

## NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION BRANCH

LABORATORY NAME:		CERT #:	
PRIMARY ANALYST:		DATE:	
NAME OF PERSON COMPLETING CHECKLIST (PRINT):			
SIGNATURE OF PERSON COMPLETING CHECKLIST:			

Parameter: **Chlorophyll a (Spectrophotometric)**  
Method: **Standard Methods 10150 B-2022**

## Equipment &amp; Reagents:

Tissue Grinder - Preferably use round-bottom grinding tubes with a matching pestle having grooves in the TFE tip.	Filters, glass fiber or membrane (0.45-µm porosity, 47-mm diameter)	Centrifuge tubes, 15-mL graduated, screw-cap.
Clinical or benchtop centrifuge (5000 to 7500 rpm) [g = (0.00001118r) (rpm), where r = centrifuge's radius].	vacuum pump	Hydrochloric acid, HCl, 0.1 N.
Cuvettes, with 1-cm path length.	Pipets, various volumes.	Solvent-resistant filter assembly (e.g., an all-glass filter-support apparatus that can be cleaned with water and organic solvents),
Aqueous acetone solution: Mix 90 parts acetone (reagent grade BP 56°C) with 10 parts saturated magnesium carbonate *	*Saturated magnesium carbonate solution: Add 1.0 g finely powdered MgCO <sub>3</sub> to 100 mL distilled water.	Spectrophotometer with a narrow band (pass) width (0.5 to 2.0 nm) because the chlorophyll absorption peak is relatively narrow. At a spectral band width of 20 nm, the chlorophyll a concentration may be underestimated by as much as 40%.

## PLEASE COMPLETE CHECKLIST IN INDELIBLE INK

Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

	GENERAL	L A B	S O P	EXPLANATION
1.	What is the most recent review/revision date of the SOP? [15A NCAC 02H .0805 (a) (7)] <b>Date:</b>			Quality assurance, quality control, and Standard Operating Procedure documentation shall indicate the effective date of the document and be reviewed every two years and updated if changes in procedures are made.  Verify proper method reference. During review notate deviations from the approved method and SOP.
2.	Are all revision dates and procedural edits tracked and documented? [15A NCAC 02H .0805 (a) (7)]			Each laboratory shall have a formal process to track and document review dates and any revisions made in all quality assurance, quality control and SOP documents.
3.	Is there North Carolina data available for review?			If not, review the annual NC WW/GW LCB Chlorophyll Round Robin data
	PRESERVATION and STORAGE	L A B	S O P	EXPLANATION
4.	What type of bottle is used for sample collection? [SM 10150 A-2022 (2)] [WW/GW LCB Chlorophyll Sample Collection, Preservation and Holding Time Policy] <b>Answer:</b>			<b>SM:</b> Use opaque containers because even brief exposure to light during storage alters chlorophyll values or wrap with aluminum foil.  <b>Policy:</b> Chlorophyll samples must be collected in bottles that protect the sample from light (e.g., brown, opaque or foil wrapped) and maintained at ≤ 6 °C, without freezing, until filtration.  Brown, wide-mouth bottles are recommended. No less than 250 mL is recommended to obtain a representative sample. Bottles should be clean and acid-free.
5.	Are samples protected from light and iced to above freezing but ≤ 6 °C during transport and storage? [WW/GW LCB Chlorophyll Sample Collection, Preservation and Holding Time Policy]			Chlorophyll samples must be collected in bottles that protect the sample from light (e.g., brown, opaque or foil wrapped) and maintained at ≤ 6 °C, without freezing, until filtration. Filtration must begin as soon as possible but not to exceed

