



DOC313.53.94312

## **EZ7300 ATP Analyser**

Method and reagent sheets

03/2022, Edition 1.01

1. Legal information.....	3
2. Analytical specifications.....	3
3. Analysis method .....	4
4. Reagents .....	6
4.1 Reagent overview and consumption .....	7
4.2 DI-water overview and consumption .....	7
4.3 Storage and quality of chemicals.....	8
4.4 EZ-ATP Water-Glo detection reagent.....	9
4.5 EZ-ATP Calibration solution.....	9
4.6 NaOH solution (1M).....	11
4.7 HCl Solution (1M).....	11
4.8 Cleaning solution .....	12

## 1. Legal information

Manufacturer: AppliTek NV/SA

Distributor: Hach Lange GmbH

The translation of the manual is approved by the manufacturer.

## 2. Analytical specifications

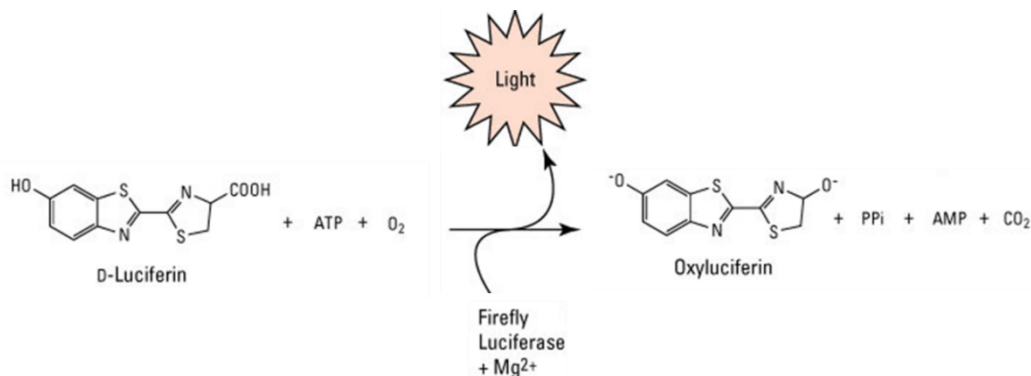
Please refer also to the respective technical datasheet at Hach Support Online.

ATP - All specifications			
<b>Analysis method</b>	Determination of adenosine triphosphate (ATP) by means of chemiluminescent reaction using luciferin and luciferase, conform with standard method ASTM D4012-81		
<b>Parameter</b>	ATP		
<b>Cycle time</b>	Standard measurement cycle time: 10 minutes (incl. sample lysis)		
<b>Limit of detection (LOD)</b>	≤ 0.05 pg/mL (0.1 pM) ATP		
<b>Precision/Repeatability</b>	Better than 4% full scale range for standard test solutions		
<b>Cleaning</b>	Automatic; 3-step; frequency freely programmable		
<b>Calibration</b>	Automatic, 2-point; frequency freely programmable		
<b>Validation</b>	Automatic; frequency freely programmable		
<b>Interferences</b>	High concentrations of Hg <sup>2+</sup> , Cu <sup>2+</sup> , Zn <sup>2+</sup> , Cd <sup>2+</sup> , Fe <sup>2+</sup> . Total salt concentrations higher than 1 g/L. pH lower than 5.5 and higher than 8.		
<b>Measuring ranges</b>	<b>% of range - Dilution</b>	<b>Low range (pg/mL)</b>	<b>High range (pg/mL)</b>
	<b>standard range</b>	<b>0.05</b>	<b>200</b>

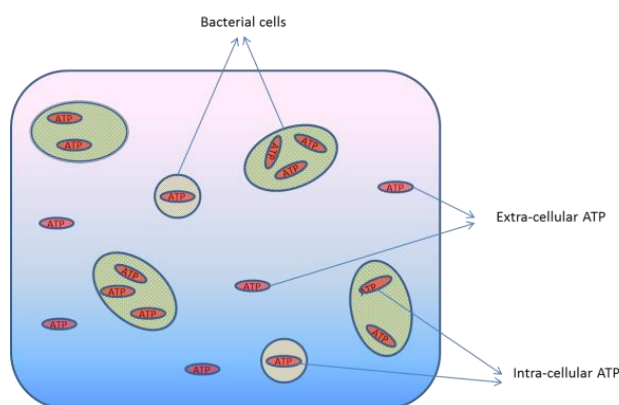
### 3. Analysis method

#### Summary

**ATP** (Adenosine Triphosphate) is used by all organisms, from the simplest bacteria to human being, as their primary energy currency. ATP transports chemical energy within cells for metabolism. The EZ-ATP® on-line analyzer quantifies the ATP concentration in the sample by counting the **light units** that are produced through reaction with luciferin and luciferase. The higher the light output, the higher the ATP content and the higher the bacterial load of the sample.



