Extraction of Lipids in Solution by the Method of Bligh & Dyer

(Bligh,E.G. and Dyer,W.J. 1959. A rapid method for total lipid extraction and purification. *Can.J.Biochem.Physiol.* 37:911-917.)

Use new (or solvent-cleaned) all-glass tubes:

1. For each 1 ml of sample, add 3.75 ml 1:2 (v/v) $CHCl_3$:MeOH and vortex well. If you will be subjecting the lipids to GC analysis, this solvent should include the final amount of internal standard (*e.g.*, 5 µg β-sitosterol).

2. Then add 1.25 ml $CHCl_3$ and vortex well.

3. Finally add 1.25 ml dH_2O and vortex well.

4. Centrifuge at 1000 RPM in IEC table-top centrifuge for 5 min at room temperature to give a two-phase system (aqueous top, organic bottom).

5. Recover the bottom phase as follows: insert Pasteur pipette through the upper phase with gentle positive-pressure (*i.e.*, gentle bubbling) so that the upper phase does not get into the pipette tip. When pipette tip is at the bottom of the tube, carefully withdraw bottom phase through the pipette, making sure to avoid interface or upper face (should only try to recover ~90% of bottom phase, not all of it).

The table below shows proportions for different volumes of sample:

Sample	**0.2 ml**	**0.5 ml**	**1 ml**	**1.5 ml**	**2 ml**	**3.5 ml**
1:2 $CHCl_3$:MeOH	0.750	1.9	3.75	5.7	7.5	13.125
$CHCl_3$	0.250	0.625	1.25	1.875	2.5	4.375
dH_2O	0.250	0.625	1.25	1.875	2.5	4.375
Total volume	1.45	3.65	7.25	10.95	14.50	25.375

6. If you need a very clean preparation, you should "wash" the recovered bottom phase with "authentic upper phase" as follows:

a. Prepare "authentic upper phase" as follows: in a large glass tube or multiple normal-sized tubes, run multiple tubes of the procedure above using dH_2O in place of sample. Collect the upper phase and store in a tube.

b. Add your recovered bottom phase from step #5 above to a tube and then add the appropriate amount (sample + dH_2O volume) of "authentic upper phase." For example, if you did a 1-ml sample prep, you add ~2.25 ml of "authentic upper phase."

c. Vortex well, centrifuge, and collect bottom phase as above.