Colorimetric Lipid Assay for Corals

THE KEY IS TO BE CAREFUL WITH PIPETTING

Lipid Extraction

Reagents:

1. CH_3OH (Methanol)
2. $CHCl_3$ (Chloroform)
3. 0.05 M NaCl in water

Folch Method:

1. Pull samples from -80 freezer and allow to thaw (maximum of 24 samples per plate in triplicate)
2. Vortex sample and transfer 600 µL of coral tissue slurry sample to labelled 1.5 mL tube
3. Pre-make 2:1 ($CHCl_3$:CH_3OH) stock every day
4. Add 400 µL of $CHCl_3$ and 200 µL of CH_3OH in a 2:1 ratio to 1.5 mL sample tube
5. Vortex then shake on plate shaker for 20 minutes
6. Add 160 µL of 0.05M NaCl ($CHCl_3$: CH_3OH : NaCl is in a 2:1:0.8 ratio – keep this ratio)
7. Invert tubes gently two times and open and reclose lid
8. Centrifuge at 3000 rpm for 5 minutes
9. Remove $CHCl_3$ (top) layer and dispose before taking 100 µL for the assay
    - Do three times for 3 replicates of 100 µL

Lipid Assay

Reagents:

1. 17% Phosphoric acid (H_3PO_4)
2. 0.2 mg/mL vanillin in 17% phosphoric acid
3. Concentrated (18M) sulfuric acid
4. CH_3OH
5. $CHCl_3$
6. Corn Oil

Standards:

1. Make a stock serial dilution in 7 1.5 mL tubes for each plate
2. Add 300 µL of $CHCl_3$ to standard tubes 2 through 7
3. Add 600 µL of 1.5 mg/ml stock to standard tube 1.
4. Pull 300 µL from tube 1 and place in tube 2. Back pipette to mix.
5. Pull 300 µL from tube 2 and place in tube 3. Back pipette to mix.
6. Repeat this process for tubes 3 through 6.
7. Discard 300 µL from tube 6 so total volume equals 300 µL.
8. Tube 7 is a blank of ONLY $CHCl_3$. *DO NOT ADD CORN OIL*

Standard	**1**	**2**	**3**	**4**	**5**	**6**	**7 (blank)**
(mg/mL)	1.5	0.75	0.375	0.188	0.094	0.047	0.000

Assay:

1. In a 96-well plate, add 100 µL of sample or standard to each well in triplicate
7. Add 50 µL of CH_3OH to each well
2. Evaporate solvent on a 90°C hotplate for 10 minutes
3. Add 100 µL H_2SO_4 to each well (*change from clear to yellow colour*)
4. Incubate on hotplate at 90°C for 20 minutes
5. Cool the plate on ice for 2 minutes
6. Transfer 75 µL of each sample or standard from the microplate to a new 96-well microplate
7. Read background absorbance at 540 nm using microplate reader (Caudill Lab 208)
8. Add 34.5 µL of 0.2 mg/mL vanillin in 17% phosphoric acid to each well
9. Incubate for 10 minutes (*should change from yellow to pink color*)
10. Read absorbance at 540 nm using microplate reader ('Lipid Colleen and JB'; Caudill Lab 208)

Standard Preparation

0.05M NaCl:

- 0.1461g NaCl in 50 mL DI water

Corn Oil:

- 245 µL of corn oil in 14.755 ml $CHCl_3$
- Information:
    - Corn oil standard: Sigma Aldrich 47112-U Corn Oil analytical standard (1000 mg)
    - Density = 0.9188 g/ml (Noureddini et al., 1992)
    - Total volume of amuple = 1 g* (1ml / 0.9188g) =1.08837614 ml
    - Known concentration of ampule= 1000mg / 1.088ml = 918.8 mg/ml (same as known density)
- Stock 1.5 mg/mL corn oil standard (15 mL):
    - 1.5 mg/mL * **15mL** = 918mg/mL * X mL

Stock 0.2 mg/mL vanillin in 17% phosphoric acid:

- 20 mL of 17% H_3PO_4
    - 4 mL 85% H_3PO_4
    - 16 mL of DI water
- Stock vanillin solution
    - 20 mL 17% H_3PO_4
    - 4 mg vanillin

Coral total lipid calculation*

1. Create standard curve with known standard concentrations and absorbance values ($y = mx + b$)
2. Using the resulting equation, convert sample absorbance to concentrations (mg/mL)
3. Multiply sample concentration (mg/mL) by total slurry volume (mL) and dilution factor (600/1360 µL), then divide by surface area (cm^2) for resulting units: mg/cm^2

** Can use associated Rmarkdown script to calculate concentrations per sample well (Lipid Calculation Script.Rmd)*