				48 hours from the time of collection. Samples with a pH < 6 S.U. must be extracted immediately after filtration. For samples with a pH ≥ 6 S.U., filters may be wrapped in foil, placed in an air-tight bag and frozen between -20 °C and -70 °C for up to 24 days.
6.	Are samples filtered within 48 hours of collection? [WW/GW LCB Chlorophyll Sample Collection, Preservation and Holding Time Policy]			Filtration must begin as soon as possible but not to exceed 48 hours from the time of collection.
7.	If sample pH ≥ 6 S.U., are filtered samples wrapped in foil, placed in airtight plastic bags, and stored frozen between -20 °C and -70 °C for no more than 24 days and the process documented? [NC WW/GW LCB Chlorophyll Sample Collection, Preservation and Holding Time Policy]  <b>Storage temperature:</b>			For samples with a pH ≥ 6 S.U., filters may be wrapped in foil, placed in an air-tight bag and frozen between -20 °C and -70 °C for up to 24 days.
8.	If sample pH < 6 S.U., are filtered samples extracted as soon as possible after filtration? [SM 10150 A-2022 (2)] [NC WW/GW LCB Chlorophyll Sample Collection, Preservation and Holding Time Policy]			<b>Method:</b> Process samples from naturally acidic water with pH < 6 promptly after filtration to prevent possible chlorophyll degradation from residual acidic water on the filter. (Naturally acidic water has a pH < 6 S.U. due to humic acid or the contents of senescent cells, not preservatives.)  <b>Policy:</b> Samples with a pH < 6 S.U. must be extracted immediately after filtration.
	<b>PROCEDURE – Filtration and Extraction</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
9.	What type of filter is used? [SM 10150 A-2022 (2) and (2) (a) (4)]  <b>Answer:</b>			Preferably, use glass fiber filters to remove algae from water. The glass fibers help break the cells during grinding, larger volumes of water can be filtered, and no precipitate forms after acidification. Inert membrane filters, such as polyester filters, may be used when these factors are irrelevant.  Filtration equipment, filters, glass fiber (Whatman GF/F (0.7 gm), GFB (1.0 gm), Gelman AE (1 gm), or equivalent) or membrane (0.45-µm porosity, 47-mm diameter)  <b>Note:</b> A filter fluorescence blank is a good idea when membrane filters are used.
10.	What volume of sample is filtered?  <b>Answer:</b>			SM does not mention sample volume.
11.	Is the sample storage container rinsed with about 20 mL organic-free lab water (which is also passed through the same sample filter to make sure all cells are collected)? [SM 10150 A-2022 (2) (b) (1)]			Rinse the sample storage container with about 20 mL organic-free lab water (which is also passed through the same sample filter to make sure all cells are collected). Note: This implies that a whole volume sample is used for filtration.
12.	Is approximately 2 mL of MgCO <sub>3</sub> solution added to the sample just before filtering process is completed? [SM 10150 A-2022 (2) (b) (1)]			Add approximately 2 mL of MgCO <sub>3</sub> solution to the sample just before the filtering process is completed. MgCO <sub>3</sub> solution acts as a pH buffer to keep chlorophyll from degrading.
13.	Are samples filtered, extracted and analyzed under subdued lighting? [SM 10150 A-2022 (2)]  <b>Describe lighting:</b>			Conduct this procedure with chlorophyll extracts in subdued or green light to avoid degradation.  The DWR laboratory uses a dark room with green lighting.
14.	Is acetone used as the extraction solvent? [SM 10150 B-2022 (2) (b) (2)]			Place the sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution, and macerate at 500 rpm for 1 min. Use a PTFE-glass grinder for a glass-fiber filter and glass grinder for a membrane filter.
15.	Are filters mechanically ground? [SM 10150 A-2022 (2) (b) (2)]  <b>Length of time:</b>  <b>Grinding RPM:</b>			See above.

16.	Describe grinding apparatus. [SM 10150 A-2022 (2) (b) (2)] <b>Answer:</b>			Use PTFE-glass grinder for a glass-fiber filter and glass-glass grinder for a membrane filter.
17.	What is the final volume of the extracted sample? [SM 10150 A-2022 (2) (b) (3)] <b>Answer:</b>			Transfer sample to a screw-cap centrifuge tube, rinse grinder with a few milliliters 90% aqueous acetone, and add the rinse to the extraction slurry. Adjust total volume to 10 mL, with 90% aqueous acetone.
18.	How long are samples allowed to steep? [SM 10150 A-2022 (2) (b) (3)] <b>Answer:</b>			Steep the samples at least 2 h at 4°C in the dark. Note: Some literature suggests that longer steeping times improve recovery. The DWR lab steeps overnight, not to exceed 24 hours.
19.	Are samples steeped in the dark above freezing and $\leq 4$ °C? [SM 10150 A-2022 (2) (b) (3)]			Steep samples at least 2 h at 4°C in the dark.
20.	After steeping, are samples filtered or centrifuged? [SM 10150 A-2022 (2) (b) (4)] <b>Filter type (if applicable):</b> <b>Centrifuge time and speed (if applicable):</b>			Clarify by filtering through a solvent-resistant disposable filter [e.g., a 0.45 $\mu$ m PTFE 13 mm syringe filter (to minimize retention of extract in filter and filter holder, force 1 to 2 mL air through filter after extract)] or by centrifuging in closed tubes for 20 min at 500 g or 3000 rpm.
21.	Is the clarified extract decanted into a clean, calibrated, 15-mL, screw-cap centrifuge tube and the total volume measured? [SM 10150 A-2022 (2) (b) (4)]			Decant clarified extract into a clean, calibrated, 15-mL, screw-cap centrifuge tube and measure total volume.
	<b>PROCEDURE – Sample Analysis</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
22.	Is 3 mL of clarified extract transferred to a 1-cm cuvette and the absorbance measured at 750 and <b>664 nm</b> ? [SM 10150 B-2022 (2) (a)]			Transfer 3 mL clarified extract to a 1-cm cuvette and read absorbance at 750 and <b>664 nm</b> .
23.	Is the extract acidified in the cuvette with 0.1 mL 0.1 M HCl, gently agitated and allowed to sit for 90 seconds? [SM 10150 B-2022 (2) (a)]			Acidify the extract in the cuvette with 0.1 mL 0.1 M HCl. Gently agitate the acidified extract and, 90 s after acidification, read absorbance at 750 and 665 nm. The volumes of extract, volume of acid, and the time after acidification are critical for accurate, consistent results.
24.	Is a cuvette with longer path length used for very dilute extracts? [SM 10150 B-2022 (2) (a)]			For very dilute extracts, use cuvettes with a longer path. If a larger cell is used, add a proportionately larger volume of acid. Correct absorbance obtained with larger cuvettes to 1 cm before making calculations.
25.	If a longer path length is used, is a proportionately larger volume of acid used? [SM 10150 B-2022 (2) (a)]			See above
26.	90 seconds after acidification, is the absorbance measured at 750 and at <b>665 nm</b> ? [SM 10150 B-2022 (2) (a)]			Gently agitate the acidified extract and, 90 s after acidification, read absorbance at 750 and at <b>665 nm</b> .
27.	Is the 750-nm absorbance value subtracted from the readings <b>before acidification (absorbance 664 nm) and after acidification (absorbance 665 nm)</b> ? [SM 10150 B-2022 (2) (a)]			The absorbance reading at 750 nm is a correction for turbidity. Subtract the 750-nm absorbance value from the other readings before using them in the calculations below.
28.	How is the chlorophyll-a concentration calculated? [SM 10150 B-2022 (2) (a)] <b>Show Calculation:</b>			Using the corrected values, calculate chlorophyll a and pheophytin a per cubic meter as follows:

