

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](http://www.luminultra.com) to learn about all the exciting opportunities that our technology can bring to your application.

Whereas traditional microbiological tests require days for feedback and measure only a fraction of the microorganisms, 2<sup>nd</sup> 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 2<sup>nd</sup> Generation ATP technology works;
- How to handle and store components of this kit;
- How to perform tests;
- How to calculate and interpret results; and
- Where to find more information and how to contact us.

## Choosing the Right Test Kit



**QGA Test Kit (QGA-100C)**

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 oilfield waters.*
- Quench-Gone Organic Modified (**QGO-M™**):  
*For low-solids organic-based samples, such as fuel, oily brine, lubricants and latex polymers.*
- Deposit & Surface Analysis (**DSA™**):  
*For measuring deposits and surfaces, including corrosion products and slimes.*
- 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 and other coatings.*
- QuenchGone21 Wastewater (**QG21W™**):  
*For wastewater and bioprocessing samples, whether influent, bioreactor or effluent.*

All test kits are designed to be used with any photomultiplier-based luminometer. New users are advised to acquire the kit or kits of their choice to pair with the Field Kit & Luminometer Package (Product # **EQP-PAC**) when getting started with LuminUltra's 2<sup>nd</sup> Generation ATP test kits.

## Where to use the QGA Test Kit



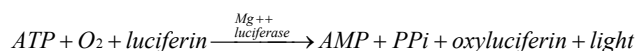
The Quench-Gone Aqueous (QGA) test kit is designed for low-solids water-based samples. Using a single analysis, you will be able to quickly measure total microbiological concentration in any aqueous sample with a wide detection range. Use QGA to detect total microbiological activity in:

- ✓ Fresh & Salt Water
- ✓ Oilfield Waters
- ✓ Cooling Water
- ✓ High-Purity Water
- ✓ Drinking Water
- ✓ Sanitary Water
- ✓ Treated Effluent
- ✓ Reclaimed Water

...and more! In general, the QGA test kit is used in samples having less than 10% free oil and/or salinity content. For samples with greater than 10% free oil and/or salinity, use the Quench-Gone Organic Modified (QGO-M) test kit.

## How Does ATP Testing Work?

LuminUltra's test kits are based on the measurement of ATP. ATP 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).



The QGA test kit utilizes a 5-minute filtration-based analysis to measure a parameter called Cellular ATP (cATP™). cATP represents ATP from living microorganisms in suspension in a fluid and therefore is a direct indication of the **planktonic** population.

While QGA is optimized to measure down to **0.1 pg ATP/mL** using standard procedures and equipment, there is essentially no limit to the sensitivity of this method. Procedural adaptations can be used to amplify the low-range sensitivity when necessary. Contact LuminUltra for more details on this option.

## 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 one of the following equipment bundles:

- Field Kit & Luminometer (**EQP-PAC**):  
*Field Case, Micropipettors, Kikkoman Lumitester.*
- Bench Materials & Luminometer (**EQP-BTM**):  
*Micropipettors, Kikkoman Lumitester, Test Tube Racks.*
- Kikkoman Lumitester™ C-110 (**EQP-LUK**):  
*Kikkoman Lumitester luminometer & accessories.*



**Field Kit & Luminometer Package (EQP-PAC)**

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

LuminUltra is sensitive to the needs of each individual customer. Our expert staff can consult with you to determine the best means by which the data collected from our test kits can be integrated into your existing data management practices. We have multiple software platforms and can even assist you to integrate ATP data directly into existing control systems, process historians, and data archives.

Getting started with QGA is easier with direct training. LuminUltra 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 QGA 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.

**QGA Test Kit Contents & Storage Conditions**

Component (LuminUltra P/N)	Store At	Shelf Life
<b>Luminase™ Dropper (Lu-5mL)</b> <i>Luciferase Enzyme Reagent, 5mL</i>	4°C*	4 mo*
<b>UltraCheck™ 1 Dropper (UC1-5mL)</b> <i>1 ng ATP/mL Standard, 5mL</i>	20°C	12 mo
<b>UltraLyse™ 7 Bottle (UL7-125mL)</b> <i>cATP Extraction Reagent, 125mL</i>	20°C	12 mo
<b>UltraLute™ Bottle (ULu-500mL)</b> <i>cATP Dilution Reagent, 500mL</i>	20°C	12 mo
Quench-Gone Syringe Filters, 25/pk <b>(DIS-SFQG-25)</b>	20°C	-
60mL Syringe, PP/Neoprene, 25/pk <b>(DIS-S60-25)</b>	20°C	-
100 to 1000µL Blue Pipette Tips, 100/rack <b>(DIS-PT1-100R)**</b>	20°C	-
1 to 5mL Natural Pipette Tips, 50/pk <b>(DIS-PT5-50)**</b>	20°C	-
12x55mm Test Tubes, 50/pk <b>(DIS-CT12-50)</b>	20°C	-
17x100mm Test Tubes, 25/pk <b>(DIS-CT17-25)</b>	20°C	-
17mm Caps, 25/pk <b>(DIS-C17-25)</b>	20°C	-

