Test Kit Instructions

QuenchGone21™ Wastewater Test Kit

Product #: QG21W-50 / QG21Wa-25

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.

QG21W Test Kit (QG21W-50C)

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 Can I Use QG21W?

QG21W test kits provide a real-time measurement of the active biomass population, stress levels, and solids viability in any biological wastewater treatment process. By isolating the living population and eliminating all interferences, the operator can maximize efficiency and stability in treatment processes such as:

- ✓ Activated Sludge
- ✓ Anaerobic Bioreactors
- ✓ Membrane Bioreactors
- ✓ MBBR Bioreactors
- ✓ Lagoons
- ✓ UASB Bioreactors
- ✓ Digesters
- ✓ Attached Growth

QG21W test kits are also designed to assess living biomass population size and stress level in untreated, incoming wastewaters and process effluents. Supplementing bioreactor measurements with upstream and downstream measurements facilitates proactive notification of process upsets.

QG21W test kits can also be used in a variety of biological production processes (including biological fermentation for alcohol or ethanol production) and bioremediation processes such as those used to rehabilitate soil.

QG21W test kits are available in two formats. Choose the best format to suit your needs from the following descriptions:

- QG21W (**QG21W-50**) provides the basic means to measure biomass concentration and health at any process location using the Total ATP (or **tATP™**: living plus dead biomass) and Dissolved ATP (or **dATP™**: dead biomass only) measurements. **QG21W-50** test kits include sufficient materials to test 50 samples.
- QG21W Advanced (**QG21Wa-25**) provides the same capabilities the QG21W test kit in addition to being able to quantify floc bulking in suspended growth aerobic reactors through the Floc-Bulking ATP (**fbATP™**) protocol, or attached growth in fixed-bed reactors, UASB digesters, trickling filters, moving-bed bioreactors (MBBR's), and soil remediation through the Attached Growth ATP (**agATP™**) protocol. **QG21Wa-25** test kits include sufficient materials to test 25 samples.

How Does ATP Testing Work?

QG21W 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).

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

QG21W test kits use two parallel 1-minute analyses (tATP and dATP) on each sample to determine three valuable pieces of information:

- **Cellular ATP (cATP™)** – represents ATP from living microorganisms and therefore is a direct indication of the living population. This information greatly facilitates inventory management and process optimization.
- **Biomass Stress Index (BSI™)** – represents the stress level experienced by the microbiological population. This quantity is very useful in monitoring for toxicity in bioreactors and upstream processes.
- **Active Biomass Ratio (ABR™)** – represents the percentage of bioreactor solids that are active microorganisms. Maximizing the ABR provides many benefits such as enhanced sludge quality and improved settling. Note that Total Suspended Solids (TSS) data are required to calculate ABR.

The QG21W Advanced test kit includes the above plus capabilities for two additional measurements:

- **Specific Floc-Bulking ATP (s-fbATP™)** – represents the quantity of ATP from bulking floc relative to microorganisms. This measurement provides an early-warning of bulking conditions, allowing operators to proactively mitigate bulking conditions in sedimentation processes.
- **Specific Attached-Growth ATP (s-agATP™)** – is a measurement of the ratio of suspended to attached microorganisms in attached-growth processes. Higher fractions of suspended relative to attached microorganisms indicate sub-optimal process conditions.

The QG21W Advanced kit provides 25 fbATP OR agATP tests (depending on your application), plus 25

tests for both tATP and dATP. We recommend always testing tATP, dATP and fbATP/agATP on the same sample for the best process information.

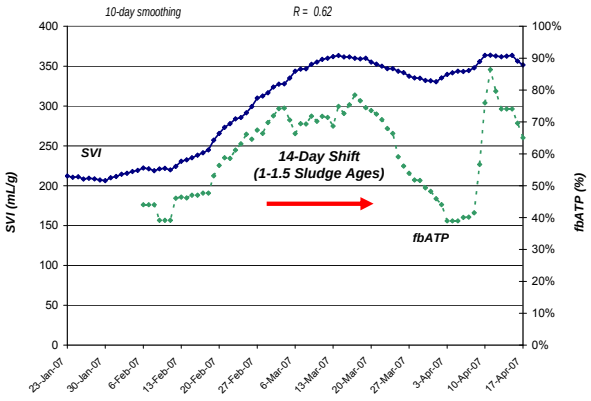
These additional parameters offer users the best preventative assessment capabilities for managing several new and unique wastewater treatment processes, as discussed below. The objective of the QG21W Advanced test kit is to enhance the power of LuminUltra's monitoring solution to address some of the most significant emerging challenges in the biological wastewater treatment sector!

Activated Sludge Bioreactors

Activated Sludge bioreactors often encounter poor settling conditions commonly referred to as **bulking**. This is generally related to the growth of filamentous organisms or the accumulation of viscous material (e.g. EPS) in the floc. Such situations are undesirable because it reduces the ability to compact biomass in clarifiers and separate out dischargeable effluent.

The fbATP measurement allows the earliest-possible warning of bulking conditions – often one sludge age or more – which allows operators to:

- Identify the causes of sludge bulking by knowing <u>when</u> the instigating conditions occur.
- Take preventative action to mitigate the onset of bulking in the early stages of growth.



