Test Kit Instructions

Deposit & Surface Analysis

Product #: DSA-25 / DSA-100

Introduction

At LuminUltra, we are committed to providing high quality test kits to anyone that needs fast and reliable results about the microbiological characteristics of any process! Visit www.luminultra.com to learn about the exciting opportunities that our solutions can provide.

Whereas traditional microbiological tests require days for feedback and measure only a fraction of the microorganisms, **2nd Generation** Adenosine Triphosphate (ATP) test kits from LuminUltra measure total microorganisms and provide feedback in minutes!

In this test kit instruction guide, you will learn...

- Where this kit can be used;
- How 2nd Generation ATP technology works;
- How to handle and store components of this kit;
- How to perform tests;
- How to calculate and interpret results; and
- How to contact us.

DSA Test Kit (DSA-25C)

Choosing the Right Test Kit

LuminUltra provides 6 core test kits for measuring total microbiological concentration via ATP, each tailored to specific applications:

- Quench-Gone Aqueous (**QGA™**):
  For low-solids water-based samples, such as drinking, cooling and process waters with less than 10% free oil and/or salinity.
- Quench-Gone Organic Modified (**QGO-M™**):
  For low-solids organic-based samples, such as fuel, bottom waters, metalworking fluids, lubricants, oily brine, and oilfield waters with more than 10% free oil and/or salinity. QGOM-XLPD is also available for samples that are more difficult to filter such as latex polymers, concrete admixtures, and personal or home care products.
- Deposit & Surface Analysis (**DSA™**):
  For measuring attached growth such as biofilm, corrosion products, slimes, and biological filter media.
- QuenchGone21™ Industrial (**QG21I™**):
  For high-solids process fluids, including paper process and other wash waters.
- QuenchGone21 Specialty (**QG21S™**):
  For chemical product testing, such as slurries, adhesives, paints, and other coatings.
- QuenchGone21 Wastewater (**QG21W™**):
  For wastewater and bioprocessing samples, whether influent, bioreactor or effluent. Also provides the capability to quantify attached growth and floc bulking sedimentation processes.

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Where to use the DSA Test Kit

The Deposit & Surface Analysis (**DSA**) test kit is designed for measurement of microorganisms on surfaces, in deposits, and on biofilm collection devices (e.g. corrosion coupons). Using a single analysis, you will be able to quickly measure total microbiological concentration on any surface or deposit sample with a wide detection range. Use DSA to detect total microbiological activity in:

- ✓ Process Surfaces
- ✓ Solid Deposits
- ✓ Biofilm Collection Devices
- ✓ Biological Filter Media

…and more!

How Does ATP Testing Work?

LuminUltra's test kits are based on the measurement of ATP, which is a direct and interference-free indicator of total living biomass. ATP is measured using the firefly luciferase assay, where a sample containing ATP is introduced to a solution containing the enzyme Luciferase, which naturally occurs in the tails of fireflies, to produce light. The light is detected in a **luminometer** as Relative Light Units (RLU).

$$ATP + O_2 + luciferin \xrightarrow{\substack{Mg++\\luciferase}} AMP + PPi + oxyluciferin + light$$

The DSA test kit utilizes a 10-minute dilution-based analysis to measure a parameter called Total ATP (tATP™). When measured on surfaces or in deposits, tATP represents the accumulation of **sessile** biomass on process equipment and therefore can indicate the presence of or potential for microbiological corrosion.

Getting Started

LuminUltra's test kits contain all of the consumable materials required to run their specified number of tests (Defined by the last 2 or 3 digits of the product code). To use these test kits, LuminUltra recommends either:

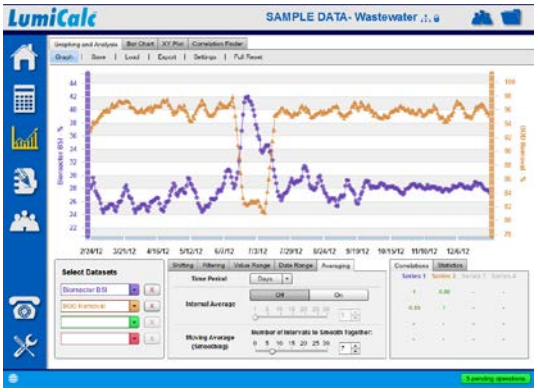
- PhotonMaster™ Luminometer & Equipment Set (**EQP-PAC-PMT**):
  Carry Case, Micropipettors, PhotonMaster Luminometer, Test Tube Racks.

