

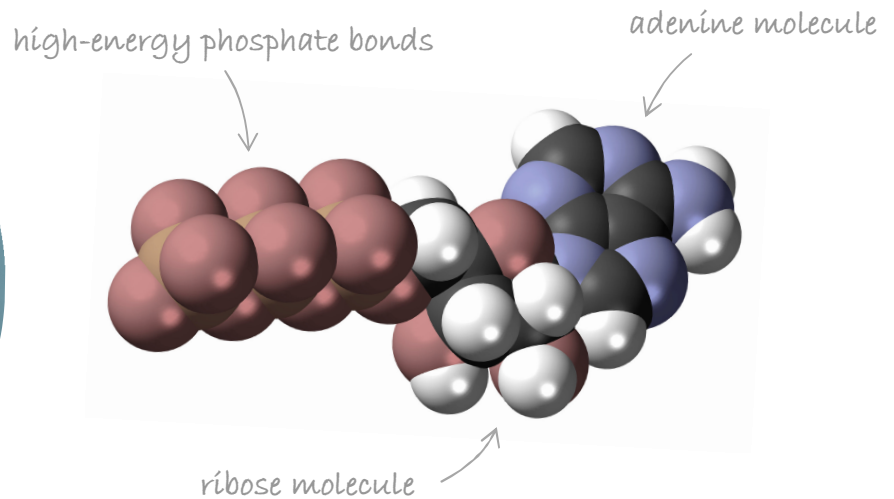
On-line Monitoring of  
Bacterial and Pathogen Load  
Through Adenosine Triphosphate.

The EZ-ATP® On-line Microbiology Analyzer.



# Facts about ATP, microbial load and water quality tests

Adenosine triphosphate or ATP is the energy currency of life on our planet. It is a high energy molecule found inside and around living cells and used to activate the biological functions of individual cells, tissues or even organs of complex organisms. ATP is in fact so fundamental to life that it can be considered an integral part of the “design specifications” of any living cell or organism.



The ASTM D4012-81 “firefly method” was developed as an alternative to traditional microbial tests such as cell tagging, plate counting and turbidity. It involves a rapid, sensitive determination of viable biomass of bacteria in water and waste water, cleaning and hygiene applications. The current need for rapid, routine monitoring of the bacterial safety of drinking water has sparked renewed interest in ATP.

An important challenge in analyzing bacterial and pathogen load in water is not as much the total ATP levels, but also differentiate properly between living and non-living cells. Total ATP numbers fail to give a clear picture of the intrinsic water safety. This can only be achieved by measuring the individual portions of intracellular - extracellular ATP using special sample pre-treatment techniques.

# EZ-ATP<sup>®</sup> — The first microbiology analyzer using the ATP firefly assay

## Features and general performance

- **Complying** with internationally accredited ASTM D4012-81 standard test method
- Measuring **total ATP** portions of any type of bacterial microorganism
- **Complete ATP recovery**: detection of intracellular, extracellular and total ATP
- **No bias** from the composition of the growth medium such as with plate counting
- **Rapid** measurement: 10 - 15 minutes (including lysis)
- **No delay** between sample take-off and measurement
- **Low cost** of analysis relative to a large number of results
- **Smart** features: automatic calibration and cleaning protocol
- **Low limit of detection** (LOD): 0.05 ng/L (0.1 pM) ATP
- **Low maintenance**, easy replaceable reagent kit
- **Multiple streams** possible (up to 8)

*Image: front view of the EZ-ATP. The transparent door allows instant inspection of the analytical part. On the left: external sample unit with lysis. Inside: long-life sample and drain pumps. Carefully acclimatized and sealed from ambient light, the black reagent chamber contains enzyme and substrate, and operates the automatic firefly assay.*



