Sawall_lab_Chla_ChlC2_coral_tissue_analysis_Carbonne

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Protocols Adapted for Sawall Lab at ASU

Quantifying Chlorophyll-*a* and Chlorophyll-*c2* Concentrations

Adapted from Dr. Hollie Putman's protocol by Dr. Chloe Carbonne

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Materials Checklist

☐ 100% acetone
☐ Flammable-safe fridge (4 °C)
☐ Quartz 96-well plate
☐ Glass plate lid (plastic lids will melt)
☐ Centrifuge
☐ 10 mL glass syringe
☐ 2 mL microcentrifuge tubes
☐ Q-tips
☐ Labeling tape
☐ Microplate reader (630, 663, 750 nm)
☐ Homogenizer (Ultra-Turrax type)
☐ Parafilm (optional)

Protocol

A. Sample Preparation

1. Prepare a **500 µL aliquot** of adult airbrush homogenate (coral slurry) in a **2 mL microcentrifuge tube**.
2. Add **1 mL of 100% acetone** to the slurry using a **10 mL glass syringe**.
3. Vortex tubes for **15 seconds**.
    - If the pellet does not dissolve or tissue chunks remain:
        - Sonicate **2 × 5 seconds**, **100% amplitude**
        - Keep tubes **on ice**
        - Avoid evaporation as much as possible
4. Place tubes **in the dark** at **4 °C** for **24 hours**.
5. After incubation, sonicate again:
    - **2 × 5 seconds**, **100% amplitude**, on ice
6. Centrifuge tubes at **2,000 rpm for 3 minutes** to pellet debris.

B. Plate Setup

1. Use the plate template to **map sample positions**.
2. Pipette:
    - **200 µL** of each sample into **triplicate wells**
    - **200 µL acetone blanks** into:
        - First 3 wells
        - Last 3 wells
3. As you fill the plate:
    - Cover every ~3 columns with the **glass lid or parafilm**
    - Use another plate (or object of equal height) to support the lid and maintain balance
4. Once the plate is complete, **fully cover with the glass lid**.

C. Measuring Absorbance

1. Measure absorbance at:
    - **630 nm**
    - **663 nm**
    - **750 nm**
2. Using Roger Lab's plate reader:
    - Open **Gen5 software**
    - The **Task Manager** will open automatically
    - Select protocol: **"Chloe_Chla"**

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3. Calculating Chlorophyll Concentration

1. Subtract **A750** from all absorbance measurements.
2. Calculate chlorophyll *a* and *c2* concentrations using equations from **Jeffrey & Humphrey (1975)**.

Path Length Correction

Because well-plate path length differs from a 1 cm cuvette, apply a correction following **Warren (2007)**.

- Path length correction factor used here: **0.6 cm**
- This value may vary by instrument

Corrected Absorbance Calculations "'r datac630.$corr < -(data$c630 - datac750)/0.6$datac663.corr <- (datac663 − $data$c750) / 0.6 Units of concentration: µg mL ^{1}

To calculate a plate-specific path length, follow the procedure described in Warren (2007).

References Jeffrey, S. W. & Humphrey, G. F. (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2

Warren, C. R. (2007) Standards for chlorophyll measurement

Synergy HTX Operating Manual

Gen5 Software Manual