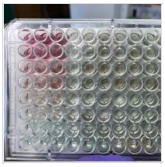

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Coral Lipid Assay for 96-well plates

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ABSTRACT

This protocol is designed to work with coral host tissue slurry that has been processed by airbrushing to remove the tissue from the skeleton and after removal of symbiont cells and homoginization. This protocol will allow quantification of lipid concentrations of samples using 96-well plates for rapid assessment.

This protocol was adapted from Folch et al 1957[1] and Cheng et al 2011[2]. For calculation of lipid concentrations, follow the directions presented in the materials section or use the custom Markdown file that can be found on my GitHub (LipidAssay_96wellplate).

1. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957 May;226(1):497-509. PMID: 13428781.
2. Cheng YS, Zheng Y, VanderGheynst JS. Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. Lipids. 2011 Jan;46(1):95-103. doi: 10.1007/s11745-010-3494-0. Epub 2010 Nov 11. PMID: 21069472.

ATTACHMENTS

Folch et al (1957) A Simple Method For The Isolation And Purification Of Total Lipides From Animal Tissues.pdf

cheng 2010.pdf

DOI

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PROTOCOL CITATION

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Coral, lipid assay, 96-well plate

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MATERIALS TEXT

Reagents:

Lipid extraction

- CH_3OH (Methanol)
- $CHCl_3$(Chloroform)
- 0.05 M NaCl in water

Lipid Assay

- 17% Phosphoric acid (H_3PO_4)
- 0.2 mg/mL vanillin in 17% phosphoric acid
- Concentrated (18M) sulfuric acid
- CH_3OH
- $CHCl_3$
- Corn Oil

Equipment:

- 96-well plates
- 1.5 mL tubes
- Vortex
- Plate shaker
- Centrifuge
- Hotplate
- Ice bucket/ice
- Fume hood
- Plate reader (can read absorbance at 540 nm)

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
4. Alternatively, you can use custom Rmarkdown script to calculate concentrations per sample well (*Lipid Calculation Script.Rmd*) found on my GitHub (LipidAssay_96wellplate)

Lipid Extraction

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All portions of this protocol should be performed in a fume hood as much as possible with appropriate safety precautions, including gloves, lab coat, and closed toe shoes.

The key with this protocol is very careful pipetting

2 Pull samples from -80 freezer and allow to thaw (maximum of 24 samples per plate in triplicate)

3 Vortex sample and transfer 600 µL of coral tissue slurry sample to labelled 1.5 mL tube

4 Pre-make new 2:1 ($CHCl_3$:CH_3OH) stock every day running plates

5 Add 400 µL of $CHCl_3$ and 200 µL of CH_3OH in a 2:1 ratio to 1.5 mL sample tube

6 Vortex then shake on plate shaker for 20 minutes

7 Add 160 µL of 0.05M NaCl ($CHCl_3$: CH_3OH : NaCl is in a 2:1:0.8 ratio – keep this ratio)

8 Invert tubes gently two times and open and reclose lid

9 Centrifuge at 3000 rpm for 5 minutes

10 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 Standard Creation

11 Make a stock serial dilution in 7 1.5 mL tubes for each plate

12 Add 300 µL of $CHCl_3$ to standard tubes 2 through 7

Add 600 µL of 1.5 mg/ml stock to standard tube 1.

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14 Pull 300 µL from tube 1 and place in tube 2. Back pipette to mix.

15 Pull 300 µL from tube 2 and place in tube 3. Back pipette to mix.

16 **Repeat this process for tubes 3 through 6.**

17 Discard 300 µL from tube 6 so total volume equals 300 µL.

18 Tube 7 is a blank of ONLY $CHCl_3$. *DO NOT ADD CORN OIL*

A	B	C	D	E	F	G	H
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

Standard concentrations table

Lipid Assay

19 In a 96-well plate, add 100 µL of sample or standard to each well in triplicate (see template below)

A	B	C	D	E	F	G	H	I	J	K	L	M
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	*Std 1*	*Std 1*	*Std 1*	S1	S1	S1	S9	S9	S9	S17	S17	S17
B	*Std 2*	*Std 2*	*Std 2*	S2	S2	S2	S10	S10	S10	S18	S18	S18
C	*Std 3*	*Std 3*	*Std 3*	S3	S3	S3	S11	S11	S11	S19	S19	S19
D	*Std 4*	*Std 4*	*Std 4*	S4	S4	S4	S12	S12	S12	S20	S20	S20
E	*Std 5*	*Std 5*	*Std 5*	S5	S5	S5	S13	S13	S13	S21	S21	S21
F	*Std 6*	*Std 6*	*Std 6*	S6	S6	S6	S14	S14	S14	S22	S22	S22
G	*Std 7*	*Std 7*	*Std 7*	S7	S7	S7	S15	S15	S15	S23	S23	S23
H	*Blank*	*Blank*	*Blank*	S8	S8	S8	S16	S16	S16	S24	S24	S24

Template of sample and standard placement in 96-well plate

20 Add 50 µL of CH_3OH to each well

21 Evaporate solvent on a 90°C hotplate for 10 minutes

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22 Add 100 µL $H_2SO_4$to each well (*wells will change from clear to yellow colour*)

23 Incubate on hotplate at 90°C for 20 minutes

24 Cool the plate on ice for 2 minutes

25 Transfer 75 µL of each sample or standard from the microplate to a new 96-well microplate (*wells may contain sticky residue and bubbles, but you should be able to avoid that to pull the required 75* µL)

26 Cover the plate and read background absorbance at 540 nm using microplate reader (*this is a baseline measure that is used for correcting the final plate absorbance*)

27 Add 34.5 µL of 0.2 mg/mL vanillin in 17% phosphoric acid to each well

28 Incubate for 10 minutes (*should change from yellow to pink color*)

29 Cover the plate again and read absorbance at 540 nm using microplate reader

30 Calculate sample concentrations following method listed under 'Materials'

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