# EZ-ATP® — Bringing automation, reliability and performance in ATP testing

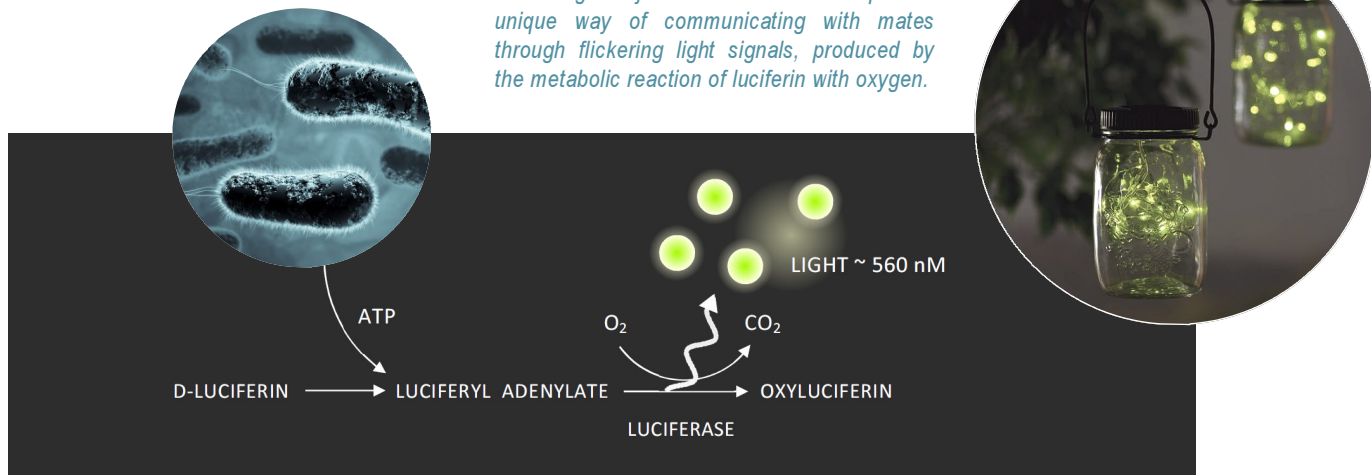
The basic analysis principle conducted by the mainframe of the **EZ-ATP®** On-line Microbiology Analyzer is comparable with ATP test kits, in a way that quantification takes place by means of a chemiluminescence reaction similar to the metabolism found in fireflies.

As shown in the schematic reaction below, luciferin is the substrate in a 2-step chemical reaction to oxidize the ATP, catalyzed by the firefly luciferase enzyme. The exothermic reaction with oxygen releases energy to produce visible light. This light output is proportional to the amount of ATP used in the reaction, thus indicating its presence in the sample. High ATP values indicate a potential risk in surpassing threshold values of certain microorganisms in the past or in the near future.

The light output of the reaction between ATP and luciferin is measured with utmost precision by the **EZ-ATP®** analyzer and expressed as Relative Light Units (RLU). Since quality reagents are key to optimum analytical performance, AppliTek has joined forces with ATP experts from Promega. Contained in the internal acclimatized reagent chamber, the proprietary reagents offer superior stability and sensitivity, resulting in seamless, convenient operation and excellent limits of detection.

- Superior Water-Glo™ Luciferin and luciferase reagents
- Reagent stability of up to 12 months
- Reagent replenishment only after 2500 full analysis cycles

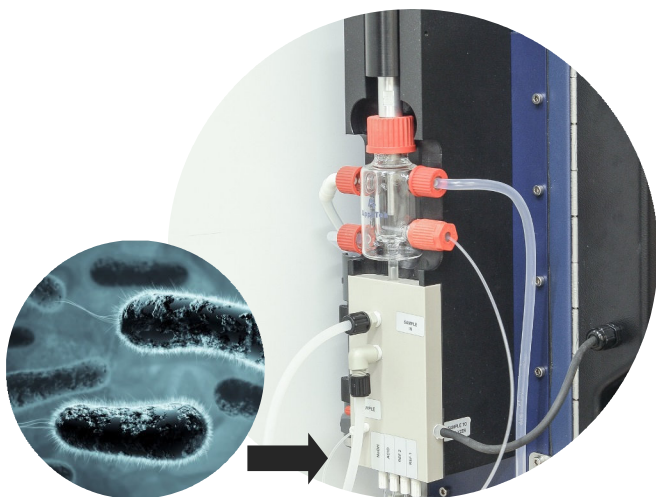
*Image: the enchanting spectacle of fireflies held in glass jars. Fireflies have adopted a unique way of communicating with mates through flickering light signals, produced by the metabolic reaction of luciferin with oxygen.*



# EZ-ATP® — Proprietary sample pretreatment for quantifying different ATP portions

The firefly assay by detection of adenosine triphosphate is capable of directly assessing living microorganisms from any other type of cell, providing a direct measure of the microbial load or contamination of your water sample: fecal coliforms (*E. coli*), sulphate reducing bacteria, nitrifying bacteria, Legionella...

Knowing the different ATP portions —extracellular (free) and intracellular— allows to follow closely abnormalities in microbial levels or even biocide control programs. For this reason AppliTek developed a proprietary sample treatment unit for the **EZ-ATP®**, mounted on the sample entry side of the mainframe (see image below), with variable lysis treatment settings. Samples are automatically taken from a source or fast loop and processed by the analyzer's controller software until calculation of results.