- Save on costly or ineffective corrective actions by making immediate evaluation (i.e. Chlorine).
- Focus on developing meaningful, proactive solutions to prevent or eliminate bulking conditions!

Moving-Bed Bioreactors (MBBRs)

Moving Bed Bio-Reactors (MBBRs) are a specialized type of biological reactor. They are very similar to a typical activated sludge bioreactor, with one key difference: the addition of (typically) plastic media. This media is designed with a maximum ratio of surface area to weight, and serves as a 'bio-carrier' to which microorganisms attach and form communities.

Microorganisms can form extremely efficient communities when provided a surface to which they can adhere; therefore, attached growth systems are often much more efficient and resistant to upsets as compared to their suspended growth counterparts. Their disadvantage however is that when biomass is lost, the community requires substantially longer to recover. By monitoring both the biomass attached on the media through the agATP measurement plus those that are suspended in the process via the standard cATP test, users can not only gain a superior estimate of the total system biomass, but also monitor and control the relative percentage of attached biomass to suspended biomass and obtain the earliest possible warning against catastrophic biomass detachment!

Attached-Growth Bioreactors

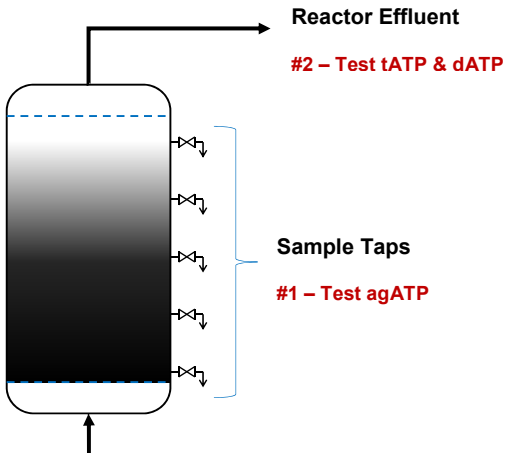
Attached growth or 'Fixed-film' wastewater treatment systems take advantage of microorganisms' natural preference to exist as part of communities attached to surfaces. These communities contain a diverse array of microorganism types that work together synergistically to sustain a healthy population. These communities are commonly referred to as 'biofilm'. Biofilms can be classified as 'biomass', made up of actual microorganisms as well as microbially-related materials such as Extracellular-Polymeric Substances (EPS). EPS can be considered to be an extension of the microbial cells, providing a solvent that allows the community to do work in the extra-cellular environment and offering them protection from external toxins.

Common types of attached growth systems include trickling filters, rotating biological contactors, and biologically-active filters. Similar to the application of the agATP method in MBBR processes, LuminUltra's agATP measurement along with the standard cATP test method provides users with a superior total system biomass indicator and an early-warning against catastrophic biomass detachment.

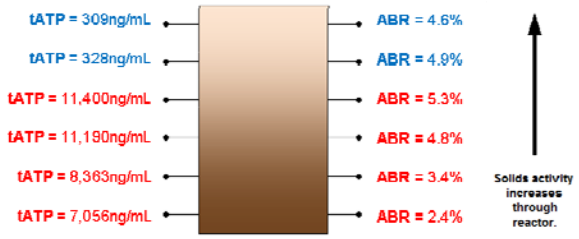
UASB Bioreactors

Upflow Anaerobic Sludge Blanket (UASB) digesters are a relatively new type of biological reactor. They are extremely efficient in converting high COD wastewater streams into a useful product (biogas) and minimal waste (sludge). Because of the nature of anaerobic microorganisms, they are also prone to debilitating upsets from which they can be very slow to recover. LuminUltra's monitoring solutions can provide UASB operators with new insight into the efficiency of these processes and can provide early warnings of potential toxicity events that can lead to such upsets.

The following simplified process diagram shows the primary locations on which to run wastewater ATP tests.



Through the application of the agATP method in the UASB fluidized bed, you will be able to gain an understanding of total biomass activity in the process.



A certain amount of biomass will be present in the reactor effluent at all times, which is due to hydraulics and bacteria life cycles. The most common failure in UASB bioreactors is sludge degranulation and subsequent departure of biomass from the reactor. This can lead to a recuperation cycle of many months and expensive re-seeding of the reactor. Although many factors can play a role, this is often caused by the entry of a toxin into the bioreactor via the feed which causes biomass stress, separation (degranulation) and subsequently depart to avoid said stress.

The beginnings of this problem can be very subtle so the sensitivity of QG21W testing is very advantageous since it can detect unhealthy bacteria and changes in the quantity departing the reactor, providing the earliest possible warning of this undesirable event!

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.

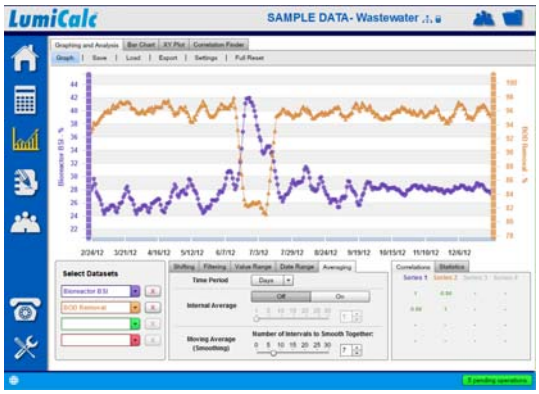
PhotonMaster Equipment Set (EQP-PAC-PMT)

- 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.