\* Luminase shelf life can be extended to 6 months when frozen, or can be left at room temperature for as long as 3 weeks during routine use. Note that the Luminase supplied in QGA kits is NOT interchangeable with other forms of Luminase (i.e. Luminase<sup>W</sup>, Luminase Lite, and Luminase<sup>XL</sup>).

\*\* Pipette tips supplied in complete test kits are compatible with most Fisherbrand and Eppendorf adjustable micropipettors.

**NOTE:** If your application requires greater sensitivity, inquire about our XL kit option.

## Preparing to Test

- New to 2<sup>nd</sup> Generation ATP technology? Before getting started, consult the training center at [www.luminultra.com](http://www.luminultra.com) for video demonstrations, use guidelines, validation guidelines, and more!

- Be certain to allow **Luminase** to reach ambient temperature prior to use!
  - For room temperature (15 to 25 °C) storage, no warming is required.
  - For refrigerator (2 to 8 °C) storage, let stand at ambient for at least 1 hour prior to testing.
  - For freezer (-10 to -20 °C) storage, let stand at ambient for at least 2 hours prior to testing.
  - **Luminase** exposure to temperatures between 30 and 40 °C should be limited to 1-2 hours. Prolonged exposure will result in accelerated activity loss. Never expose to temperatures > 40°C.
  - For more information on **Luminase** storage and handling, consult the **Luminase** insert.
- If you are new to the use of micropipettors, consult the Micropipetting Fundamentals training materials on [www.luminultra.com](http://www.luminultra.com).
- Avoid analysis contamination by always using a new pipette tip for each pipetting step.
- Avoid usage of expired test kit components. Contact LuminUltra to replace expired components.
- Because ATP and bacteria are present on skin, do not touch the surface of pipette tips.
- Ensure that all assay tubes are clean inside and outside. Do not mark on assay tubes as this may impact light detection by the luminometer.
- Microbiological characteristics of most samples will begin to change immediately upon collection. Analyze samples **within 2 hours of collection** whenever possible.
- If samples cannot be tested within 2 hours of collection, store refrigerated (2 to 8 °C) and test within 24 hours of collection. Ensure that samples are first allowed to reach ambient temperature prior to testing.

- 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. Obtain MSDS and other product documentation from [www.luminultra.com](http://www.luminultra.com).
- Typical  $RLU_{bg}$  when using a Kikkoman Lumitester C-100 or C-110 are  $\leq 10$ . If high  $RLU_{bg}$  are consistently observed, repeat assays in an area out of direct sunlight or intense lighting. If problems still occur, contact LuminUltra for assistance.
- A single  $RLU_{bg}$  may be used for multiple analyses much like a single UltraCheck 1 RLU ( $RLU_{UC1}$ ).

## Diagnostics and Troubleshooting

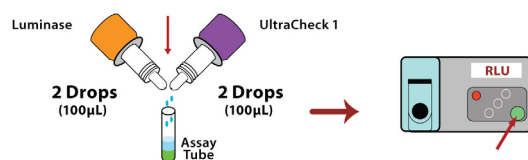
- LuminUltra's test kits are the most robust and quantitative ATP test kits available. If you suspect you are encountering interferences in your application of these kits, first check to ensure you are using the correct kit type for your sample. If you are unsure or feel you have unique testing requirements, contact LuminUltra's support staff for assistance.
- If you encounter difficulty achieving complete filtration of your fluid sample, consider pre-diluting the sample in sterile water or buffer prior to Step 2 of the QGA cATP Analysis, or use a kit more suitable for the sample type. Fluid samples with high solids content or complex nature may require pre-dilution prior to analysis to ensure reliable filtration and recovery of ATP. Contact LuminUltra for recommendations on pre-dilution or use one of our pre-dilution kits for maximum compatibility with the analysis procedure.
- When testing samples that yield low RLU values (i.e.  $RLU_{cATP} \leq 50$  when using a Kikkoman Lumitester C-100 or C-110), it is recommended that you account for background noise in the test procedure prior to the final calculations.
  - To assess background noise, simply follow the procedure without adding any of the ATP-containing sample into the analysis.
  - Correct for background noise by subtracting the background RLU ( $RLU_{bg}$ ) from the measured RLU ( $RLU_{cATP}$ ) prior to executing calculations.