PhotonMaster Equipment Set (EQP-PAC-PMT)

- Lumitester™ C-110 Luminometer & Equipment Set (**EQP-PAC-C110**):
  Carry Case, Micropipettors, Lumitester C-110 Luminometer, Test Tube Racks.

NOTE: LuminUltra's test kits can be used with the majority of photomultiplier tube–based luminometers. Contact LuminUltra to confirm compatibility of your luminometer.

In addition to test kits and equipment, LuminUltra also recommends the use of **LumiCalc™** software. This powerful platform allows you to calculate, store, and analyze your data to maximize your experience with 2nd Generation ATP testing. Plus, it provides a stable and secure ability to share data and collaborate with your peers!



LumiCalc Software (LC-SOFT-M/A/L)

LuminUltra is sensitive to the needs of each individual customer. We can supply you with on-site auditing and training services, web-based training, and one-on-one consultation to get your process improvement program off the ground. Contact us today to learn more!

Test Kit Contents and Storage

When you receive your test kit, utilize the following guidelines for material storage. Note that the presence and quantity of each item listed below will depend on test kit size and type. Avoid freezing of all product components except where noted, and avoid usage of expired test kit components.

DSA Test Kit Contents & Storage Conditions

Component (LuminUltra P/N)	Storage	Shelf Life
Luminase™ Enzyme & Buffer Vials (**Lu-3mL-FD**) *Luciferase Enzyme Reagent, 3mL*	4 to 25°C	6 to 12 mo*
UltraCheck™ 1 Dropper Bottle (**UC1-2.5mL**) *1 ng ATP/mL Standard, 2.5mL*	4 to 25°C	18 mo
UltraLyse™ 7 (Extraction) Tube, 5mL (**UL7-5mL-25R**) *ATP Extraction Reagent, 5mL*	4 to 25°C	18 mo
UltraLute™ (Dilution) Tube, 9mL (**ULu-9mL-25R**) *ATP Dilution Reagent, 9mL*	4 to 25°C	18 mo
LumiSolve™ Bottle (**LS-30mL**) *Surface Swabbing Reagent, 30mL*	4 to 25°C	18 mo
Sterile Swabs, 25/pk (**DIS-SWAB-25**)	-	-
100 to 1000µL Blue Pipet Tips, 100/rack (**DIS-PT1-100R**)	-	-
10 to 200µL Yellow Pipet Tips, 96/rack (**DIS-PT01-96R)**	-	-
12x55mm Test Tubes, 50/pk (**DIS-CT12-50**)	-	-

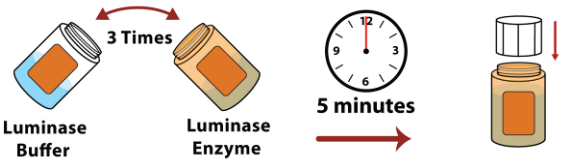
* Luminase is manufactured and shipped in matching bottles of freeze-dried powder and liquid buffer. The stated shelf life is for the freeze-dried form; store refrigerated for the best possible shelf life. Following rehydration, the reagent will be stable for 3 months when refrigerated and 6 months when frozen. Note that the Luminase supplied in DSA kits is NOT interchangeable with other forms of Luminase (i.e. Luminase^{W}, Luminase Lite, and Luminase^{XL}).

General Tips

- New to 2nd Generation ATP technology? Before getting started, consult www.luminultra.com for video demonstrations, use guidelines, validation guidelines, other product documentation, and more!
- Microbiological characteristics of most samples will begin to change immediately upon collection. If samples cannot be tested within 2 hours of collection, store refrigerated (2 to 8°C) and test within 24 hours of collection. Allow samples to reach ambient temperature prior to testing, and perform ATP analyses on the same sample used for measuring other parameters for reliable interpretation.
- Waste reagent can be discarded as general waste in most cases. Consult MSDS for more information. Contact LuminUltra for copies of MSDS.
- All materials in this test kit including pipet tips and test tubes are single-use only. Because ATP and bacteria are present on skin, do not to touch the surface of pipet tips. Ensure that all pipet tips and test tubes are clean inside and outside prior to use. Do not mark on assay tubes as this may impact light detection by the luminometer.
- Avoid taking multiple luminometer readings on the same assay. The light output from ATP assays is relatively constant and at a maximum for the first 15-30 seconds after mixing, after which the output will decline.
- When testing samples that yield low RLU values (i.e. $RLU_{ATP} \leq 50$), it is recommended to account for background noise. Simply follow the procedure without adding any of the ATP-containing sample into the analysis and record this value as RLU_{bg}. Typical RLU_{bg} when using a PhotonMaster or Lumitester C-110 are ≤ 10. If high RLU_{bg} are consistently observed, repeat assays in an area out of direct sunlight or intense lighting. A single RLU_{bg} may be used for multiple analyses much like a single UltraCheck 1 RLU (RLU_{ATP1}).