QG21W Test Kit Contents & Storage Conditions

Component (Part Number)	Store At	Shelf Life
Luminase™ W Enzyme & Buffer Vials (**LuW-3mL-FD**) *Luciferase Enzyme Reagent, 3mL*	4 to 25ºC	6 to 12 mo*
UltraCheck™ 1 Dropper Bottle (**UC1-5mL**) *1 ng ATP/mL Standard, 5mL*	4 to 25ºC	18 mo
UltraLyse™ 30[21] (Extraction) Tube, 2mL (**UL30(21)-2mL-50R**) *tATP Extraction Reagent, 2mL*	4 to 25ºC	18 mo
UltraLute™/Resin (Dilution) Tube, 8mL (**ULuR-8mL-50R**) *tATP Dilution Reagent, 8mL*	4 to 25ºC	18 mo
LumiSolve™ (Stabilizer) Tube, 10mL (**LS-10mL-50R**) *dATP Stabilizing Reagent, 10mL*	4 to 25ºC	18 mo
UltraLyse™ 30[21] (Extraction) Tube, 10mL (**UL30(21)-10mL-25R**) ** *tATP Extraction Reagent, 10mL*	4 to 25ºC	18 mo
100 to 1000µL Blue Pipet Tips, 100/rack (**DIS-PT1-100R**)	-	-
100 to 1000µL Wide-Mouth Pipet Tips, 100/rack (**DIS-PT1WM-100R**)	-	-
10 to 200µL Yellow Pipet Tips, 96/rack (**DIS-PT01-96R**)	-	-
12x55mm Assay Tubes, 50/pk (**DIS-CT12-50**)	-	-
2" x 2" 250µm Mesh Squares, 25/pk ** (**DIS-MESH-25**)	-	-
1.5" x 1.5" Weigh Boat, 25/pk ** (**DIS-WD-25**)	-	-
Scissor-Type Forceps, 1/pk** (**EQP-FOR**)	-	-
Specimen Container, 120mL, 25/pk ** (**DIS-CONT-25**)	-	-

* Luminase[W] 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[W] supplied in QG21I kits is NOT interchangeable with other forms of Luminase (i.e. Luminase Lite, Luminase, and Luminase[XL]).

** Materials are included as part of QG21W Advanced (QG21Wa-25) test kit only.

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 8ºC) 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.

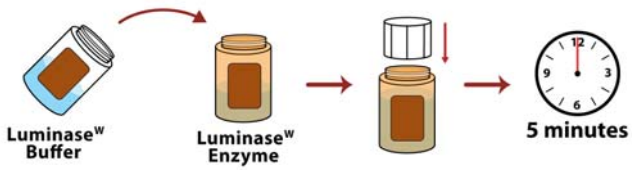
- Avoid taking multiple luminometer readings on the same assay. The light output from ATP assays is relatively constant and at a maximum for the first 15-30 seconds after mixing, after which the output will decline.
- When testing samples that yield low RLU values (i.e. $RLU_{ATP} \leq 50$), it is recommended 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}).

Tips for Wastewater System Testing

- For all samples – including influent, effluent, bioreactor and return sludge samples – always measure tATP and dATP. When using the QG21Wa test kit, utilize fbATP and/or agATP measurements only on specific sample types in addition to tATP and dATP on their associated surrounding fluids.
- For bioreactor samples, it is recommended that the same samples as those used for measuring MLSS and/or MLVSS are used for QG21W tests.
- When testing influent samples for toxicity, it is recommended that raw samples be mixed 1:1 with bioreactor biomass and then the mixture tested using this test kit. Consult the QG21W training section at www.luminultra.com or contact us for more detailed instructions on this procedure.

Handling Luminase

- **Luminase^W** 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^W Rehydration Process

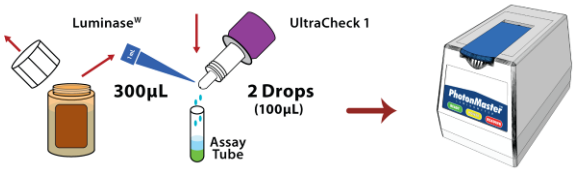
- Rehydrated **Luminase^W** can be stored in the refrigerator for up to 3 months (or freezer for up to 6 months with unlimited freeze-thaw cycles) following rehydration. Always bring cold rehydrated **Luminase^W** to ambient temperature prior to use. 1 hour is generally sufficient for this purpose.
-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

Step 1 – ATP Standard Calibration

Included in QG21W and QG21Wa test kits.

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^W**) 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 300µL of **Luminase^W** 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} ≤ 500 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.

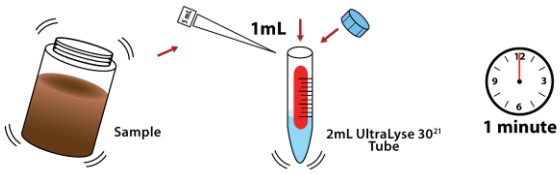
Step 2 – tATP™ Analysis

Included in QG21W and QG21Wa test kits.

