Method Sheet 06

Using a multichannel pipette to seed mammalian cell cultures into 96-well microplates for screening assays

Overview

This method sheet explains how to accurately dispense suspensions of adherent mammalian cells into a 96-well microplate using a multichannel pipette, with the aim of using the plates to screen natural extracts for their capacity to alter cell viability or other parameters. 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.

Equipment

- Sterile, clear plastic 96-well microplate(s) with lids
- Multichannel pipette (8- or 12-channel) capable of dispensing 100 - 200 µl
- Sterile pipette tips (compatible with the multichannel pipette)
- Reservoir(s) for media containing bacterial cells to set up the plate
- Tissue culture suite with a Class 2 biosafety cabinet and an incubator capable of maintaining a 5% CO_2 atmosphere at 37^{o}C
- Waste container for used tips

Reagents

- Complete tissue culture medium appropriate for culture of your cell-line of choice (e.g. Dulbecco's Modified Eagle Medium, DMEM, containing 10% foetal calf serum, 100 units/mL of penicillin and 100 µg/mL of streptomycin)
- An adherent mammalian cell-line of choice growing as a healthy culture in a T75 flask or larger (e.g. PC-3 prostate cancer cell-line)

Method

1) This protocol assumes you have been trained in the basics of mammalian tissue culture techniques - please seek such training if you have not received it already before attempting to follow the method.
2) Perform the following work within a biosafety cabinet and wearing appropriate PPE.
3) Once your cell-line reaches 80-90% confluence, perform the normal passaging process for that cell-line by seeding a new flask to maintain the stock culture.
4) Do not discard the remaining cells, these will be used to seed the microplates.
5) Use a haemocytometer or similar device to measure the concentration of the remaining cell suspension.
6) Prepare a dilution of the cell suspension to a concentration of 2 x 10^5 cells per ml in a separate tube (note, this value can be adjusted depending on your cell-line of choice).

7) Aliquot a sufficient volume (typically 11 ml per plate) of the cell suspension into a suitable sterile reservoir (if unavailable, the lid of a separate, sterile 96-well plate can be used).
8) Set the multi-channel pipette to dispense a volume of 99 µl (standard for most assays, but can change for your own experiment).
9) Firmly press the pipette into a row of sterile pipette tips (8 or 12, depending on the type of pipette), ensuring all are securely attached.
10) If the tips are loose or fall off during use, use gloved finger and thumb to pull up and seat firmly each tip individually, being very careful to touch only the upper part of the tip, and not the lower part of the tip which must remain sterile before use.
11) Push the plunger on the pipette down to the first stop (not all the way to the second stop).
12) Insert the tips into the liquid in the reservoir, ensuring all tips are below the surface of the liquid.
13) Slowly release your thumb to allow the plunger to return to the top position.
14) Look carefully across all the tips to ensure the level of liquid is the same in each tip, if not, dispense the suspension back into the reservoir and try again.
15) Likewise, if there are any large air bubbles in any of the tips, dispense back into the reservoir and try again.
16) Move the pipette to the open sterile plate and dispense all the liquid into the wells of an empty column, pipetting past the first stop all the way to the second stop of the plunger this time.
17) If the tips become loose during this process, you can repeat the tightening of the tips to the pipette as above.
18) Because the same liquid is being pipetted into every well, there is no requirement to change tips between rows or columns when preparing the plate initially (note, you must change tips each time when it comes to the challenge step).
19) If any tips are lost, it may be easier to discard the tips and replace with fresh tips.
20) Repeat this process until every well of the plate contains the cell suspension.
21) Discard the tips in the waste tips container.
22) Repeat for the number of plates you intend to prepare for your experiment.
23) Place the microplates in a 37^{o}C incubator with 5% CO_2 atmosphere as soon as pipetting is complete.
24) Allow the cells to settle and adhere to the plastic at the bottom of the microplate wells before challenging - most researchers will normally allow an overnight incubation to perform this step, but it may also be complete and ready for challenge by late afternoon if plating in the morning.
25) Once the cells have adhered, proceed to challenging the cells with the *Phytotitre* plant extract library and appropriate controls (follow Method Sheet 07).

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.
- Technically, only 9.6 ml of cell suspension is necessary to set up a 96-well plate containing 100 µl of suspension per well, but it is standard practise to always make ~10% more than the minimum since some liquid always remains unrecoverable from the reservoir.
- This protocol applies to the use of adherent cell-lines - the assays that follow will not work well with suspension cell-lines, so modifications of the protocols would be necessary to screen using such cell-lines.