EZ-ATP 29/10/2015 16:50:48				
Parameter	CH	Result	Unit	Alarm
ATP free		0	pg/mL	OK
ATP total		0	pg/mL	OK
ATP bact		0	pg/mL	OK
Dark Count Free		0	RLU	OK
PMT Direct	Direct	0	RLU	OK

1	E-Stop		
User: Automatic			
1. ATP free	2. ATP total	3. Man free	

The entire analysis cycle (10 - 15 minutes) consists of two consecutive measurements, one with a non-chemical lysis to desintegrate all living cells and determine the ATP from living cells. The intracellular portion of ATP is calculated as [total ATP – extracellular ATP].

Additional features include:

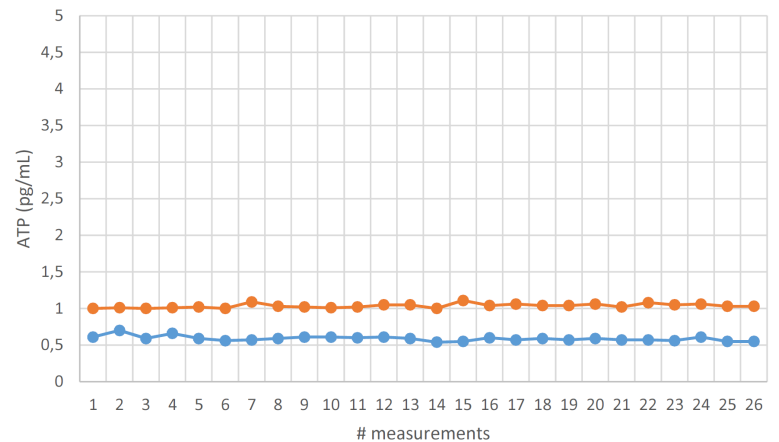
- Multiple streams possible (up to 8)
- Leak protection
- 3-step cleaning sequence to remove biofilm formation and residual ATP
- Sampling either by direct injection or from atmospheric overflow vessel
- Extra option for grab sample analysis

# Test data

With the development of the **EZ-ATP®** On-line Microbiology Analyzer, AppliTek aims to present a unique alternative to current manual analysis procedures in microbiology, and adding a new dimension to the possibilities of the standard ASTM firefly assay. In a process environment or for any process where the microbiological water quality is critical, corrective actions depend on timely and accurate data.

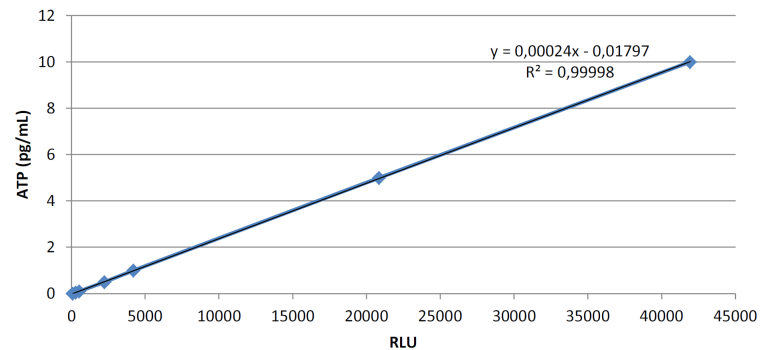
In the first graph on the right, results are displayed from a test performed on regular tap water sampled at the point-of-use. Exemplary low-level detection of ATP (in pg/mL), with safe levels of free (*blue*) and total ATP (*orange*) as per local water regulations. Each discrete point of measurement follows a 10 - 15 minutes interval.

ATP Free vs ATP Total on tap water



The second graph displays the factory calibration of the analyzer with ATP standard solution. Excellent correlation is obtained between actual and expected results in the measuring range of 0 - 10 pg/mL. This highlights the importance of using quality standard solutions in any given application when it comes to optimum analytical performance.