The Total ATP (**tATP**) analysis measures ATP from both living and dead cells. Perform one tATP analysis on each sample you wish to test.

2.1 – EXTRACTION

Using a new wide-mouth pipet tip, add 1mL of well-mixed sample to a **2mL UltraLyse 30[21] (Extraction) Tube**. Cap and invert three times to mix. Allow at least 1 minute for incubation.



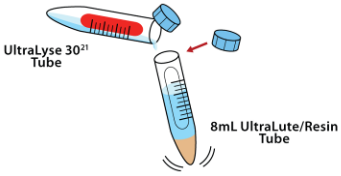
NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

NOTE: At this point, the contents of the Extraction Tube can be capped and stored refrigerated between 2-8ºC for up to 1 week prior to 2.2.

TIP: Wide-mouth pipet tips are those that have sufficiently large openings to prevent tip plugging by sample particles. In general, wide-mouth 100-1000µL pipet tips are sufficient for most samples. If required, increase the bore size of a tip using a clean pair of scissors.

2.2 – DILUTION

Pour the **UltraLyse 30[21] (Extraction) Tube** contents into a new **8mL UltraLute/Resin (Dilution) Tube**. Transfer the mixture back and forth between the two tubes several times for best mixing accuracy. Cap and invert three times to mix. Allow beads to settle.



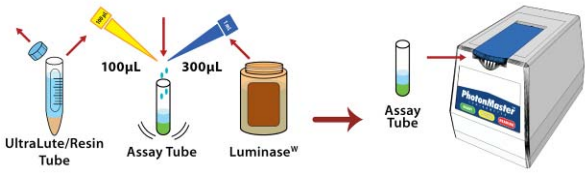
NOTE: At this point, the contents of the Dilution Tube are stable at room temperature for up to 4 hours.

TIP: If beads do not settle or settle slowly, tap the Dilution Tube gently to assist settling.

TIP: If the Extraction Tube cannot be poured into the Dilution Tube, simply pour the Dilution Tube contents into the Extraction Tube to liquefy its contents.

2.3 – ASSAY

Using a new pipet tip, add 100µL of the **UltraLute/Resin (Dilution) Tube** contents and another new pipet tip to add 300µL of **Luminase[W]** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record RLU_{tATP} manually, or directly in LumiCalc.



NOTE: If RLU_{tATP} ≤ 10 on a PhotonMaster or Lumitester C-110, you are below the low–detection limit. Report tATP (ng ATP/mL) = 0 in calculations.

NOTE: When RLU_{tATP} ≤ 50 on a PhotonMaster or Lumitester C-110, it is recommended that you measure and subtract RLU_{bg} from your measurement.

TIP: If "Scale Over" is returned, repeat the tATP analysis using 100µL of sample in 2.1 (EXTRACTION) and modify the dilution factor in the calculations as noted.

Step 3 – dATP™ Analysis

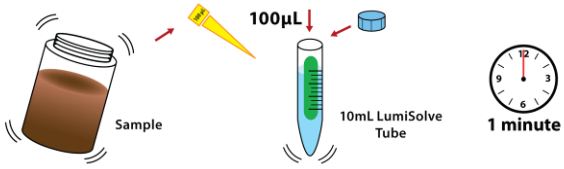
Included in QG21W and QG21Wa test kits.

The Dissolved ATP (**dATP**) analysis measures ATP from only dead cells. Perform one dATP analysis on each sample you wish to test.

3.1 – DILUTION

Using a new pipet tip, add 100µL of well-mixed sample to a **10mL LumiSolve (Stabilizer) Tube**. Cap and

invert three times to mix. Allow at least 1 minute for incubation.

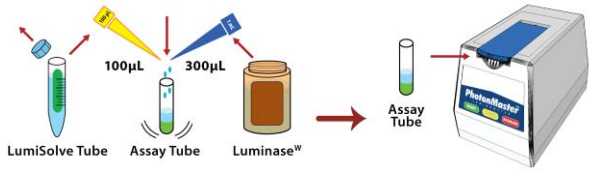


NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

NOTE: At this point, the LumiSolve Tube can be capped and stored between 2 to 8°C for up to 1 week prior to Part 3.2.

3.2 – ASSAY

Using a new pipet tip, add 100µL of the **LumiSolve (Stabilizer) Tube** contents and another new pipet tip to add 300µL of **Luminase^{W}** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record RLU_{dATP} manually, or directly in LumiCalc.



NOTE: If RLU_{dATP} ≤ 10 on a PhotonMaster or Lumitester C-110, you are below the low–detection limit. Report tATP (ng ATP/mL) = 0 in calculations.

NOTE: When RLU_{dATP} ≤ 50 on a PhotonMaster or Lumitester C-110, it is recommended that you measure and subtract RLU_{bg} from your measurement.