- If dropper bottles become plugged or you encounter difficulty dispensing drops, remove and discard the dropper tip and use a pipettor to measure and dispense the reagent.

## Step 1 – UltraCheck 1 Calibration

The **UltraCheck 1 (UC1)** Calibration converts luminometer RLU values into actual ATP concentrations. Perform one **UltraCheck 1** calibration per day or for each set of samples analyzed at the same time. Be sure that **Luminase** is allowed to reach ambient temperature prior to use.

**PROCEDURE:** Add 2 drops (100 $\mu$ L) of **UltraCheck 1** and 2 drops (100 $\mu$ L) of **Luminase** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record  $RLU_{UC1}$  for use in the final calculations.



**NOTE:** If  $RLU_{UC1} \leq 5,000$  using a Kikkoman Lumitester C-100 or C-110, it is recommended to obtain a new bottle of Luminase for maximum sensitivity.

**NOTE:**  $RLU_{UC1}$  will fall over time for the same batch of Luminase. This is because of decreased luciferase enzyme activity. When followed, the guideline above ensures that there is sufficient activity to meet the specified detection limit.

## Step 2 – QGA cATP™ Analysis

The QGA Cellular ATP (cATP) analysis measures ATP from living cells only. Perform one cATP analysis on each water-based sample you wish to test.

### 2.1 – SELECT SAMPLE VOLUME

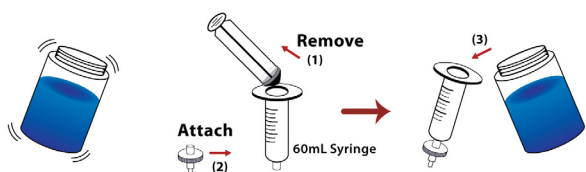
The QGA method allows flexibility in the amount of sample used for analysis. Use the following table to select the quantity of sample to be filtered in 2.2. As a general rule of thumb, the greater your sample volume, the greater the sensitivity you will be able to achieve!

**QGA Sample Volume Recommendations**

Sample Type	Recommended Volume (mL)
Cooling Water	10 to 25
Oilfield, Fuel Tank Bottom Water	10 to 25
Fresh, Brackish & Salt Water	25 to 50
Reclaimed Water, Effluents	25 to 50
Drinking & Sanitary Water	50 to 100
High-Purity Water	≥ 100

### 2.2 – MEASURE SAMPLE VOLUME

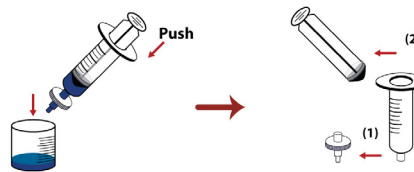
Mix your sample to ensure homogeneity. Remove the plunger from a 60mL syringe and attach a filter. Pour the appropriate volume of sample from 2.1 into the barrel of the syringe.



**TIP:** Alternatively, the syringe tip can be immersed into the sample and drawn into the barrel before attaching the filter, rather than pouring the sample into the barrel after attaching the filter. Be sure the syringe tip is clean if this mechanism is used.

### 2.3 – FILTRATION

Slowly push the entire sample volume through the filter and into a waste receptacle. Push the plunger far enough to filter the sample and stop to ensure that the filter remains wet. Detach the filter and remove the plunger.

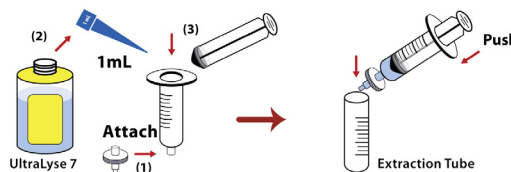


**NOTE:** If the full volume of sample could not be filtered, record the actual volume processed.

**TIP:** If increased sensitivity is desired, filter additional sample by repeating 2.2 and 2.3 using the same syringe and filter.

### 2.4 – EXTRACTION

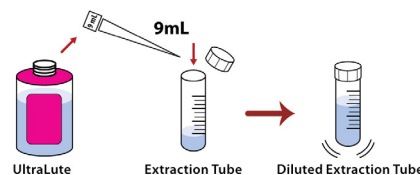
Re-attach the filter to the syringe barrel. Use the micropipettor to add 1mL of **UltraLyse 7** to the barrel. Pass the **UltraLyse 7** through the filter to dryness and collect in an unused 17x100mm test tube. This is called the Extraction Tube.



