

### 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](http://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, **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
- How to contact us.



QGA Test Kit (QGA-100C)

### 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.*

## Where to use the QGA Test Kit

**NOTE:** The QGA test kit is compliant with ASTM Standard D4012 for Measurement of Adenosine Triphosphate (ATP) Content of Microorganisms in Water.



The Quench-Gone Aqueous (**QGA**) test kit is designed for low-solids

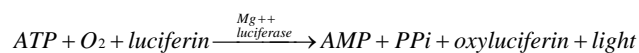
water-based samples. Using a single analysis, you will be able to quickly measure total microbiological concentration in any aqueous sample with a wide detection range. Use QGA to detect total microbiological activity in:

- ✓ Fresh & Salt Water
- ✓ Process Water
- ✓ 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, 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).



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 these options.

## 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.*

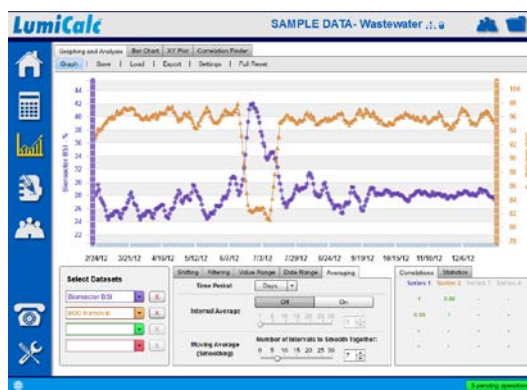


PhotonMaster Equipment Set (EQP-PAC-PMT)

- 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 2<sup>nd</sup> 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.

QGA Test Kit Contents & Storage Conditions

Component (LuminUltra P/N)	Storage	Shelf Life
<b>Luminase™</b> Enzyme & Buffer Vials (Lu-3mL-FD) <i>Luciferase Enzyme Reagent, 3mL</i>	4 to 25°C	6 to 12 mo*
<b>UltraCheck™ 1</b> Dropper Bottle (UC1-5mL) <i>1 ng ATP/mL Standard, 5mL</i>	4 to 25°C	18 mo
<b>UltraLyse™ 7</b> Bottle (UL7-125mL) <i>ATP Extraction Reagent, 125mL</i>	4 to 25°C	18 mo
<b>UltraLute™ (Dilution) Tube, 9mL</b> (ULu-9mL-50R) <i>ATP Dilution Reagent, 9mL</i>	4 to 25°C	18 mo
Quench-Gone Syringe Filters, 25/pk (DIS-SFQG-25)	-	-
60mL Syringe, PP/Neoprene, 25/pk (DIS-S60-25)	-	-
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 QGA kits is NOT interchangeable with other forms of Luminase (i.e. Luminase<sup>W</sup>, Luminase Lite, and Luminase<sup>XL</sup>).

## General Tips

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

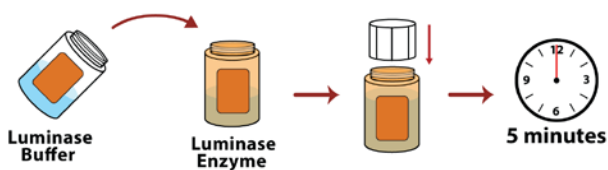
guidelines, other product documentation, and more!

- 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 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  $\leq 10$ . 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.



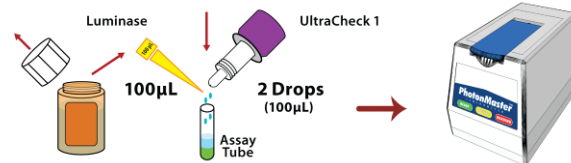
Luminase Rehydration Process

- 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  $\geq 30^{\circ}\text{C}$  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 (100 $\mu\text{L}$ ) of **UltraCheck 1** and use a new pipet tip to dispense 100 $\mu\text{L}$  of **Luminase** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record  $\text{RLU}_{\text{ATP1}}$  manually, or directly in LumiCalc.



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

**NOTE:**  $\text{RLU}_{\text{ATP1}}$  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 – 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.

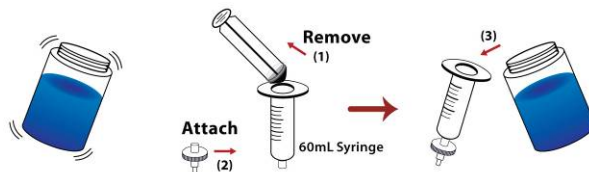
### 2.1 – MEASURE SAMPLE VOLUME

The QGA method allows flexibility in the amount of sample used for analysis. Use the following table to select the quantity of sample for your application. 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 or Process 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	$\geq 100$

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



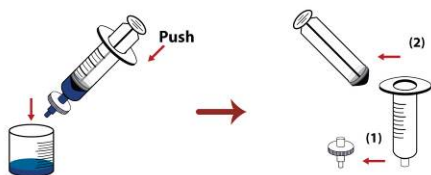
**TIP:** Alternatively, the syringe tip can be immersed into the sample and drawn into the barrel before attaching the filter, rather than pouring the sample into the barrel after attaching



the filter. Be sure the syringe tip is clean if this mechanism is used.