				$\text{Chlorophyll } a, \text{ mg/m}^3 = \frac{26.7 (664_b - 665_a) \times V_1}{V_2 \times L}$ $\text{Pheophytin } a, \text{ mg/m}^3 = \frac{26.7 [1.7(665_a - 664_b) \times V_1]}{V_2 \times L}$ <p>where:</p> <p><math>664_b, 665_a</math> = absorbance of 90% acetone extract before and after acidification, respectively.</p> <p><math>V_1</math> = volume of extract (L),</p> <p><math>V_2</math> = volume of sample (m<sup>3</sup>), and</p> <p><math>L</math> = light path length or width of cuvette (cm).</p>
	QUALITY ASSURANCE	L A B	S O P	EXPLANATION
29.	Does the laboratory analyze duplicate samples at a rate of 5% or greater? [15A NCAC 02H .0805 (a) (7)]			Except where otherwise specified in an analytical method, laboratories shall analyze five percent of all samples in duplicate to document precision. Laboratories analyzing fewer than 20 samples per month shall analyze one duplicate during each month that samples are analyzed.
30.	What is the acceptance criterion for sample duplicates? [15A NCAC 02H .0805 (a) (7) (A)] <b>Answer:</b>			Establish laboratory control limits.  Unless specified by the method or this Rule, each laboratory shall establish performance acceptance criteria for all quality control analyses.
31.	What corrective action does the laboratory take if the duplicate samples results are outside of established control limits or method precision limits? [15A NCAC 02H .0805 (a) (7) (B)] <b>Answer:</b>			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible. If the sample cannot be reanalyzed, or if the quality control results continue to fall outside established limits or show an analytical problem, the results shall be qualified as such.
32.	[Recommended] Is a reference standard analyzed to test wavelength accuracy? <b>State standard type and frequency:</b>			
33.	[If applicable] What is the acceptance criterion for the reference standard? [15A NCAC 02H .0805 (a) (7) (A)] <b>Answer:</b>			Establish laboratory control limits.  Unless specified by the method or this Rule, each laboratory shall establish performance acceptance criteria for all quality control analyses.
34.	[If applicable] What corrective action is taken when the reference standard recovery exceeds the acceptance criterion? [15A NCAC 02H .0805 (a) (7) (B)] <b>Answer:</b>			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible. If the sample cannot be reanalyzed, or if the quality control results continue to fall outside established limits or show an analytical problem, the results shall be qualified as such.
35.	Are results qualified to indicate quality control failures or sample anomalies when reporting results? [15A NCAC 02H .0805 (e) (5)]			Reported data associated with Quality Control failures, improper sample collection, holding time exceedances, or improper preservation shall be qualified as such.

Additional Comments:

Inspector: \_\_\_\_\_ Date: \_\_\_\_\_