Lipid Extraction

Phospho- and sphingolipids can be extracted from fully established 3D epidermal models and visualised on 2D TLC plates using the following method.

Use glass tubes throughout, to avoid any potential contamination or distortion of the results that can arise from plasticware.

Protocol

1. Remove the 3D sample from the insert using a dispase treatment
2. Resuspend the samples in H2O/MeOH/CHCl3 (1/3.32/1.66 vol/vol) and vortex. Extraction volume should be proportional to starting tissue weight, for example use approximately 1 mL of mixture for 30 mg of tissue.
3. Sonicate for 4x1 min (in waterbath).
4. Allow to extract overnight at 4°C.
5. Add H2O/CHCl3 (1.66/1.66 vol/vol). Volume is proportional to starting tissue weight, use approx 550 uL per 30 mg of starting tissue.
6. Centrifuge for 10 min at approx 2'000 g to achieve phase separation.
7. Discard the upper aqueous phase and the protein layer at the phase interface.
8. Evaporate the lower organic phase at 40°C.
9. Resuspend in CHCl3/MeOH (4/1 vol/vol), use approx 100 uL per 30 mg starting tissue weight.
10. Spot 10 uL per lane on 2D thin layer chromatography (TLC) plates.
11. Lipids can then be separated using an appropriate solvent system. For example phospho- and sphingolipids can be separated using the mixture CHCl3/MeOH/25% NH4OH (32.5/12.5/2.25 vol/vol)

In case of further questions, please email out scientists directly: scientist@cellntec.com