Calibration curve ATP



RLU = Relative Lights Units

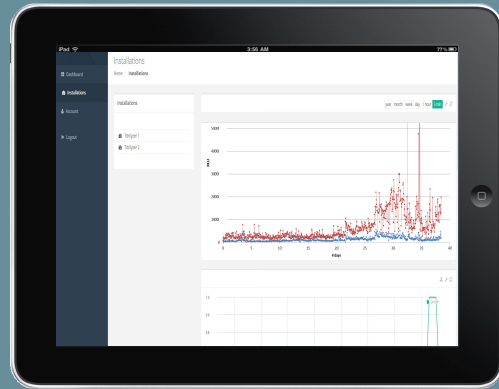
## Technical specifications

<b>Analytical data</b>	<b>Reagents</b>	<b>Environmental data</b>
<b>Analysis method</b> Standard method ASTM D4012-81 Determination of adenosine triphosphate (ATP) by means of chemiluminescent reaction using luciferine and luciferase	<b>Reagent containers (included)</b> Outside cabinet: 4 Containers come with torqueless screw caps.	<b>Ambient operating conditions</b> 10 °C – 30 °C +/- 4 °C deviation at 5 - 95% relative humidity non-condensing (50 °F – 86 °F +/- 7.2 °F deviation)
<b>Parameter</b> Microbial load as ATP (free, total and intracellular)	<b>Reagent solutions</b> Luciferine ≤ 150 mL* / 28 days Luciferase ≤ 150 mL* / 28 days * Based on 1 analysis result/30min	<b>Reagent temperature</b> Keep between 10 °C - 30 °C (50 °F - 86°F)
<b>Measuring ranges</b> 0.5 - 200 pg/mL (0.1 - 400 pM) ATP Higher ranges on request.	<b>Calibration solutions</b> Trish HCl buffer + ATP standard Consumption depends on pre-programmed sequence; ≤ 250 mL / 28 days	<b>Sample pressure</b> By external overflow vessel Or < 3 bar by direct injection
<b>Cycle time</b> 10 - 15 minutes (including lysis)	<b>Cleaning solutions</b> Sodium hydroxide + hydrochloric acid Consumption depends on pre-programmed sequence; ≤ 5 L / 28 days	<b>Sample flow rate</b> Fast loop sample supply required: 200 - 300 ml/min
<b>Calibration</b> Factory calibrated (2-point) Typically 45 minutes	<b>Utilities</b>	<b>Sample particulates</b> Maximum size 200 µm, < 0.1 g/l
<b>Cleaning</b> Automatic, free adjustable sequence	<b>Power</b> 110 - 240 VAC, 2 A, 50 Hz Max. power consumption: 120 VA	<b>Control and communication</b>
<b>Precision / Repeatability</b> 1 pg/mL (range 0.5 - 10 pg/mL) On standard solutions	<b>Instrument air (purging)</b> Dry and oil free according to ISA-S7.0.01-1996 quality standard for instrument air	<b>User interface / controller</b> Industrial PC with 5.7" TFT colour user interface, compact flash memory
<b>Mechanical data</b>	<b>Tap water</b> For rinsing, calibration and/or dilution	<b>Data handling, logging and security</b> <ul style="list-style-type: none"> <li>• Standard Ethernet 10 M (RJ45) NE 2000</li> <li>• Communication ports supporting Ethernet connectivity to MODBUS TCP/IP</li> <li>• Log files with 1,000 values/results are stored</li> <li>• Easy export to spreadsheet files</li> <li>• Sealed USB port for data download/upload</li> <li>• User interface with administrator access and menu keys activated/inactivated</li> <li>• Data retention in case of power failure, initialization program for safe status after restart</li> </ul>
<b>Protection class</b> Analyzer cabinet: IP55 / Panel PC: IP65	<b>Drain</b> Atmospheric pressure, vented, min. Ø 64 mm	<b>Analogue outputs</b> Total: 8, active 4 –20 mA Max. load 500 Ohm
<b>Cabinet and materials, hinged part</b> Thermoform ABS / Door: plexiglass	<b>Earth connection</b> Dry and clean earth pole with low impedance (< 1 ohm) using an earth cable of > 2.5 mm <sup>2</sup>	<b>MODBUS TCP/IP, MODBUS-RS232 -RS485</b> Optional
<b>Cabinet and materials, wall section</b> Galvanized steel, powder coated		
<b>Dimensions (H X W X D)</b> 69 cm (27.2") x 46.5 cm (18.3") x 33 cm (13")		
<b>Total weight</b> 35 kg (77 lbs.)		

# Take control over water quality and safety in your process

Current microbiological standards are still based on time-consuming laboratory methods, which have been used for many years to measure the total microbial population in water samples. Not surprisingly, the need for real-time water quality data has sparked new interest in the use of the well-known ATP assay.

Enter AppliTek's **EZ-ATP®** On-line Microbiology Analyzer, providing you results in minutes and giving an objective, measurable basis for actions against sudden changes in bacterial and pathogen levels. Knowing that their vulnerable water source is permanently monitored, is truly reassuring for operators, engineers, plant managers and last but not least the general public.



*Example of EZ-ATP data communicated by secure VPN connection to mobile network and accessed by a tablet PC.*

**Application fields include: Drinking water - Bottling - Raw water intake - Industrial cooling systems - Data center HVAC - RO demineralization - Desalination - Oil & gas**



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