

ERGOSTEROL EXTRACTION

(from Brodie et al., 2003, FEMS Microbiol. Ecol. 45 :105-114)

1. Weigh 5g of fresh sieved (2mm) soil
2. Place in a 50ml centrifuge tube
3. Add 15ml of cold methanol and 5ml of a fresh KOH solution (40g KOH / L ethanol)
4. Vortex for 30sec and sonicate (in our case : 1min output 6 on a Cell disruptor Model W-370, Heat Systems-Ultrasonics, Inc.)
5. Place the tubes in a water bath pre-heated at 85°C
6. Remove after 15min, mix manually for 1min, and replace for a further 15min
7. Cool in a fridge for 20min
8. Add 10ml of HPLC grade pentane, and hand shake for 1min
9. Centrifuge at 3000xg for 3min (to separate the pentane layer from soil)
10. Remove the pentane layer and transfer to a new tube
11. Carry out steps 8-10 three times for each sample and combine the pentane extracts
12. Dry extracts under a stream of nitrogen
13. At that point, dry extracts can be either stored at -20°C until analysis or redissolved in HPLC grade methanol