<https://www.protocols.io/view/coral-lipid-assay-for-96-well-plates-q26g789pqlwz/v1>

Lipid extraction

1. Vortex sample & transfer 600 uL of coral tissue slurry into 1.5ml tube
2. Pre-make 2:1 chloro:Methanol solutions
3. Add 600 uL of chloro:meth solution
4. Vortex then shake on plate shaker for 20 mins
5. Add 160 uL of NaCL to each tube
6. Invert tubes gently two times and open and reclose lid
7. Centrifuge at 3000 rpm for 5 min
8. Remove the top layer and dispose
9. Pipette 3 replicates 100 uL of remaining slurry into wells

Standards

1. Make stock serial dilution in 7 1.5mL tubes for each plate
2. Add 300 uL of CHcl3 to standard tubes 2-7
3. \*\*\*\* a bunch of detail on making the standard ratios that I don’t feel like typing

Lipid Assay

1. Prepare 96 well plate of 100 uL of each sample in triplicates
2. Add 50 uL of CH3OH to each well
3. Evaporate solvent on 90˚ hotplate for 10 mins
4. Add 100 uL H2SO4 to each well
5. Evaporate solvent on 90˚ hotplate for 20 mins
6. Transfer 75uL of each sample to new 96 well plate (avoid residue & bubbles)
7. Read background absorbance at 540nm using microplate reader
8. Add 34.5 uL of 0.2 vanillin in 17% phosphoric acid to each well
9. Incubate 10 mins
10. Cover plate again and read absorbance at 540 nm using microplate reader

Corn oil from sigma aldritch

Avoid sticky around the edges

Remake standards every day – especially vanillin in phosphoric acid

Vortex all reagents before re-use

Be careful with chemicals in plate reader

How to tell if things aren’t working

* Extractions should result in two layers
* Serial dilution can be easily messed up
* Assay should have color changes
  + Sulfuric acid should turn yellow, if that doesn’t happen maybe don’t finish it
  + Second color change – yellow to pink is critical (pink is what’s being picked up)

If my samples are HIGHER than the standard, make a standard that is within that range

(if 1.5 isn’t high enough)