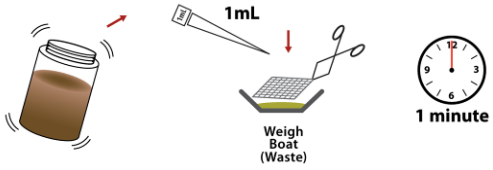
Step 4a – fbATP™ Analysis

Included in QG21Wa test kit only.

The Floc-Bulking ATP (**fbATP**) analysis measures only ATP from bulking floc. Perform fbATP measurements only on suspended growth aerobic bioreactor samples (e.g. Mixed Liquor), in addition to testing tATP and dATP on the same samples.

4a.1 – FILTRATION

Using the supplied forceps, place a new piece of 2”x2” 250µm mesh over a new 1.5”x1.5” weigh boat. Using a new wide-mouth pipet tip, slowly and carefully pipet 1mL of well-mixed sample onto the top of the mesh and allow the filtrate to be collected in the weigh boat. Allow 1 minute for filtration to occur.



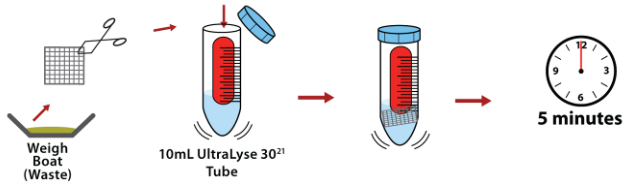
NOTE: If no filtrate passes through the mesh after 1 minute, this can be a sign of viscous bulking in your sample.

TIP: The filtrate and used weigh boat should be disposed following this step. The mesh is used in step 4a.2, and the forceps are reused for the life of the test kit.

TIP: Wide-mouth pipet tips are those that have sufficiently large openings to prevent tip plugging by sample particles. In general, wide-mouth 100-1000µL pipet tips are sufficient for most samples. If required, increase the bore size of a tip using a clean pair of scissors.

4a.2 – EXTRACTION

Use forceps to carefully transfer the 2” x 2” 250µm mesh into a new **10mL UltraLyse 30^{21} (Extraction) Tube**. Cap and mix the container thoroughly to ensure complete contact between **UltraLyse 30^{21}** and the mesh. Allow at least 5 minutes of incubation.



NOTE: If you are unable to easily insert the mesh into the 10mL Extraction Tube, place the mesh in one of the supplied 120mL specimen containers and pour the 10mL of UltraLyse 30^{21} into the container for extraction.

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

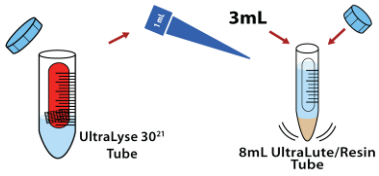
NOTE: At this point, the contents of the Extraction Tube can be capped and stored refrigerated between 2-8°C for up to 1 week prior to 4a.3.

TIP: If the mesh sticks to the walls of the Extraction Tube, simply place the container on its side during the incubation period with the mesh immersed in UltraLyse 30^{21}.

4a.3 – DILUTION

Using a new pipet tip, transfer 3mL (3 x 1mL) of the contents from the **UltraLyse 30^{21} (Extraction) Tube**

into a new **8mL UltraLute/Resin (Dilution) Tube**. Cap and invert three times to mix. Allow beads to settle.

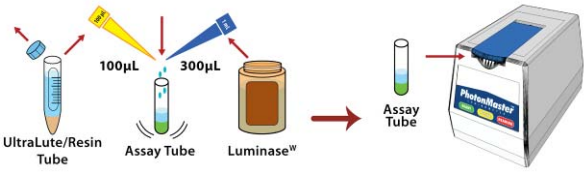


NOTE: At this point, the contents of the Dilution Tube are stable at room temperature for up to 4 hours.

TIP: If beads do not settle or settle slowly, tap the Dilution Tube gently to assist settling.

4a.4 – ASSAY

Using a new pipet tip, add 100µL of the **UltraLute/Resin (Dilution) Tube** contents and another new pipet tip to add 300µL of **Luminase^{W}** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record RLU_{fbATP} manually, or directly in LumiCalc.



NOTE: If $RLU_{fbATP} \leq 10$ on a PhotonMaster or Lumitester C-110, you are below the low–detection limit. Report fbATP (ng ATP/mL) = 0 in calculations.

NOTE: When $RLU_{fbATP} \leq 50$ on a PhotonMaster or Lumitester C-110, it is recommended that you measure and subtract RLU_{bg} from your measurement.

Step 4b – agATP™ Analysis

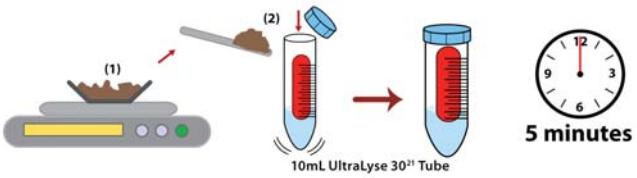
Included in QG21Wa test kit only.

The Attached Growth ATP (**agATP**) analysis measures ATP from attached microorganisms in fixed-bed reactors, UASB digesters, trickling filters, moving-bed bioreactors (MBBR's), and soil remediation. Perform agATP measurements only on samples collected from such systems, and measure tATP and dATP on the surrounding fluid.

