Method Sheet 10

Resuspending solvent-free plant extracts for further experimentation

Overview

This method sheet explains how to resuspend solvent-free (dry) plant extracts for further experimentation. A common step taken after the discovery of hits during a screening programme is to seek to replicate the main findings using fresh extracts of the hit plants. These can be prepared in house, or purchased from our company. As the *Phytotitre* project kit comprises only non-polar extracts (the main kit also contains 400 polar extracts of the same plants), all have been extracted using the solvent dichloromethane. After filtration and evaporation, the extract is typically resinous and sticky, making it difficult to remove using a spatula for weighing out. This method sheet explains how to suspend such extracts directly in the vial with a suitable solvent (typically DMSO) for follow-on *in vitro* experiments.

Reagents

- Vial containing solvent-free plant extract prepared by dichloromethane extraction of dried plant parts
- Solvent for extract resuspension (typically dimethyl-sulphoxide, DMSO)

Equipment

- Pipettes and suitable pipette tips

Method

1) Take a note of the mass of extract printed on the vial label, or the mass you have left in your evaporation vessel if preparing the dry extract yourself.
2) To prepare a 10 mg/ml stock (a typical concentration for stock extract suspensions), divide the mass of the extract in milligrammes by 10 (let's call this number 'x').
3) Add 'x' millilitres of DMSO directly into the vial, or your evaporation vessel.
4) For example, if the vessel contains 80 mg of extract, add 8 ml of DMSO.
5) Replace the cap and vortex the vial until the extract is completely resuspended.
6) Aliquot the stock extract into labelled 1.5 ml tubes and freeze at -20ºC for further use.

Notes

- It is generally difficult to use a spatula to remove and weigh non-polar extracts because of their resinous, sticky nature.
- Polar extracts of plants, in contrast, are generally a fine, dry powder and are easy to remove by spatula for weighing.
- It is also possible to purchase ready-dissolved extracts in DMSO at 10 mg/ml if preferred.

- Stock extract solutions are typically prepared at 10 mg/ml, but you can resuspend at higher or lower concentrations as preferred by adjusting the volumes accordingly.
- Other non-polar solvents can be used for resuspension of the extracts, but DMSO is the one most commonly used because it is compatible with living cells and capable of dissolving compounds covering a wide range of polarities, including the non-polar compounds that dominate in the *Phytotitre* project kit extracts.
- Attempting to resuspend in an aqueous solvent directly, such as bacterial growth medium or mammalian cell tissue culture medium, is likely to result in poor solubility and inefficient resuspension, since most of the most of the molecules in the *Phytotitre* project kit extracts are non-polar.
- If the suspension is still quite viscous after resuspension, it can be pipetted more easily by cutting the end of the pipette tip off using scissors - the resulting larger aperture permits easier pipetting of viscous liquids.
- Try to minimise the number of freeze-thaw cycles of each aliquot after resuspension.

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