



Designation: D4012 – 23a

Standard Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water¹

This standard is issued under the fixed designation D4012; 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 This test method covers a protocol for capturing, extracting and quantifying the cellular adenosine triphosphate (cATP) content associated with microorganisms normally found in laboratory cultures and waters in plankton and periphyton samples from waters.

1.2 The ATP is measured using a bioluminescence enzyme assay, whereby light is generated in amounts proportional to the concentration of ATP in the samples. The light is produced and measured quantitatively as relative light units (RLU) which are converted by comparison with an ATP standard and computation to pg ATP/mL.

1.3 This method does not remove all known chemical interferences, known to either luminesce in the 530 nm $\pm$ 20 nm range, or to quench light emitted in that range. It should not be used to determine ATP concentrations in samples with dissolved organic compounds, heavy metals or >10 000 ppm total dissolved solids. Alternative methods have been developed for determining ATP concentrations in fluids samples likely to contain such interferences (Test Methods [D7687](#) and [E2694](#)).

1.4 Knowledge of the concentration of ATP can be related to viable biomass or metabolic activity of microorganisms ([Appendix X1](#)).

1.5 This test method offers a high degree of sensitivity, rapidity, accuracy, and reproducibility.

1.6 The analyst should be aware that the precision statement pertains only to determinations in reagent water and not necessarily in the matrix being tested.

1.7 This test method is equally suitable for use in the laboratory or field.

1.8 The method normally detects cATP concentrations in the range of 0.1 pg cATP/mL (-1.0Log_{10} [pg cATP/mL]) to 4 000 000 pg cATP/mL (6.6Log_{10} [pg cATP/mL]) in 50 mL water samples.

1.9 Providing interferences can be overcome, bioluminescence is a reliable and proven method for qualifying and quantifying ATP, although the method does not differentiate between ATP from different sources, for example, from different types of microorganisms, such as bacteria, fungi, algae and protozoa.

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

1.11 *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.*

1.12 *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](#)

[D1601 Test Method for Dilute Solution Viscosity of Ethylene Polymers](#)

[D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants](#)

[D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis](#)

[D6161 Terminology Used for Microfiltration, Ultrafiltration, Nanofiltration, and Reverse Osmosis Membrane Processes](#)

[D6300 Practice for Determination of Precision and Bias Data for Use in Test Methods for Petroleum Products, Liquid Fuels, and Lubricants](#)

[D7687 Test Method for Measurement of Cellular Adenosine](#)

¹ This test method is under the jurisdiction of ASTM Committee [D19](#) on Water and is the direct responsibility of Subcommittee [D19.24](#) on Water Microbiology.

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