The ATP measurement consists of two parts, a free and a total ATP measurement. In a water sample, ATP can be located freely in the water mass, coming from disrupted cells. This is called extracellular ATP or **free ATP**. Another possibility is the presence of ATP inside bacteria and other cells. This is called **intercellular ATP**. In order to have a clear picture of the biological load, it is important to differentiate between these two types of ATP. The portion of intracellular ATP correlates with the living bacterial biomass and the portion of free ATP can indicate previous cell growth. The **total** amount of **ATP** is the sum of free and intracellular ATP.



#### Analysis steps

The analyzer is designed and programmed to perform each analysis procedure automatically. This procedure involves flushing, cleanings, sampling, analyzing the sample (addition of reagents, ultrasonic treatment and measurement) and data processing. The bioluminescent reaction takes place in a measuring cell and is detected with a built-in photon multiplier tube (PMT). The reaction is temperature controlled by use of a heating/cooling system. Lysis of the cell, to release intracellular ATP, is performed by use of an ultrasonic probe.


### **Calibration**


The calibration procedure measures a REF1 solution (channel 9, REF1 valve) and a REF2 solution (channel 10, REF2 valve) to adapt the slope and offset factors by means of a two-point calibration.

### **Cleaning**

A built-in cleaning can be performed at programmable intervals to avoid biological growth in the flowcell and tubing. The 3-step cleaning procedure includes a HCl flushing step, followed by a NaOH and de-ionized water flush. The total cleaning time is 15 minutes.

## 4. Reagents

<b>⚠ CAUTION</b>	
	Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Read the safety data sheet from the supplier before bottles are filled or reagents are prepared. For laboratory use only. Make the hazard information known in accordance with the local regulations of the user.

<b>⚠ CAUTION</b>	
	Chemical exposure hazard. Dispose of chemicals and wastes in accordance with local, regional and national regulations.

## 4.1 Reagent overview and consumption

In the tables below, the products that are needed to prepare the reagents are listed. The product name, the formula, the molecular weight, the CAS No. and the amount needed to prepare 1 liter of the reagents is given. Check the consumption of the reagents (28 days) to adapt the volumes needed.

Product	Consumption	Consumption/28 days A rata 1 analysis/10 min	Recommended containers
Detection reagent	~ 0.1 mL / analysis	~ 405 mL	Plastic – 250 mL
NaOH solution	~ 25 mL / cleaning	Depending on frequency of cleaning	Plastic – 5 L
HCl solution	~ 25 mL / cleaning	Depending on frequency of cleaning	Plastic – 5 L
REF1 solution	~ 100 mL/calibration	Depending on frequency of calibration	Plastic – 100 mL
REF2 solution	~ 100 mL/calibration	Depending on frequency of calibration	Plastic – 100 mL

## 4.2 DI-water overview and consumption

	Rinse water (mL/analysis) Type I	Dilution water (mL/analysis) Type I	Total (mL/analysis)	Consumption/28 days A rata 1 analysis / 10 min
0	N.A.	N.A.	N.A.	N.A.

## 4.3 Storage and quality of chemicals

### Quality of chemicals

All chemicals should be of Reagent grade, ACS grade or better (\*). The use of pro analysis chemicals is recommended. Poor quality of the reagents can affect the analyser performance.

(\* Analytical Reagent (AR), Guaranteed Reagent (GR), UNIVAR, AnalaR, Premium Reagent (PR), ReagentCertified ACS reagent, ACS Plus reagent, puriss p.a. ACS reagent, ReagentPlus®, TraceCERT®, Suprapur®, Ultrapur®, or better are also possible.

### Quality of DI-water

All EZ analysers are tested with standard solutions, reagents and dilution water prepared using type I water or better as defined by ASTM D1193-91.

To achieve the specifications as stated on the data sheet, method and reagents sheet and acceptance test reports, the same water quality (or better) must be used for the preparation of the standard solutions, reagents and dilution water.

Additionally the water used for the preparation of the standard solutions for an EZ analyser must be free of the parameter or any of the interferences for the method of that EZ analyser.

