



Standard Test Method for Measurement of Adenosine Triphosphate in Water-Miscible Metalworking Fluids¹

This standard is issued under the fixed designation E2694; 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 provides a protocol for capturing, extracting and quantifying the adenosine triphosphate (ATP) content associated with microorganisms found in water-miscible metalworking fluids (MWF).

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

1.4 The test method detects ATP concentrations in the range of 4.0 pg ATP/mL to 400 000 pg ATP/mL.

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

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

1.7 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:²

D1129 Terminology Relating to Water

¹ This test method is under the jurisdiction of ASTM Committee E34 on Occupational Health and Safety and is the direct responsibility of Subcommittee E34.50 on Health and Safety Standards for Metal Working Fluids.

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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.

D4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water

D4840 Guide for Sample Chain-of-Custody Procedures

D6161 Terminology Used for Microfiltration, Ultrafiltration, Nanofiltration and Reverse Osmosis Membrane Processes

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

E1326 Guide for Evaluating Non-culture Microbiological Tests

E1497 Practice for Selection and Safe Use of Water-Miscible and Straight Oil Metal Removal Fluids

E2523 Terminology for Metalworking Fluids and Operations

2.2 Government Standards:³

29 CFR 1910.1000 Occupational Safety and Health Standards; Air contaminants

29 CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories

3. Terminology

3.1 *Definitions:* For definition of terms used in this method, refer to Terminology standards **D1129**, **D6161**, and **E2523**.

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

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

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

3.5 *bioluminescence*, *n*—the 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.

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

³ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, <http://www.access.gpo.gov>.

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