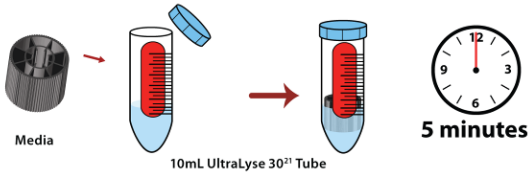
4a.2 – EXTRACTION

Acquire a representative quantity of your attached growth microorganisms from which you will measure tATP. Because these communities are largely living biomass, it is not necessary to measure dATP of the attached biomass. In other words, you can assume that tATP = cATP in this test.

For fixed-bed systems (such as UASB digesters) or biofilm-driven systems, acquire a sample of the bioreactor bed or scrape a portion of the biofilm. Measure approximately 1g of sample and quantitatively add to a **10mL UltraLyse 30^{21} (Extraction) Tube**. Cap and mix the container thoroughly to ensure complete contact between **UltraLyse 30^{21}** and the sample. Allow at least 5 minutes of incubation.



Or, when testing engineered attached growth systems such as MBBR's, acquire a sample of the media ('discs'). Add 1-2 pieces of media to a **10mL UltraLyse 30^{21} (Extraction) Tube**. Cap and mix the container thoroughly to ensure complete contact between **UltraLyse 30^{21}** and the media. Allow at least 5 minutes of incubation.

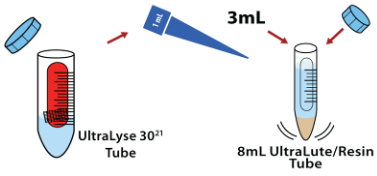


NOTE: When using the bulk-format version, you must dispense your own reagent into a clean test tube.

NOTE: At this point, the contents of the Extraction Tube can be capped and stored refrigerated between 2-8°C for up to 1 week prior to 4b.2.

4b.2 – DILUTION

Using a new pipet tip, transfer 3mL (3 x 1mL) of the contents from the **UltraLyse 30^{21} (Extraction) Tube** into a new **8mL UltraLute/Resin (Dilution) Tube**. Cap and invert three times to mix. Allow beads to settle.

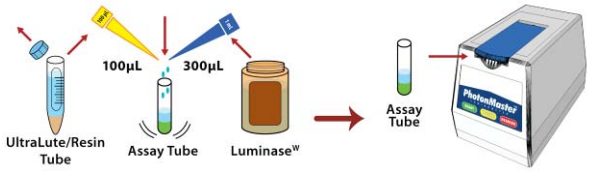


NOTE: At this point, the contents of the Dilution Tube are stable at room temperature for up to 4 hours.

TIP: If beads do not settle or settle slowly, tap the Dilution Tube gently to assist settling.

4b.3 – ASSAY

Using a new pipet tip, add 100µL of the **UltraLute/Resin (Dilution) Tube** contents and another new pipet tip to add 300µL of **Luminase^{W}** to a new 12x55mm test tube (the Assay Tube), swirl gently five times, immediately insert into the luminometer and measure. Record RLU_{agATP} manually, or directly in LumiCalc.



NOTE: If RLU_{agATP} ≤ 10 on a PhotonMaster or Lumitester C-110, you are below the low–detection limit. Report agATP (ng ATP/mL) = 0 in calculations.

NOTE: When RLU_{agATP} ≤ 50 on a PhotonMaster or Lumitester C-110, it is recommended that you measure and subtract RLU_{bg} from your measurement.

Calculations

Following completion of QG21W analyses, RLU values must be converted to ATP concentrations using the following calculations. For easy calculations, utilize **LumiCalc** software. Calculate each value for each sample tested before determining Key Process Indicators.

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

   NOTE: If 100µL of sample was used in the tATP analysis, replace the dilution factor "11" with "101".

   $$tATP\,(ng\ ATP/mL) = \frac{RLU_{tATP}}{RLU_{ATP1}} \times 11\,(ng\ ATP/mL)$$

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

2. Dissolved ATP (**dATP**) – represents ATP within a sample that has been released from dead cells.

   $$dATP\,(ng\ ATP/mL) = \frac{RLU_{dATP}}{RLU_{ATP1}} \times 101\,(ng\ ATP/mL)$$

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

3. Floc-Bulking ATP (**fbATP**) – measures ATP associated with bulking floc in suspended growth aerobic bioreactor samples.

   $$fbATP\,(ng\ ATP/mL) = \frac{RLU_{fbATP}}{RLU_{ATP1}} \times 36.7\,(ng\ ATP/mL)$$

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

4. Attached Growth ATP (**agATP**) – measures ATP associated with attached microorganisms in attached growth systems.

   $$agATP\,(ng\ ATP/\text{units}) = \frac{RLU_{agATP}}{RLU_{ATP1}} \times \frac{36.7\,(ng\ ATP)}{1g\ or\ \#\ media}$$

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

Use these results to determine key process indicators shown in the next section.

Key Process Indicators

For monitoring basic biomass concentration and health at any process location, the following parameters are used. For easy calculations, utilize **LumiCalc** software.

