Method Sheet 38

Kinetic analysis of mammalian cell viability

Overview

This method sheet explains how to challenge mammalian cell cultures to explore the kinetics of cell killing in response to a cytotoxic natural extract. Performing this experiment should give some insight into the optimum amount of time necessary for challenge of cells to observe the most cell killing.

Reagents

- One or more hit natural extracts at 10 mg/ml in DMSO

Equipment

- Multichannel pipette (8- or 12-channel) capable of dispensing 1 - 10 µl
- Sterile pipette tips (compatible with each pipette)
- Static (non-shaking) 37°C CO_2 incubator
- Waste container for used tips

Method

1) Passage the stock cell culture on Monday (ideally in the morning), and resuspend the remaining cells to a concentration of 2 x 10^5 cells/ml in complete medium.
2) Seed every well of a 96-well plate with 99 µl per well of this cell suspension (equivalent to 1.98 x 10^4 cells per well).
3) Allow the cells to settle for 1 to 2 hours before challenging.
4) Defrost the hit *Phytotitre* extract(s) and a control vial of sterile DMSO alone.
5) Challenge two wells in column 6 of the plate with 1 µl of DMSO, and a further two wells in the same column with 1 µl of the hit extract.
6) The plate map below shows which wells should be treated and on which days:

Column 3: No treatment; Treat cells on: column 4 Wednesday, column 5 Tuesday, column 6 Monday

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			DMSO 0	DMSO 1 day	DMSO 2 days	DMSO 3 days						
C			DMSO 0	DMSO 1 day	DMSO 2 days	DMSO 3 days						
D			Extract 0	Extract 1 day	Extract 2 days	Extract 3 days						
E			Extract 0	Extract 1 day	Extract 2 days	Extract 3 days						
F												
G												
H												

7) These volumes yield a final concentration of 1% DMSO, and 100 µg/ml extract, respectively.
8) Return the plate to the incubator for 24 hours.
9) The next day (Tuesday) retrieve the plate from the incubator and challenge column 5 with the same treatments at the same time of day the cells were challenged the day before.
10) On Wednesday, at the same time of day, do the same for four wells in column 4 of the plate.
11) On Thursday, at the same time of day, add 1 µl of PBS or medium to the cells in column 3.
12) Immediately after this, process the plate for MTT assay (following Method Sheet 08) or crystal violet assay (following Method Sheet 09) of cellular viability, as preferred.
13) If assessing cellular viability by MTT assay, the SDS should be added to every well 4 hours after treatment with MTT, then the plate should be read the next day (i.e. Friday).
14) If assessing cellular viability by crystal violet assay, the plate can be stained and read on the same day (i.e. Thursday).

Notes

- This method shows how to challenge mammalian cells in 96-well plate format for kinetic analysis using one extract - if you are testing more than one hit extract, there is space available on the same plate to examine up to two additional extracts at the same time within the same experiment - to do so simply adjust the plate map accordingly.
- Remember to complete this experiment at least three times to enable statistical analysis of the data.

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