Method Sheet 07

Challenging mammalian cell-lines with extracts from the *Phytotitre* natural product library

Overview

This method sheet explains how to challenge mammalian cell cultures in 96-well plate format with extracts from the *Phytotitre* natural product library using a multichannel pipette, with the aim of exploring the impact of individual extracts on cellular proliferation and/or viability.

Reagents

- Defrosted *Phytotitre* or *Puretitre* library

Equipment

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

Method

1) Fully defrost a *Phytotitre* or *Puretitre* library plate in a 37ºC incubator (follow Method Sheet 01).
2) Prepare a 96-well plate containing mammalian cell cultures at 99 µl per well (follow Method Sheet 06).
3) Carefully remove the cap mat from the library plate and place with the round domes facing upwards (do not discard the cap mat).
4) Column 1 of the library plate is left empty to allow space for negative controls - you can make use of this by pipetting 100 µl of sterile DMSO into each well of this column in the library plate to use as a vehicle only control.
5) Place both library and culture plates side by side and ensure both are in the correct orientation (i.e. check that well A1 is in the top left position).
6) Set a small volume multichannel pipette to dispense 1 µl (this is a common starting point, but can vary depending on your own experiment, see notes below).
7) Attach tips to the pipette and ensure all are firmly seated as in the previous method.
8) Work from left to right, starting at column 1 and moving across, one pipetting cycle at a time, to column 11.
9) It can be difficult to keep track of which column is being pipetted from and to when challenging plates for screening, so you can cover the wells that have already been completed with a sterile 96-well plate lid to help keep your place as you move along the plate from column to column.
10) Push the plunger down to the first stop (not all the way to the second stop).

11) Place the tips below the surface of the liquid in each well of the respective column of the library plate (containing stock extracts).
12) Slowly allow the plunger to return to the top position.
13) Look carefully across all the tips to ensure the level of liquid in each tip is the same, if not, dispense the suspension back into the correct wells of the library plate and try again.
14) Likewise, if there are any large air bubbles in any of the tips, dispense and try again.
15) Move the pipette to the plate containing the mammalian cell culture and dispense all the liquid into the respective wells, being careful to pipette into the liquid and not onto the side of the well.
16) When dispensing, push the plunger past the first stop all the way to the second stop.
17) Discard the tips after pipetting into each column (this is essential to prevent cross-contamination of the library contents).
18) Place fresh tips on the pipette before moving onto the next column.
19) Move the plate lid marker one column to the right to make it easier to see which column is next to work from.
20) Continue until every well of the plate between columns 1 and 11 have been challenged.
21) Column 12 is available for you to pipette positive control compounds (if desired, these will be specific to each type of experiment).
22) For a simple positive control for a compound that induces 100% cell death, you can pipette 1 µl of 10% SDS into every well of column 12 using a single channel pipette.
23) Be careful not to splash any SDS into neighbouring wells, as this will cause false positive results for cell killing in those wells.
24) Move the culture plate backwards and forwards, then side to side, gently several times to mix the compound into the suspension.
25) Avoid jarring movements that may splash liquid between wells and cause cross-contamination.
26) Place the culture plate in a 37°C incubator with 5% CO2 atmosphere to allow the cells to grow.
27) Cell viability or growth assays can then be performed 18 to 48 hours after challenge (see Method Sheet 08 and 09).

Plate map

	1	2	3	4	5	6	7	8	9	10	11	12
A	DMSO	01	09	17	25	33	41	49	57	65	73	SDS
B	DMSO	02	10	18	26	34	42	50	58	66	74	SDS
C	DMSO	03	11	19	27	35	43	51	59	67	75	SDS
D	DMSO	04	12	20	28	36	44	52	60	68	76	SDS
E	DMSO	05	13	21	29	37	45	53	61	69	77	SDS
F	DMSO	06	14	22	30	38	46	54	62	70	78	SDS
G	DMSO	07	15	23	31	39	47	55	63	71	79	SDS
H	DMSO	08	16	24	32	40	48	56	64	72	80	SDS

1 µl DMSO (vehicle control) — 1 µl each plant extract or stock compound — 1 µl 10% SDS (positive control)

Notes

- The protocol assumes that the user is trained in the basic principles and techniques of mammalian cell culture - please seek such training if you have not received it already before attempting to follow the method.
- Column 1 of the library plate is left empty to allow space for negative controls - you can make use of this by pipetting 100 µl of sterile DMSO into each well of this column in the library plate to use as a vehicle only control.
- Column 12 of the library plate is also left empty to allow space for customers to insert their own positive controls of interest (if available, for examples chemotherapy drugs or other compounds with established cellular toxicity for the cell-line of interest).
- When dispensing compound, ensure the liquid goes directly into the larger culture volume, and does not touch and then adhere to the plastic side of the well.
- If using the *Phytotitre* library, the stock plant extracts are at 10 mg/ml, so transfering 1 µl of stock extract into 99 µl of culture represents a 1:100 dilution, which means the final concentration of plant extract in each culture will be 100 µg/ml.
- Accordingly, the final concentration of DMSO (the solvent, also called vehicle, used to dissolve the plant extracts) will be 1% (wt:vol) when following this protocol.
- If using the *Puretitre* collection instead, the stock compounds are at 10 mM, which means the final concentration of each compound in each culture will be 100 µM.
- Mammalian cells can typically tolerate up to 1% DMSO with little impact on growth or viability, but higher concentrations can have more pronounced effects.
- If there are too many hits per screen, or the 1% DMSO control exhibits toxicity for your particular cell-line, it is possible to halve the effective concentration (i.e. to 50 µg/ml extract and 0.5% DMSO) by plating the cells in a total volume of 199 µl before addition of 1 µl extract or DMSO per well.
- Further dilutions are also possible using intermediate plates containing complete medium (e.g. 1 µl extract into 24 µl DMEM per well in plate 1, then 5 µl from plate 1 onto 95 µl of each cell culture in plate 2, for a 1:500 dilution from stock).