

## Chlorophyll-A Extraction Protocol

### Reagents:

- Saturated magnesium carbonate solution
  - 1 gram magnesium carbonate (finely powdered)
  - 100 ml of distilled water
- 90% aqueous acetone
  - 90 parts acetone (reagent grade)
  - 10 parts saturated magnesium carbonate solution
  - There are special instructions for this solution in the extraction portion of the protocol
- 0.1 N hydrochloric acid (HCl) solution (0.1N is equivalent to 0.1M)
  - Dilute the acid to 0.1N HCl appropriately based on stock acid concentration

### Filtration:

1. **Turn lights off due to chlorophyll degradation**
2. Büchner funnel is attached to a side arm flask connected either to an aspirator attached to the sink or a pump. (\*if using faucet aspirators use clear plastic flex tubing that will not collapse while suctioning)
3. Whatman glass filter (55mm diameter, 1.2um) paper is placed into the Büchner funnel and needs to be dampened with distilled water.
4. Sample water (no more than 1000mL) is then run through the filter. Ideally the full liter of the sample should be taken and filtered. However, due to clogging, as much sample that is able to be filtered should be filtered and that exact amount should be noted on the conical tubes.

### Time Restriction Directions:

5. Once filtered, paper can be removed and doused in saturated magnesium carbonate solution and stored in a **properly labeled** plastic bag. This is to prevent acidification of the chlorophyll and if need be stored in a dark, -20 degrees Celsius fridge for up to 28 days. If time allows, you may proceed straight to the next step.

### Extraction:

1. **Turn lights off due to chlorophyll degradation**
2. Filter paper is torn into smaller pieces and added to a **properly labeled** conical screw cap tube.
3. **Do not mix or disturb the aqueous acetone.** The powder  $MgCO_3$  will settle to the bottom. Only obtain the clear liquid from the top for step 4.
4. Fill the conical tube, used in step 1 of extraction, with 90% aqueous acetone solution up to the 10mL mark. Make sure the filter paper is completely covered by the solution.
5. Completely wrap the conical tube in foil.
6. Place samples upright in a test tube holder.

7. Place tube in a dark, 4 degree Celsius fridge for at least four hours to steep.
8. Spin tubes in a balanced centrifuge for 5-10 minutes at top speed. 10 minutes is preferred.
9. Liquid present in the tube (supernatant) is then ready to be placed into the cuvettes for spectrophotometric reading.

Spectrophotometry:

1. Familiarize yourself with the general spectrophotometer directions. (15 minute warm-up period)
2. The "blank" used in this procedure is distilled water. Fill the cuvette  $\frac{3}{4}$  of the way full. Each spectrophotometer must be calibrated with the dH<sub>2</sub>O blank after the warm-up period. If the wavelength is adjusted then the machine must be calibrated again.
3. Set up three spectrophotometers to read at 750 nanometers, 664 nanometers, and 665 nanometers. Place these close together as the readings are time sensitive.
4. 2 ml (0.002L) of supernatant is taken from the spun down conical screw cap tubes and added to a cuvette.
5. **The following steps are time sensitive and data must be recorded on the spreadsheet provided. Be sure to kimwipe the cuvette each time.**
6. Run the cuvette from step 4 at 750nm and record the absorbance (not transmittance). This is your 750b value.
7. Immediately run the cuvette from step 6 in the spec set to 664nm. Record the absorbance, this is your 664b value.
8. 100 microliters of HCl is added to the cuvette from step 7 to begin acidification.
9. The cuvette from step 8 is then run at 665nm after adding HCl. Record the absorbance, this is your 665a value.
10. The cuvette from step 9 is then run at 750nm after adding HCl. Record the absorbance, this is your 750a value.
11. Repeat steps 4-10 for each sample.

Equation:

- $$\frac{26.7(664b - 665a) \times V_1}{V_2 \times L}$$
  - 26.7: constant
  - Corrected 664b: spectrophotometric value **before** acidification. (664b - 750b)
  - Corrected 665a: spectrophotometric value **after** acidification. (665a - 750a)
  - $V_1$  (liters): measurement of the liquid taken off of the top of the filtered, spun down sample.
  - $V_2$  (m<sup>3</sup>): total amount of water filtered through the filter.  
Conversion: 1mL = 0.000001m<sup>3</sup> (example 1000mL filtered = 0.001)
  - L: width of cuvette is constant at 1.2cm
- Use this equation and data gathered to determine the chlorophyll-a concentration.

## Data Table

Date	Site/ Location	750b (nm)	664 b (nm)	Corrected 664b (nm)	665 a (nm)	750a (nm)	Correcte d 665a (nm)	V <sub>1</sub> (constant 2mL)	V <sub>2</sub> (m <sup>3</sup> ) Filtered volume	Length of cuvette (constant cm)	Chl-a value
	1							0.002		1.2	
	2							0.002		1.2	
	3							0.002		1.2	
	4							0.002		1.2	
	5							0.002		1.2	