Handling Luminase

- **Luminase** is manufactured using a process called freeze-drying. This maximizes product stability prior to use. Before using this product, it must first be rehydrated by mixing freeze-dried powder with liquid buffer and then allowed to incubate for at least 5 minutes. Take care to avoid contamination when removing the glass vial stopper.



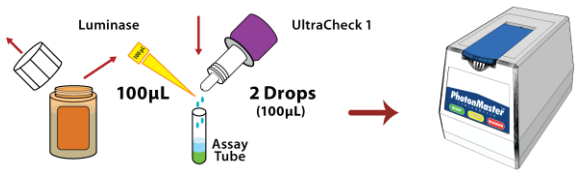
Luminase Rehydration Process

- Rehydrated **Luminase** can be stored in the refrigerator for up to 3 months (or freezer for up to 6 months with unlimited freeze-thaw cycles) following rehydration. Always bring cold rehydrated **Luminase** to ambient temperature prior to use. 1 hour is generally sufficient for this purpose.
- Never expose rehydrated **Luminase** to ≥30°C for longer than 1-2 hours.
- In general, it is recommended that **Luminase** only be rehydrated as required. In other words, rehydrate on the day of testing rather than in advance.
- Never attempt to partition portions of freeze-dried **Luminase** enzyme and/or the supplied buffer into smaller quantities.
- If you begin utilizing a new bottle of **Luminase** during your testing, make sure to collect a new calibration result for that bottle. Alternatively, mix bottles of **Luminase** for all testing at one time.

Step 1 – ATP Standard Calibration

The ATP Standard Calibration (**ATP1**) converts luminometer RLU values into actual ATP concentrations. Perform one calibration per day or for each set of samples analyzed at the same time. Be sure that all reagents (especially rehydrated **Luminase**) are allowed to reach ambient temperature prior to use.

PROCEDURE: Add 2 drops (100µL) of **UltraCheck 1** and use a new pipet tip to dispense 100µL of **Luminase** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record RLU_{ATP1} manually, or directly in LumiCalc.



NOTE: If RLU_{ATP1} ≤ 5,000 using a PhotonMaster or Lumitester C-110 rehydrate a new bottle of Luminase for maximum sensitivity.

NOTE: RLU_{ATP1} will fall over time for the same batch of Luminase. This is due to decreased luciferase enzyme activity. When followed, the guideline above ensures that there is sufficient activity to meet the specified detection limit.

Step 2 – Sample Preparation

The DSA test kit provides three options to collect and prepare samples:

A. Surface Swab – A measured area of the surface to be tested is swabbed to collect microbial particles. ATP is then extracted and measured from the swab.

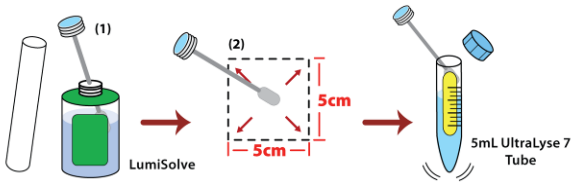
B. Measured Deposit – A deposit is collected and a precise mass or volume is measured. ATP is then extracted and measured from the deposit.

C. Biofilm Collector – A biofilm collection device (e.g. corrosion coupon) in directly immersed into **UltraLyse 7** to extract and measure ATP.

Choose from any one of methods A, B or C, and then proceed to Step 3 (tATP Analysis). In general, option B is preferred as it is the most quantitative.

Option A – SURFACE SWAB

Obtain an unused Sterile Swab and wet with **LumiSolve**. Swab an approximately 5x5cm (2x2in) surface area. Insert swab in a **5mL UltraLyse 7 (Extraction) Tube**. Cap and mix the contents of the container.

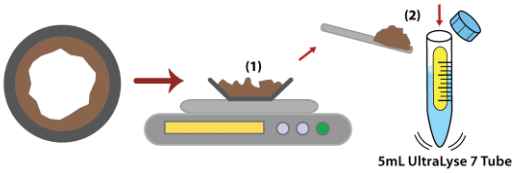


NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

TIP: To increase analysis sensitivity, increase the swabbed surface area.

