Method Sheet 01

Defrosting the *Phytotitre* or *Puretitre* kits

Overview

This method sheet explains how to defrost and repackage the *Phytotitre* or *Puretitre* kits before and after use in experiments.

Reagents

- *Phytotitre* plant extract library (400E) or *Puretitre* natural compound library (PT200)

Equipment

- Dry 37°C incubator (a CO_2 incubator can also be used if convenient)

Method

1) The *Phytotitre* and *Puretitre* libraries are stored at -20°C or -80°C, and will therefore require defrosting before use.
2) Separate the individual plates from the frozen kit.
3) Place the individual plates side by side, not on top of each other, in a 37°C incubator.
4) It is best not to defrost the stack as a whole, since this will take a very long time to defrost.
5) Allow all wells of the plate to thaw completely before use (typically takes 30-40 minutes).
6) Carefully remove the plate from the incubator to your workspace and gently dry the outside of the plate with tissue if moisture present.
7) Keep the plate facing upwards, and do not turn upside down at any point (if this happens, the well contents can be lost by adherence to the cap mat).
8) Wearing gloves, hold the outer skirt of the plate down with one hand, and gently peel back the cap mat from one corner with the other hand.
9) Remove the flexible cap mat completely and store in a safe place with the round well cap projections facing upwards (do not discard).
10) Use the plate contents for your experiment (e.g. a screening assay).
11) To replace the cap mat, align the cutout on the cap mat with the same shape on the base of the plate.
12) Press gently with your thumbs to seat the cap mat onto the wells.
13) Complete the cap sealing by placing the white, rectangular plastic pressing piece that ships with the kit on top of the cap mat.
14) Press down firmly with the heel of your hand onto the plastic pressing piece until the cap mats are firmly seated on each well of the plate.
15) Return the plate to the freezer if using again.

Notes

- The natural extracts and compounds are dissolved in dimethyl sulphoxide (DMSO), which is the standard for drug library screening.
- DMSO has a melting point of 18^{o}C, so the kits will not thaw well at room temperature.
- As the outer wells will thaw more quickly than the inner wells of the plate, it is best to check that the central wells are fully thawed before beginning the experiment.
- It is possible to accelerate the thawing process by placing the plate gently within a very shallow dish containing warm (37^{o}C) water, such that it does not reach more than half way up the skirt of the plate, however, be very careful to prevent contamination of the well contents with the water, and dry the plates well on tissue before opening and further use.
- Repeated freeze-thaws will eventually reduce the bioactivity of some of the extracts or natural compounds, so it is best to minimise freeze-thaw cycles if possible.