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

   $$cATP\,(ng\ ATP/mL) = tATP\,(ng\ ATP/mL) - dATP\,(ng\ ATP/mL)$$

   NOTE: When the computed dATP (pg/mL) is greater than tATP (pg/mL), first confirm that the result is not due to inhibition by re-testing tATP and dATP using 0.1mL of sample rather than 1mL. If the result persists, report dATP (pg/mL) = tATP (pg/mL). Occurrences of dATP > tATP are most often the result of a combination of test method and instrumentation sensitivity and are to be considered normal.

   NOTE: It is important to stress that in situations of dATP (ng/mL) = tATP (ng/mL), it does not mean that the entire microbiological population is **dead** and are therefore incapable of performing work functions (e.g. BOD removal). What it does mean is that in their current state, the microorganisms are severely compromised to the degree that their weakened cell membranes are lysed and their ATP is released even when exposed to a mild buffer such as LumiSolve. Occurrences of dATP (ng/mL) = tATP (ng/mL) should be taken as an alert to take action

immediately to correct the stress (e.g. catastrophic loss of nutrients or oxygen, severe toxicity). Sustained stress at this level can result in complete failure of a bioreactor.

2. Active Volatile Suspended Solids (**AVSS**) – represents the total mass of living microorganisms contained in the sample. The conversion factor of 0.5 is an established factor to convert from ng ATP/mL to mg Solids/L

$$AVSS(mg\ Biomass/L) = cATP\ (ng\ ATP/mL) \times 0.5$$

NOTE: For more information on the conversion of ng cATP/mL to mg Active Biomass/L, visit www.luminultra.com or contact support.

3. Active Biomass Ratio (**ABR**) – represents the percentage of total suspended solids that are living microorganisms.

NOTE: Calculate only if TSS data is available.

$$ABR\ (\%) = \frac{AVSS\ (mg\ Biomass/L)}{TSS\ (mg/L)} \times 100\%$$

NOTE: If ABR > 100%, it may be an indication that severe deflocculation has occurred and not all biomass has been captured in the TSS analysis.

4. Biomass Stress Index (**BSI**) – provides a measure of the stress level (quality) of the microbiological community.

$$BSI\ (\%) = \frac{dATP\ (ng\ ATP/mL)}{tATP\ (ng\ ATP/mL)} \times 100\%$$

NOTE: If dATP (ng/mL) > tATP (ng/mL) as discussed above, the BSI value will exceed 100%. If these values persist after re-testing, report BSI = 100%.

5. When the fbATP analysis is also performed, utilize the following calculation to obtain the s-fbATP control parameter.

   Specific fbATP (**s-fbATP**) – provides the relative quantity of bulking floc to total floc. As this number increases, the risk of bulking conditions increases.

   NOTE: If fbATP (ng/mL) > tATP (ng/mL), bypass these calculations and report s-fbATP = 100%.

$$s-fbATP\ (\%) = \frac{fbATP\ (ng\ ATP/mL)}{tATP\ (ng\ ATP/mL)} \times 100\%$$

6. When the agATP analysis is also performed, utilize the following calculation to obtain the s-agATP control parameter.

   Specific agATP (**s-agATP**) – provides the relative quantity of attached microorganisms to total microorganisms. As this number decreases, the risk of process failure due to biomass detachment increases.

$$s-agATP\ (\%) = \frac{agATP\ (ng\ ATP/mL)}{tATP\ (ng\ ATP/mL) + agATP\ (ng\ ATP/mL)} \times 100\%$$

NOTE: For the greatest accuracy, it is recommended to compute the total mass of suspended and attached biomass when performing the above calculation. In other words, multiply suspended concentration by total system volume, and attached concentration by the total surface area, # of media or size of the bed. Consult support for assistance.

Interpretation Guidelines

Once cATP, BSI, ABR (if available), s-fbATP (if available) and s-agATP (if available) are calculated, true microbial control can be evaluated. ATP-based measurements are extremely sensitive to differences in microbial quantity and quality. In general, microorganisms are in their best condition when **cATP is maintained**, **BSI is minimized**, **ABR is maximized**, **s-fbATP is minimized**, and **s-agATP is maximized**. For the easiest interpretation, utilize **LumiCalc** software.

QG21W test kits can be used to audit microbial quantity and quality to reveal differences at different process locations — for example, monitoring mixing efficiency or toxicity. For process control, daily monitoring using QG21W test kits will give you true microbial quantity and quality parameters to trend over time against process characteristics and performance.

When utilizing QG21W 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 adjustments. To get started, LuminUltra provides the following guidelines:

QG21W Interpretation Guidelines

Location	Parameter	Good Control	Preventive Action Required	Corrective Action Required
Influent	BSI	< 50	50 to 75	> 75
Bioreactors	cATP	* Process Specific		
	BSI	< 30	30 to 50	> 50
	ABR	> 25	10 to 25	< 10
Activated Sludge	s-fbATP	< 30	30 to 50	> 50
Attached Growth	s-agATP	> 90	75 to 90	< 75
Effluent	cATP	< 50	50 to 250	> 250

* The magnitude of cATP will depend on bioreactor configuration. In general, deviation from typical values by +/- 25% to 50% should be considered a preventative guideline and +/- 50% or greater should be considered corrective.

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.