Option B – MEASURED DEPOSIT

Obtain a portion of the deposit and weigh 1g of sample. Add this to a **5mL UltraLyse 7 (Extraction) Tube**. Cap and mix the contents of the tube vigorously to disperse the deposit throughout the fluid.



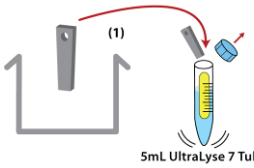
NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

TIP: To increase analysis sensitivity, increase the amount of deposit added to the UltraLyse 7 Tube.

TIP: A measured volume of deposit (e.g. 1mL) can also be used instead of a weighed amount.

Option C – BIOFILM COLLECTOR

Obtain a biofilm collection device from the process and shake gently to remove residual fluid. Place the device into a **5mL UltraLyse 7 (Extraction) Tube**. Cap and mix the contents of the tube vigorously to fully expose the device to the fluid.



NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

TIP: Attempt to test the biofilm collection device as quickly as possible following removal from process fluid. If the device is not to be tested immediately, it is preferred to store the device in a container containing process fluid until such time that it can be tested.

TIP: If the device is too large to fit into the supplied UltraLyse 7 Tube, obtain a larger vessel and ensure the device can be fully immersed in UltraLyse 7.

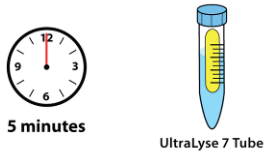
TIP: Because microorganisms and other materials collected on the device will be destroyed upon immersion in UltraLyse 7, it is preferred to have more than one device available for multiple analyses. If only one device is available, perform all other tests before performing ATP test.

Step 3 – DSA tATP™ Analysis

The DSA Total ATP (**tATP**) analysis measures all ATP within a sample, including ATP from living cells in addition to ATP that has been released from dead cells.

3.1 – INCUBATION

Allow at least 5 minutes for incubation of the **UltraLyse 7 (Extraction) Tube** to proceeding to 3.2.

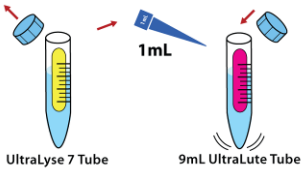


NOTE: At this point, the contents of the Extraction Tube can be capped and stored refrigerated between 2-8°C for up to 1 week prior to 3.2 and 3.3.

TIP: When using the biofilm collector method, place the Extraction Tube in an orientation to maximize immersion of the device in UltraLyse 7 during incubation.

3.2 – DILUTION

Use a micropipettor to transfer 1mL from the **UltraLyse 7 (Extraction) Tube** to a **9mL UltraLute (Dilution) Tube**. Cap and invert three times to mix.



NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

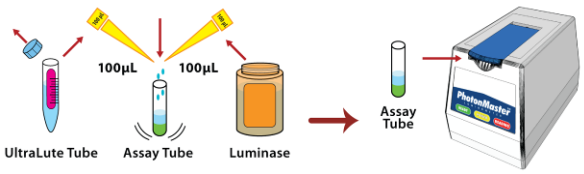
NOTE: At this point, the contents of the Dilution Tube are stable at room temperature for up to 4 hours.

NOTE: If there are significant quantities of solids in the dilution tube following this step, allow them to separate (i.e. settle of float) and sample from the cleanest possible supernatant in the next step.

3.3 – ASSAY

Using a new pipet tip, transfer 100µL from the **UltraLute (Dilution) Tube** to a new 12x55mm test tube (the Assay Tube), and use another new pipet tip to add 100µL of **Luminase**, swirl gently five times,

immediately insert into the luminometer and measure. Record RLU_{tATP} manually, or directly in LumiCalc.



NOTE: If $RLU_{tATP} \leq 10$ on a PhotonMaster or Lumitester C-110, you are below the low–detection limit. Report tATP (pg ATP/mL) = 0 in calculations, or select a larger volume in Step 2 and repeat the analysis.

NOTE: When $RLU_{tATP} \leq 50$ on a PhotonMaster or Lumitester C-110, it is recommended that you measure and subtract RLU_{bg} from your measurement. When possible, repeat the test procedure with a larger volume of sample to achieve a higher RLU_{tATP} and greater accuracy.

TIP: If "Scale Over" is returned, repeat the analysis using a smaller sample in Step 2.

