Neutral Lipid Extraction by the Method of Bligh-Dyer

(Bligh and Dyer, Can. J. Biochem. Physiol. 37, 922 (1959))

Solutions Needed:

Phosphate-buffered saline (PBS)
Methanol:H_2O (2:0.8)
$CHCl_3$

Procedure:

This procedure is scaled to use with 60 mm diameter plates. Scale up or down according to number of cells (ie. surface area).

1. Wash cells in cold PBS (3 ml/plate)
2. Add 3 ml of the MeOH/H_20 solution and scrape the cells into the buffer
3. Transfer cell material into large glass tube
4. Add 1 ml $CHCl_3$. Vortex 30 sec and allow tubes to sit until phases separate. The rate of phase separate can be increased by spinning tubes for 1 min at low speed.
5. Remove top layer and transfer to a new glass tube. Add 1 ml $CHCl_3$ and re-extract as above by vortexing and phase separation.
6. Aspirate the top layer from this second extraction and combine the two lower $CHCl_3$ phases.
7. Backwash the $CHCl_3$ phase by adding 3 ml MeOH/H_20. Vortex 30 sec and let phases separate. If phases fail to separate, add a little water until the system becomes biphasic.
8. Aspirate the top layer and transfer the bottom solution to a small glass tube.
9. Evaporate to dryness in a Speed Vac (~ 1 hr) and store under vacuum until analysis.