**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 2.5 and 2.6.

### 2.5 – DILUTION

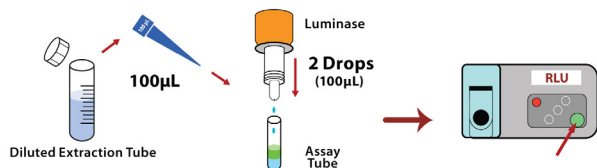
Add 9mL of **UltraLute** into the Extraction Tube. Cap and invert three times to mix. This is now called the Diluted Extraction Tube.



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

## 2.6 – ASSAY

Using the micropipettor, transfer 100µL of the Diluted Extraction Tube contents to a new 12x55mm test tube (the Assay Tube), add 2 drops (100µL) of **Luminase**, swirl gently five times, immediately insert into the luminometer and measure. Record  $RLU_{cATP}$  for use in the final calculations.



**NOTE:** If  $RLU_{cATP} \leq 10$  on a Kikkoman Lumitester C-100 or C-110, you are below the low-detection limit. Report  $cATP$  ( $pg\ ATP/mL$ ) = 0 in the final calculations, or select a larger volume in 2.1 and repeat the analysis.

**NOTE:** When  $RLU_{cATP} \leq 50$  on a Kikkoman Lumitester C-100 or C-110, it is recommended that you measure and subtract  $RLU_{bg}$  from your measurement. See Diagnostics and Troubleshooting. When possible, repeat the test procedure with a larger volume of sample to achieve a higher  $RLU_{cATP}$  and greater accuracy.

**TIP:** If “Scale Over” is returned, repeat the analysis using a smaller sample volume in 2.1

## Final Calculations

Following completion of QGA analyses, RLU values must be converted to ATP concentrations using the following calculations. For easy calculations, utilize **LumiCapture™** software available on [www.luminultra.com](http://www.luminultra.com). For manual calculations, see below.

Cellular ATP (**cATP**) represents the amount of ATP contained within living cells and is a direct indication of total living biomass quantity.

$$cATP (pg\ ATP / mL) = \frac{RLU_{cATP}}{RLU_{UC1}} \times \frac{10,000 (pg\ ATP)}{V_{Sample} (mL)}$$

**NOTE:** For pre-diluted samples, multiply the  $cATP$  concentration in  $pg\ ATP/mL$  by the appropriate pre-dilution factor prior to proceeding. For example, a 1 part sample in 9 parts of UltraLute pre-dilution would have a dilution factor of 10.

**NOTE:** When applicable, subtract  $RLU_{bg}$  from  $RLU_{cATP}$  prior to executing the above calculation.

To communicate results on the same basis as traditional culture tests,  $cATP$  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.

$$cATP (ME / mL) = cATP (pg\ ATP / mL) \times \frac{1\ ME}{0.001\ pg\ ATP}$$

**NOTE:** For more discussion on the quantity of ATP per cell, visit [www.luminultra.com](http://www.luminultra.com).

Because many traditional culture-based methods report results in a similar fashion, it is sometimes convenient to report  $cATP$  results in ME/mL using Scientific Notation (i.e. **## x 10<sup>#</sup>**) or on a **Log<sub>10</sub>** format for comparison purposes.

## Interpretation Guidelines

Once QGA  $cATP$  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 **cATP is minimized**.

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 in units of **pg cATP per mL**:

## QGA cATP Interpretation Guidelines

Application	Good Control (pg cATP/mL)	Preventive Action (pg cATP/mL)	Corrective Action (pg cATP/mL)
High-Purity Water	< 0.1	0.1 to 1.0	> 1.0
Water for Consumption (Potable, Sanitary)	< 0.5	0.5 to 10	> 10
Raw Make-up Water (Fresh, Brackish, Salt, Reclaimed)	< 10	10 to 100	> 100
Treated Process Water (Cooling, Bottom Water, Oilfield) Oxidizing Biocides *	< 10	10 to 100	> 100
Treated Process Water (Cooling, Bottom Water, Oilfield) Non-Oxidizing Biocides or Non-Chemical Treatment **	< 100	100 to 1,000	> 1,000

\* Oxidizing biocides include chlorine, bromine, ozone, and others.

\*\* Given their different mechanism of kill, non-oxidizing biocides and non-chemical treatments will typically have a higher residual cATP when processes are well controlled.

