cells in -80^{0}C

Figure 1

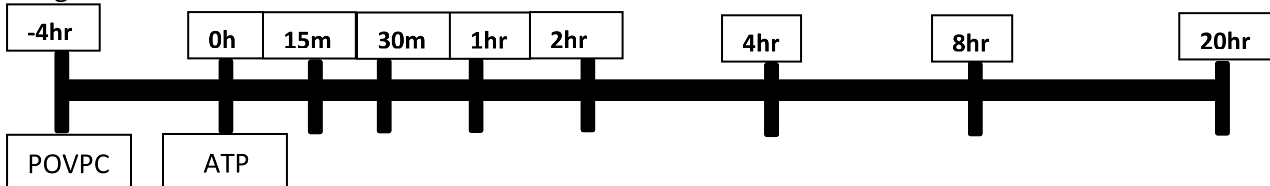

Table 1

Sample #	Pretreatment hour	Treatment hour	Treatment
1	0	-4	-POVPC-ATP
2	4	0	-POVPC-ATP
3	4	0	+POVPC-ATP
4	4	15m	-POVPC-ATP
5	4	15m	+POVPC-ATP
6	4	15m	-POVPC+ATP
7	4	15m	+POVPC+ATP
8	4	30m	-POVPC-ATP
9	4	30m	+POVPC-ATP
10	4	30m	-POVPC+ATP
11	4	30m	+POVPC+ATP
12	4	1hr	-POVPC-ATP
13	4	1hr	+POVPC-ATP
14	4	1hr	-POVPC+ATP
15	4	1hr	+POVPC+ATP
16	4	2hr	-POVPC-ATP
17	4	2hr	+POVPC-ATP
18	4	2hr	-POVPC+ATP
19	4	2hr	+POVPC+ATP
20	4	4hr	-POVPC-ATP
21	4	4hr	+POVPC-ATP
22	4	4hr	-POVPC+ATP
23	4	4hr	+POVPC+ATP
24	4	8hr	-POVPC-ATP
25	4	8hr	+POVPC-ATP
26	4	8hr	-POVPC+ATP
27	4	8hr	+POVPC+ATP
28	4	20hr	-POVPC-ATP
29	4	20hr	+POVPC-ATP
30	4	20hr	-POVPC+ATP
31	4	20hr	+POVPC+ATP