## 2.2 – SAMPLE FILTRATION

Slowly push the entire sample volume through the filter and into a waste receptacle at a rate of approximately 3-5mL per second. 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.



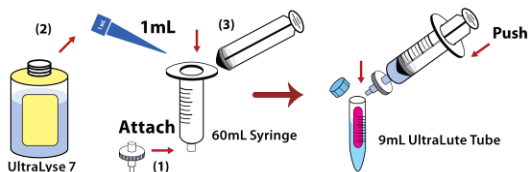
**NOTE:** If the full volume of sample could not be filtered, record the actual volume processed.

**NOTE:** If you encounter difficulty achieving complete filtration of your fluid sample, consider changing to a test kit more suitable for the sample type. Contact LuminUltra for recommendations.

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

## 2.3 – 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** slowly through the filter to dryness and collect in a new **9mL UltraLute (Dilution) Tube**. Cap and invert three times to mix.



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

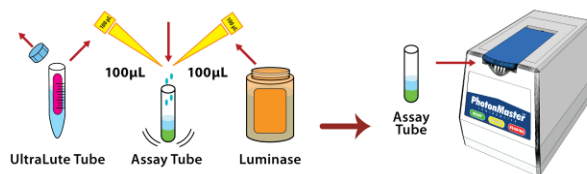
**NOTE:** When using the bulk-format version, you must dispense your own reagent into a clean test tube.

**TIP:** For longer stability, collect the 1mL extract in a separate, empty tube and store between 2-8°C for up to 1 week prior to dilution.

## 2.4 – 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  $RLU_{cATP}$  manually, or directly in LumiCalc.



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

**NOTE:** When  $RLU_{cATP} \leq 50$  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  $RLU_{cATP}$  and greater accuracy.

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

## 2.5 – CALCULATIONS

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

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

$$cATP (pg\ ATP / mL) = \frac{RLU_{cATP}}{RLU_{ATP1}} \times \frac{10,000 (pg\ ATP)}{V_{Sample} (mL)}$$

**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.

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

**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**. 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 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)
<b>High-Purity Water</b>	<0.1	0.1 to 1.0	>1.0
<b>Water for Consumption</b> (Potable, Sanitary)	<0.5	0.5 to 10	>10
<b>Raw Make-up Water</b> (Fresh, Brackish, Salt, Reclaimed)	<10	10 to 100	>100
<b>Cooling &amp; Process Water</b> <i>Oxidizing Biocides *</i>	<10	10 to 100	>100
<b>Cooling &amp; Process Water</b> <i>Non-Oxidizing Biocides or Non-Chemical Treatment **</i>	<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 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. QGA is available in five formats:

Description	Part #
QGA, 100 Tests, Complete *	QGA-100C
QGA, 100 Tests, Reagents Only	QGA-100
QGA, 100 Tests, Bulk Format **	QGA-100B
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.

\*\* 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.

### LuminUltra Technologies Ltd.

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- 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.

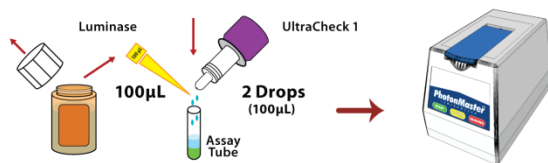
*Lumitester is a trademark of Kikkoman Corporation. All other trademarks are the property of LuminUltra Technologies Ltd.*





### 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 - Cellular ATP (cATP™) Analysis

#### 2.1 – MEASURE SAMPLE VOLUME

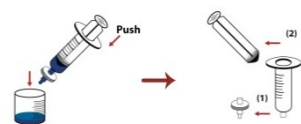
Determine volume and filter sample.

Sample Type	Volume (mL)
Cooling or Process 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



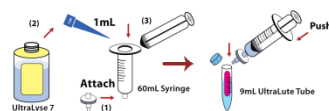
#### 2.2 – FILTRATION

Filter sample.



#### 2.3 – EXTRACTION

Extract ATP from filter & dilute.



#### 2.4 – ASSAY

Measure ATP concentration.



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

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

### Calculations

Cellular ATP (cATP) Calculation):

$$cATP \text{ (pg ATP / mL)} = \frac{RLU_{cATP}}{RLU_{ATP1}} \times \frac{10,000 \text{ (pg ATP)}}{V_{\text{Sample}} \text{ (mL)}}$$

Microbial Equivalent (ME/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 (1fg) 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
Cooling & Process Water (Oxidizing Biocides)	< 10	10 to 100	> 100
Cooling & Process Water (Non-Oxidizing Biocides or Non-Chemical Treatment)	< 100	100 to 1,000	> 1,000

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