**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 2<sup>nd</sup> generation ATP technology? Start by ordering the Field Kit & Luminometer Package (Product # **EQP-PAC**) and the test kit(s) of your choice.
- When reordering materials for testing, it is preferred to order complete kits. QGA is available in four formats:

Description	Part #
QGA, 100 Tests, Complete *	QGA-100C
QGA, 100 Tests, Reagents Only	QGA-100
QGA, 25 Tests, Complete *	QGA-25C
QGA, 25 Tests, Reagents Only	QGA-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.

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

### LuminUltra Technologies Ltd.

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[www.luminultra.com](http://www.luminultra.com)

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



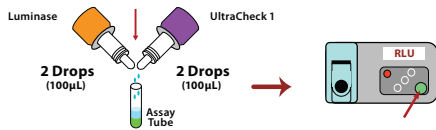
- Orders generally ship within 3 business days. You will receive order confirmation via Fax or Email.

Lumitester is a trademark of Kikkoman Corporation, all other trademarks are the property of LuminUltra Technologies Ltd.

**NOTE: Please refer to test kit instructions during first product use and for additional details including legal statements.**

### Step 1 – UltraCheck™ 1 Calibration

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



**NOTE: If  $RLU_{UC1} \leq 5000$  using a Kikkoman Lumitester™ C-100 or C-110, obtain a new bottle of Luminase.**

### Step 2 – QGA™ cATP™ Analysis

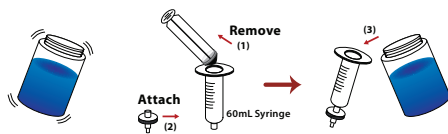
#### 2.1 - SELECT SAMPLE VOLUME

Determine volume of sample to filter.

Sample Type	Recommended Volume (mL)
Cooling Water	10 to 25
Oilfield, Fuel Tank Bottom Water	10 to 25
Fresh, Brackish & Salt Water	25 to 50
Reclaimed Water, Effluents	25 to 50
Drinking and Sanitary Water	50 to 100
High Purity Water	> 100

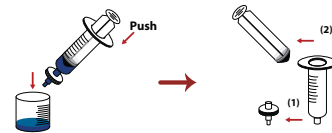
#### 2.2 - MEASURE SAMPLE VOLUME

Add sample to syringe.



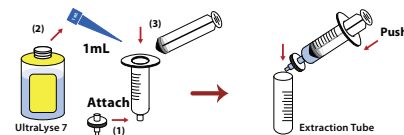
#### 2.3 - FILTRATION

Filter sample to concentrate microorganisms.



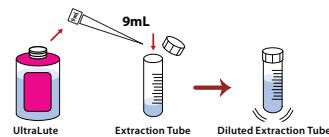
#### 2.4 - EXTRACTION

Extract ATP from filter.



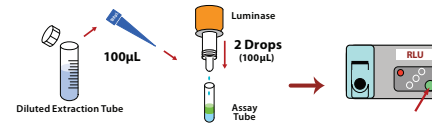
#### 2.5 - DILUTION

Dilute out interferences.



#### 2.6 - ASSAY

Measure ATP concentration.



**NOTE: If  $RLU_{cATP} \leq 10$  on a Kikkoman Lumitester C-100 or C-110, you are below the low-detection limit.**

**NOTE: If  $RLU_{cATP} \leq 50$  on a Kikkoman Lumitester C-100 or C-110, consider accounting for  $RLU_{bg}$ . See Test Kit Instructions.**

### Final Calculations

$$cATP \text{ (pg ATP / mL)} = \frac{RLU_{cATP}}{RLU_{UC1}} \times \frac{10,000 \text{ (pg ATP)}}{V_{Sample} \text{ (mL)}}$$

$$cATP \text{ (ME / mL)} = cATP \text{ (pg ATP / mL)} \times \frac{1 \text{ ME}}{0.001 \text{ pg ATP}}$$

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

### Interpretations Guidelines

Application	Good Control (pg cATP/mL)	Preventative Action (pg cATP/mL)	Corrective Action (pg cATP/mL)
High Purity Water	< 0.1	0.1 to 1.0	> 1.0
Water for Consumption (Potable, Sanitary)	< 0.5	0.5 to 10	> 10
Raw Make-up Water (Fresh Brackish, Salt, Reclaimed)	< 10	10 to 100	> 100
Treated Process Water (Cooling, Bottom Water, Oilfield) Oxidizing Biocides	< 10	10 to 100	> 100
Treated Process Water (Cooling, Bottom Water, Oilfield) Non-Oxidizing Biocides or Non-Chemical Treatment	< 100	100 to 1,000	> 1,000

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