3.4 – CALCULATIONS

Following completion of DSA analyses, RLU values must be converted to ATP concentrations using the following calculations. For easy calculations, utilize **LumiCalc** software.

The Total ATP (**tATP**) analysis measures all ATP within the deposit, including ATP from living cells in addition to ATP that has been released from dead cells. Calculate tATP according to the option selected in Step 2:

A – Surface Swab (Default A_{Sample} = 25cm²):

$$tATP\left(pg\ ATP/cm^2\right) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times \frac{50{,}000\left(pg\ ATP\right)}{A_{Sample}\left(cm^2\right)}$$

B – Measured Deposit (Default m_{Sample} = 1g):

$$tATP\left(pg\ ATP/g\right) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times \frac{50{,}000\left(pg\ ATP\right)}{m_{Sample}\left(g\right)}$$

C – Biofilm Collector:

$$tATP\left(pg\ ATP/device\right) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times \frac{50{,}000\left(pg\ ATP\right)}{1\ device}$$

NOTE: When applicable, subtract RLU_{bg} from RLU_{tATP} prior to executing the above calculations.

To communicate results on the same basis as traditional culture tests, tATP results are converted into Microbial Equivalents (**ME's**). This is based on the established conversion that 1 E. coli-sized bacteria contains 0.001 pg (1 fg) of ATP.

A – Surface Swab:

$$tATP\left(ME/cm^2\right) = tATP\left(pg\ ATP/cm^2\right) \times \frac{1\,ME}{0.001\,pg\ ATP}$$

B – Measured Deposit:

$$tATP\left(ME/g\right) = tATP\left(pg\ ATP/g\right) \times \frac{1\,ME}{0.001\,pg\ ATP}$$

C – Biofilm Collector:

$$tATP\left(ME/device\right) = cATP\left(pg\ ATP/device\right) \times \frac{1\,ME}{0.001\,pg\ ATP}$$

NOTE: For more discussion on the quantity of ATP per cell, visit www.luminultra.com.

Because many traditional culture-based methods report results in a similar fashion, it is sometimes convenient to report tATP results in ME/quantity using Scientific Notation (i.e. **#.# x $10^{\#}$**) or on a **Log_{10}** format for comparison purposes.

Interpretation Guidelines

Once DSA tATP results are calculated, microbial control can be evaluated. ATP-based measurements are extremely sensitive to changes in total microbial quantity. In general, processes will have the best microbial control when **tATP is minimized.** For the easiest interpretation, utilize **LumiCalc** software.

LuminUltra's ATP test kits can be used to audit microbial quantity to reveal differences at different process locations in an effort to quickly assess the 'hot spots' within a process that require more immediate attention.

For process control, daily monitoring using ATP test kits will give you true total microbial quantity parameters to trend over time against process characteristics and performance.

When utilizing ATP test kits it is important to remember that every process is different. During **audits**, relative comparisons from point to point are a reliable means to assess your process, while for **daily monitoring** it is important to establish a baseline trend before making control decisions. To get started, LuminUltra provides the following guidelines as the ratio of deposit tATP to bulk fluid cATP as measured by other LuminUltra test kits:

DSA tATP Interpretation Guidelines

Application	Good Control	Preventive Action	Corrective Action
Surfaces, Deposits & Coupons *	<10x	10x to 100x	>100x

* Guidelines are provided as a ratio of ATP on your surface/deposit/collector to bulk fluid ATP.

NOTE: These interpretation guidelines are designed for generic risk management guidance **only**. Users are encouraged to establish their own control ranges on which to base process decisions. LuminUltra and its affiliates do not accept any liability for any decision or assessment taken or made as a consequence of using this test kit.

Ordering Information

- New to 2nd generation ATP technology? Start by ordering the Luminometer Package (Product # **EQP-PAC-PMT** or **EQP-PAC-C110**) and the test kit(s) of your choice.
- When reordering materials for testing, it is preferred to order complete kits. DSA is available in five formats:

Description	Part #
DSA, 100 Tests, Complete *	DSA-100C
DSA, 100 Tests, Reagents Only	DSA-100
DSA, 100 Tests, Bulk Format **	DSA-100B
DSA, 25 Tests, Complete *	DSA-25C
DSA, 25 Tests, Reagents Only	DSA-25

* Complete kits include LuminUltra-manufactured reagents plus all consumables (tips, tubes, filters, syringes) required to run analysis. If you supply your own consumables, reagent only kits are available.