You can find examples of how QG21W products have been used to solve problems for various customers at www.luminultra.com!

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. QG21W is available in seven formats:

Description	Part #
QG21W Standard, 50 Tests, Complete *	QG21W-50C
QG21W Standard, 50 Tests, Reagents Only	QG21W-50
QG21W Standard, 50 Tests, Bulk Format **	QG21W-50B
QG21W Advanced, 50 Tests, Complete *	QG21Wa-25C
QG21W Advanced, 50 Tests, Reagents Only	QG21Wa-25
QG21W Advanced, 50 Tests, Bulk Format **	QG21Wa-25B
QG21W Complete, 75 Tests, Complete *	QG21Wc-75C

* 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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Fredericton, New Brunswick
Canada E3B 6G3
Tel: +1-506-459-8777
Fax: +1-506-453-9860
sales@luminultra.com
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

QuenchGone21™ Wastewater Test Kit

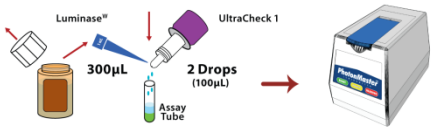
Product #: QG21W-50 / QG21Wa-25

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.



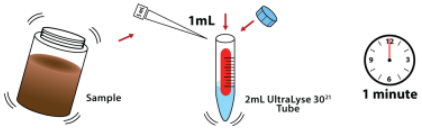
NOTE: If $RLU_{ATP1} \leq 500$ using a PhotonMaster or Lumitester C-110, rehydrate a new bottle of Luminase™ for maximum sensitivity.

Step 2 – Total ATP (tATP™)

Included in QG21W and QG21Wa test kits.

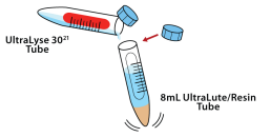
2.1 - EXTRACTION

Add sample to extract ATP.



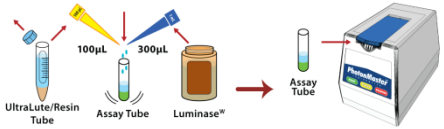
2.2 – DILUTION

Dilute out interferences.



2.3 – ASSAY

Measure ATP concentration.



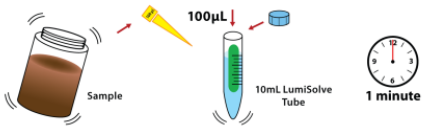
NOTE: If $RLU_{tATP} \leq 10$ using a PhotonMaster or Lumitester C-110, you are below the low-detection limit.

Step 3 – Dissolved ATP (dATP™)

Included in QG21W and QG21Wa test kits.

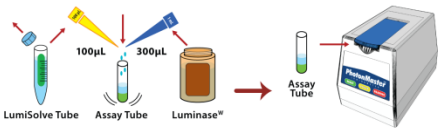
3.1 – DILUTION

Add sample to recover ATP.



3.2 – ASSAY

Measure ATP concentration.



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

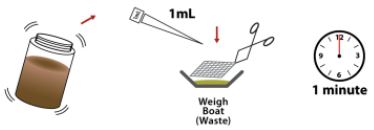
CHOOSE ONE METHOD FROM:

STEP 4a – Floc Bulking ATP (fbATP™)

Included in QG21Wa test kit only.

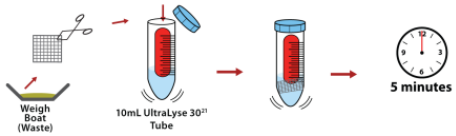
4a.1 - FILTRATION

Filter sample to separate bulking floc.



4a.2 - EXTRACTION

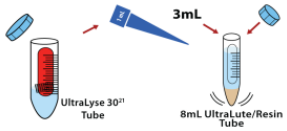
Extract ATP from mesh.



NOTE: If unable to place mesh into the Extraction tube, use one of the supplied 120mL specimen containers and pour the 10mL of UltraLyse 30^{21} onto the mesh.

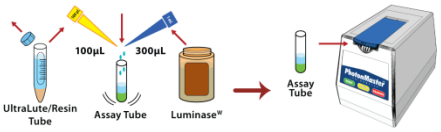
4a.3 - DILUTION

Dilute out interferences.



4a.4 - ASSAY

Measure ATP concentrations.



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

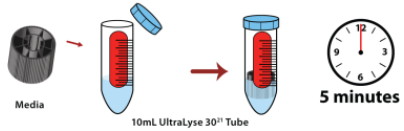
STEP 4b – Attached Growth ATP (agATP™)

Included in QG21Wa test kit only.

4b.1 - EXTRACTION

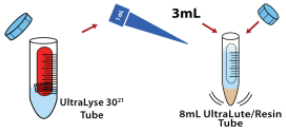
Extract ATP from sample.

OR



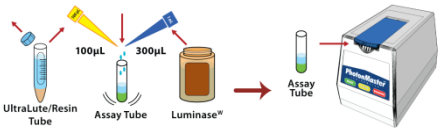
4b.2 - DILUTION

Dilute out interferences.



4b.3 - ASSAY

Measure ATP concentrations.



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

Please refer to Test Kit Instructions for calculations and interpretation advice, or use LumiCalc software.