



Designation: D3731 – 24

Standard Practices for Measurement of Chlorophyll Content of Algae in Surface Waters¹

This standard is issued under the fixed designation D3731; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope*

1.1 These practices include the extraction and the measurement of chlorophyll *a*, *b*, and *c*, and pheophytin *a* in freshwater and marine plankton and periphyton. Three practices are provided as follows:

1.1.1 Spectrophotometric, trichromatic practice for measuring chlorophyll *a*, *b*, and *c*.

1.1.2 Spectrophotometric, monochromatic practice for measuring chlorophyll *a* corrected for pheophytin *a*; and for measuring pheophytin *a*.

1.1.3 Fluorometric practice for measuring chlorophyll *a* corrected for pheophytin *a*; and for measuring pheophytin *a*.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 8.

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*²

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

3. Terminology

3.1 *Definitions:*

¹ These practices are under the jurisdiction of ASTM Committee D19 on Water and are the direct responsibility of Subcommittee D19.24 on Water Microbiology.

Current edition approved Nov. 1, 2024. Published November 2024. Originally approved in 1979. Last previous edition approved in 2020 as D3731 – 20. DOI: 10.1520/D3731-24.

² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at www.astm.org/contact. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.1.2 *chlorophyll, n*—a class of pigments involved in photosynthesis.

3.1.3 *periphyton, n*—microorganisms growing on submerged objects, commonly consisting of bacteria, algae, protozoa, and rotifers.

3.1.4 *pheopigments (phaeopigments), n*—a non-photosynthetic pigment which is the degradation product of algal chlorophyll pigments.

3.1.5 *plankton, n*—nonmotile or weakly swimming organisms, usually microscopic, that drift or are carried along by currents in surface waters, commonly consisting of bacteria, algae, protozoa, rotifers, and microcrustacea.

4. Summary of Practices

4.1 The chlorophyll and related compounds are extracted from the algae with 90 % aqueous acetone. The concentration of the pigments is determined by measuring the light absorption or fluorescence of the extract.

5. Significance and Use

5.1 Data on the chlorophyll content of the algae have the following applications:

5.1.1 To provide estimates of algal biomass and productivity.

5.1.2 To provide general information on the taxonomic composition (major groups) of the algae, based on the relative amounts of chlorophyll *a*, *b*, and *c*, and the physiological condition of algal communities, which is related to the relative abundance of pheopigments.

5.1.3 To determine long-term trends in water quality.

5.1.4 To determine the trophic status of surface waters.

5.1.5 To detect adverse effects of pollutants on plankton and periphyton in receiving waters.

5.1.6 To determine maximum growth rates and yields in algal growth potential tests.

6. Interferences and Special Considerations

6.1 *Pigment Extraction*—The chlorophylls are only poorly extracted, if at all, from some forms of algae, such as the coccoid green algae, unless the cells are disrupted, whereas other algae, such as the diatoms, give up their pigments very

*A Summary of Changes section appears at the end of this standard