# **Standard protocol for coral chlorophyll *a* content**

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1. Materials
   1. 100% acetone
   2. 15 mL falcon tubes
   3. Vortex
   4. Homogenizer
   5. Table-top centrifuge
   6. Forceps
   7. Nitrile gloves
   8. Glass cuvettes (1 cm pathlength)
   9. Spectrophotometer
2. Protocol
   1. Airbrush coral fragment withddH2O (0.2 μm), homogenize blastate, and note final volume
   2. Vortex 50 mL blastate tube and aliquot out two 1.5 ml samples of tissue blastate into two pre-labeled 1.5 ml centrifuge tubes. Samples may be frozen at  
      -20°C or -80°C for future analysis.
   3. Using your unfrozen samples, centrifuge each of the samples at ca. 3,450 rpm for ca. 3 min. This will pellet all symbiont along with some host debris in each sample.
   4. Transfer pellet and host debris into 15 mL falcon tube
   5. Remove supernatant and discard in a waste container using pipette
   6. Make sure all lights are turned off in the lab. Wrap aluminum foil over falcon tubes. Then, add 5 ml of 100% acetone to each of the pellets, make sure you cover the falcon tubes COMPLETELY with aluminum foil. Equations from Jeffrey and Humphrey specify 100% acetone for dinoflagellates. 6.Disturb pellet by poking, grinding it with acetone-cleaned forceps to aid in mixing and breaking apart pellet. Clean the forceps after each use with chemwipes. Note, chlorophyll is not stable and degrades in the presence of light. Make sure to keep samples/tube rack in aluminum foil to maintain darkness.
   7. Extract chlorophyll for 36 hours in darkness
3. <<< following period of extraction >>
   1. Spin down samples at ca. 3,450 rpm for 3 minute. Take care not to disturb pellet.
   2. Pipette out (2.5mL) for each sample and read absorbance on spectrophotometer at 663 nm and 630 nm and 750 nm. The 750 nm is an internal blank. Use 100% acetone blanks between each. (reference spectrophotometer protocol)
   3. Turbidity of the sample and solvent absorbance can be corrected for by reading absorbance at 750 nm. Chlorophyll a absorbance peak is at 663 nm; chlorophyll c2 peak is at 630 nm.
   4. Once analyzed, dispose of acetone into a labeled glass waste container and rinse cuvette with 100% acetone and ddH2O water.
   5. Calculate chlorophyll (μg/ml) a concentrations using equations below: (1cm cuvette) Chlorophyll a = 11.43 (A663 - A750  
      / PL) – 0.64(A630 – A750/PL)

where PL = pathlength in cm.  
 \*The pathlength of a cuvette = 1 cm

\*Normalize to denominator of choice (protein, surface area, biomass…)

1. References
   1. Jeffrey, S., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2  
      in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz, 167(19), 1–194.
   2. Fitt, W., McFarland, F., Warner, M., & Chilcoat, G. (2000). Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnology and Oceanography, 677–685.
   3. Wall, C. 2014. Chlorophyll a / c2 in Symbiodinium protocol. Dr. Ruth Gates’ Laboratory Hawaii Institute of Marine Biology, University of Hawaii.