** Bulk test kits contain all reagents supplied in bulk format and require the user to dispense individual quantities as required.

- To obtain pricing information, inquire about other products and services, or to place an order, contact LuminUltra or your authorized representative.

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www.luminultra.com

- Major credit cards (Visa, MasterCard, AMEX) are accepted. Contact LuminUltra by phone to place credit card orders.

- Orders generally ship within 3 business days. You will receive order confirmation via Fax or Email.

Quick Reference Guide

Deposit and Surface Analysis Test Kit

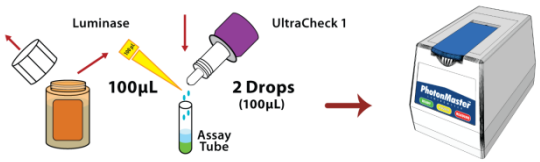
Product #: DSA-25 / DSA-100

NOTE: Please refer to Test Kit Instructions during first product use and for additional details including legal statements.

ISO 9001

Step 1 - UltraCheck™ 1 Calibration

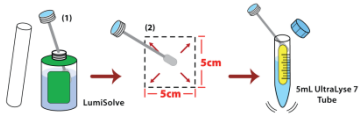
Perform one UltraCheck 1 calibration per day or per each set of samples analyzed.



NOTE: If $RLU_{ATP1} \leq 5{,}000$ using a PhotonMaster or Lumitester C-110, rehydrate a new bottle of Luminase for maximum sensitivity.

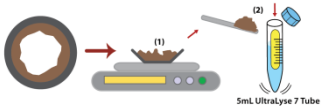
Step 2 - Sample Preparation → Select one of the following options:

Option A - SURFACE SWAB



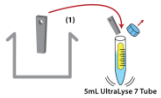
OR

Option B: MEASURED DEPOSIT



OR

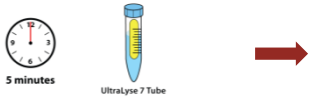
Option C: BIOFILM COLLECTOR



Step 3 – Total ATP (tATP™) Analysis → Then perform the following steps:

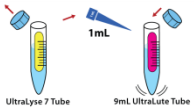
3.1 – INCUBATION

Allow time for complete extraction.



3.2 – DILUTION

Dilute out interferences.



3.3 – ASSAY

Measure ATP concentration.

NOTE: If $RLU_{tATP} \leq 10$ using a PhotonMaster or Lumitester C-110, you are below the low- detection limit.

NOTE: If $RLU_{tATP} \leq 50$ using a PhotonMaster or Lumitester C-110, consider accounting for background (RLU_{bg}). See Test Kit Instructions for guidance.

Calculations → Carry out calculations that correspond to the selected preparation method:

A - Surface Swab (Default $A_{sample} = 25cm^2$):

$$tATP\left(pg\ ATP/cm^2\right) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times \frac{50{,}000\left(pg\ ATP\right)}{A_{Sample}\left(cm^2\right)}$$

OR

$$tATP\left(\frac{ME}{cm^2}\right) = tATP\left(\frac{pg\ ATP}{cm^2}\right) \times \frac{1\ ME}{0.001\ pg\ ATP}$$

B - Measured Deposit (Default $m_{sample} = 1g$):

$$tATP\left(pg\ ATP/g\right) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times \frac{50{,}000\left(pg\ ATP\right)}{m_{Sample}\left(g\right)}$$

OR

$$tATP\left(\frac{ME}{g}\right) = tATP\left(\frac{pg\ ATP}{g}\right) \times \frac{1\ ME}{0.001\ pg\ ATP}$$

C - Biofilm Collector:

$$tATP\left(pg\ ATP/device\right) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times \frac{50{,}000\left(pg\ ATP\right)}{1\ device}$$

$$tATP\left(\frac{ME}{device}\right) = cATP\left(\frac{pg\ ATP}{device}\right) \times \frac{1\ ME}{0.001\ pg\ ATP}$$

NOTE: 1 ME (Microbial Equivalent) assumes 0.001 pg (1fg) ATP per cell.

Interpretations Guidelines

Application	Good Control (pg cATP/mL)	Preventative Action (pg cATP/mL)	Corrective Action (pg cATP/mL)
Surface, Deposits, Coupons*	< 10x	10x to 100x	> 100x

*Guidelines are provided as a ratio of ATP on your surface/deposit/collector to bulk fluid ATP.

NOTE: Interpretation Guidelines provided for general guidance. For best results, establish your own baseline and control levels.