### Storage of Reagents

While operating the instrument, keep in mind the reagent requirements as stated in the reagent overview, the chapters below and/or in the data sheet of the instrument.

#### CAUTION



For longer-term storage: Store the reagents cold; Store the reagents in the dark;  
If applicable: Store the reagents in a fridge during operation

#### CAUTION



Refresh the reagents after one month (unless stated differently in the chapters below).  
Do not mix old reagents with freshly prepared reagents. Remove old reagents from the container before adding freshly prepared reagents.



## 4.4 EZ-ATP Water-Glo detection reagent

### Storage

Store the detection reagent always in the foil pouch in which the reagent arrives to prevent degradation due to light.

For long term storage, store the reagent at -20°C. Please note the expiration date(s) of the packaging and use in appropriate order if there are different expiration dates to guarantee optimal functionality.

Before use, store the reagent at 4°C and make sure that the reagent is completely thawed before use in the analyser.

When new detection reagent is needed, transfer a new reagent bottle to the cooling unit inside the analyzer and connect the pump tubing. The priming function can be used to flush the pump tubing with fresh reagent. It is highly recommended to perform a cleaning cycle after the priming function and before starting a calibration or analysis.

## 4.5 EZ-ATP Calibration solution

### Storage

For long term storage, store the calibration kit at -20°C. Please note the expiration date(s) of the packaging and use in appropriate order if there are different expiration dates to guarantee optimal functionality.

Before use, store the reagent at 4°C and make sure that the reagent is completely thawed before use in the analyser.

## Sourcing of reagents and standards for the EZ7300 ATP

### US

Customers need to go directly in contact with:

Promega Corporation  
2800 Woods Hollow Road  
Madison, WI 53711 USA  
[COD@promega.com](mailto:COD@promega.com)

### Germany, France, Italy, Netherlands, Spain, Sweden, Switzerland, UK

Customers can order directly via Hach.

Item numbers for the reagents:

#### Germany:

APPAZX000511 EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack

APPAZX000513 EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

#### France:

MERFR.APPAZX511 EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack

MERFR.APPAZX513 EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

#### Italy:

MERIT.APPAZX511 EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack

MERIT.APPAZX513 EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

#### Netherlands:

MERNL.APPAZX511 EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack

MERNL.APPAZX513 EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

#### Spain:

MERES.APPAZX511 EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack

MERES.APPAZX513 EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

#### Sweden:

MERSE.APPAZX511 EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack

MERSE.APPAZX513 EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

**Switzerland:**

MERCH.APPAZX511	EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack
MERCH.APPAZX513	EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

**UK:**

MERGB.APPAZX511	EZ-ATP Water-Glo Detection Reagent, 250mL reagents pack
MERGB.APPAZX513	EZ-ATP calibration kit 200pg/mL, 3x Blank + 3x ATP standard

**Other European countries**

Please go in contact with:

Promega GmbH  
 Schildkroetstr. 15  
 DE-68199 Mannheim  
[ar-service@promega.com](mailto:ar-service@promega.com)

**4.6 NaOH solution (1M)**

Products	Formula	MW (g/mol)	CAS No.	1 litre solution
Sodium hydroxide	NaOH	40	1310-73-2	40 g

**Preparation**

Dissolve approximately 40 g of sodium hydroxide (NaOH) in 500 mL de-ionized water.  
 Dissolve completely and to 1 litre with de-ionized water.

**4.7 HCl Solution (1M)**

Products	Formula	MW (g/mol)	CAS No.	1 litre solution
Hydrochloric acid 32%	HCl	36.46	7647-01-0	83 mL

**Preparation**

Take 83 mL of concentrated hydrochloric acid (HCl, 32%) and dilute to 1 litre with de-ionized water.

## 4.8 Cleaning solution

The cleaning procedure should prevent any build-up of biological growth in the flowcell and tubing. The 3-step cleaning procedure includes a HCl flushing step (§4.7), followed by a NaOH (§4.6) and di-ionized water flush. Total cleaning time is 15 minutes.

<b>Change Information</b>	
Date: 30/03/2022	Previous version: Edition 6 to Edition 1.01
<b>Reason for Change</b>	
<ul style="list-style-type: none"><li>- Addition of water consumption</li><li>- Addition of information reagents</li></ul>	
<b>Description of Change</b>	
<ul style="list-style-type: none"><li>- Addition of estimated consumption of water for rinse and dilution (chapter 4.2)</li><li>- Addition of extra information regarding storage and quality of reagents (chapter 4.3)</li></ul>	