



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.

Current edition approved Nov. 1, 2023. Published November 2023. Originally approved in 1981. Last previous edition approved in 2023 as D4012 – 23. DOI: 10.1520/D4012-23A.

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.

Triphosphate in Fuel and Fuel-associated Water With Sample Concentration by Filtration

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E2694 Test Method for Measurement of Adenosine Triphosphate in Water-Miscible Metalworking Fluids

F1671 Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System

3. Terminology

3.1 Definitions:

3.1.1 For definitions of terms used in this standard, refer to Terminologies **D1129** and **D4175**.

3.1.2 *adenosine monophosphate (AMP)*, *n*—molecule formed by the removal of two molecules of phosphate (one pyrophosphate molecule) from ATP.

3.1.3 *adenosine triphosphate (ATP)*, *n*—molecule comprised of a purine and three phosphate groups that serves as the primary energy transport molecule in all biological cells.

3.1.4 *aseptic, adj*—sterile, free from viable microbial contamination.

3.1.5 *background RLU*, *n*—quantity of relative light units resulting from running the test method without incorporation of the sample.

3.1.6 *bioluminescence*, *n*—production and emission of light by a living organism as the result of a chemical reaction during which chemical energy is converted to light energy.

3.1.7 *biomass*, *n*—any matter which is or was a living organism or excreted from a microorganism. **D6161**

3.1.8 *cellular adenosine triphosphate (cellular-ATP; c-ATP)*, *n*—ATP present in whole cells, whether they are living or dead.

3.1.8.1 *Discussion*—Cellular-ATP is released upon intentional lysis of microbial cells during the sample preparation process. Microbially infected fluids contain both cellular (cell-associated/cell-bound) and extra-cellular ATP.

3.1.9 *culturable, adj*—(microorganisms that are) able to proliferate as indicated by the formation of colonies on solid growth media or the development of turbidity in liquid growth media under specific growth conditions.

3.1.10 *extra-cellular, adj*—(molecules or substances that are either) excreted by living cells or released from microbial cells that have lysed (see **3.1.14**) in the sample.

3.1.10.1 *Discussion*—Extra-cellular ATP is ATP that has been released from microbial cells that have either fully or partially lysed in the sample, the upstream fluid (fuel or water phase), or both.

3.1.10.2 *Discussion*—Lysis can occur due to natural life cycle process, antimicrobial treatment or a combination of these factors. Extra-cellular ATP can under certain circumstances persist for periods greater than 24 h after cell lysis depending on physical/chemical conditions.

3.1.11 *luciferase, n*—general term for a class of enzymes that catalyze bioluminescent reactions.

3.1.12 *luciferin, n*—general term for a class of light-emitting biological pigments found in organisms capable of bioluminescence.

3.1.13 *luminometer, n*—instrument capable of measuring light emitted as a result of nonthermal excitation.

3.1.14 *lysis, n*—disintegration or destruction of whole bacterial cells. **F1671**

3.1.15 *relative light unit (RLU)*, *n*—instrument and assay specific unit of measurement reflecting the number of photons emitted by the Luciferin-Luciferase driven hydrolysis of ATP to AMP plus pyrophosphate.

3.1.15.1 *Discussion*—RLU is not an SI unit, however, RLU are proportional to ATP concentration.

3.1.16 *viable microbial biomass, n*—metabolically active (living) microorganisms.

3.2 Acronyms:

3.2.1 *AMP*, *n*—adenosine monophosphate.

3.2.2 *ATP*, *n*—adenosine triphosphate.

3.2.3 *HDPE*, *n*—high density polyethylene.

3.2.4 *PP*, *n*—polypropylene.

3.2.5 *pg*, *n*—picogram (1×10^{-12} g).

3.2.6 *RLU*, *n*—relative light unit.

4. Summary of Test Method

4.1 A control assay is performed using 100 μ L of 1.0 ng $\pm$ 0.05 ng ATP/mL standard to produce RLU_{ctrl} .

4.2 A 50 mL sample of water is placed into a syringe and then pressure-filtered through a 0.7 μ m, glass-fiber, in-line, depth filter.

4.3 A lysing reagent is used to release cellular-ATP from microbial cells that have been captured on the glass-fiber filter, and the filtrate is dispensed into an unused culture tube.

4.4 The filtrate is diluted 1 to 10 with a buffer solution.

4.5 A 100 μ L volume of diluted filtrate is transferred to an unused culture tube into which 100 μ L of Luciferin-Luciferase reagent has been previously dispensed.

4.6 The culture tube is placed into a luminometer and the light intensity is read as RLU_{obs} .

4.7 RLU_{obs} is normalized to an actual pg ATP/mL concentration through an equation that accounts for the result of the control assay (RLU_{ctrl}), the volume of the sample processed, and the method dilution factor.

NOTE 1—Optionally, for condition monitoring purposes, pg ATP/mL are converted to Log_{10} [pg ATP/mL] of sample by computation.

5. Significance and Use

5.1 A rapid and routine procedure for determining biomass of the living microorganisms in cultures, waters, wastewaters, and in plankton and periphyton samples taken from surface waters is frequently of vital importance. However, classical techniques such as direct microscope counts, turbidity, organic