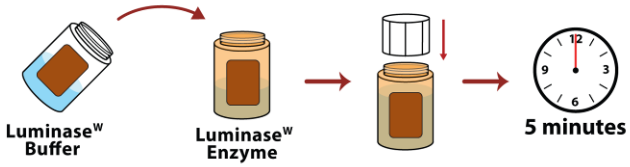
LUMINULTRA microbial monitoring

Test Kit Instructions

QuenchGone21™ Wastewater Advanced (QG21Wa)

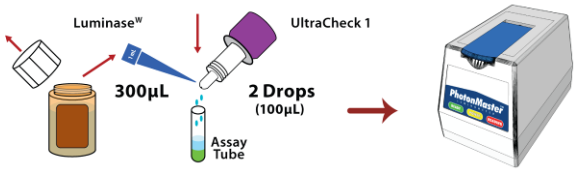
Rehydrating Luminase

- Gently mix the buffer and **Luminase^{W}** enzyme**.**
- Wait 5 minutes for solution to dissolve.



1. ULTRACHECK CALIBRATION (RLU_{ATP1})

- Hold the UltraCheck1 bottle vertical, add 2 drops (100µL) of **UltraCheck1** to a 12x55mm test tube.
- Pipet 300µL of **Luminase^{W}** into the tube.
- Swirl the tube and take reading within 10 seconds.

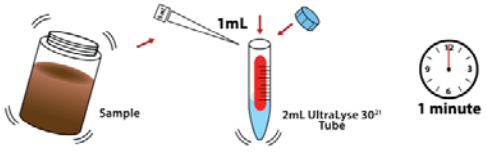


***** If RLU_{ATP1} ≤ 500 rehydrate a new bottle of Luminase^{W}.

2. TOTAL ATP ANALYSIS (RLU_{tATP})

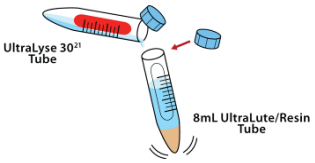
2.1 EXTRACTION

- Mix sample well and test before sample settles.
- Using a wide-mouth pipet tip, add 1mL of sample to a **2mL UltraLyse 30^{21} (Extraction) Tube**.
- Cap, mix and allow 1 minute for incubation.



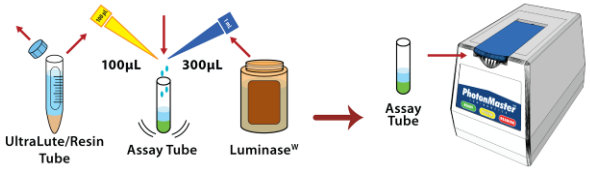
2.2 DILUTION

- Pour the **UltraLyse 30^{21} (Extraction) Tube** contents into a new **8mL UltraLute/Resin (Dilution) Tube**.
- Transfer the mixture between the tubes several times. Cap, mix and allow beads to settle.



2.3 ASSAY

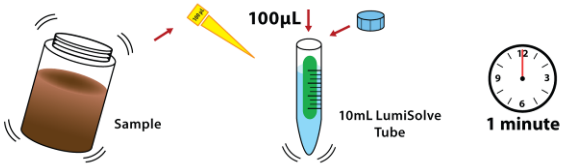
- Add 100µL of the **UltraLute/Resin (Dilution)** solution to a 12x55mm test tube.
- Use a new pipet tip to add 300µL of **Luminase^{W}**.
- Swirl the tube and take reading within 10 seconds.



3. DISSOLVED ATP ANALYSIS (RLU_{dATP})

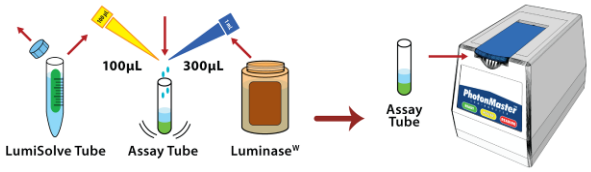
3.1 DILUTION

- Gently mix the sample and test before the sample settles.
- Using a wide-mouth pipet tip, add 100µL of sample to a **10mL LumiSolve Tube**.
- Cap, mix and allow 1 minute for incubation.



3.3 ASSAY

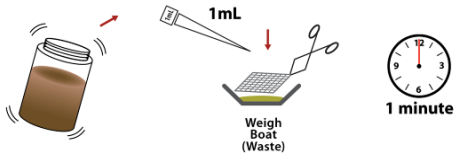
- Add 100µL of the **10mL LumiSolve** solution to a 12x55mm test tube.
- Use a new pipet tip to add 300µL of **Luminase^{W}**.
- Swirl the tube and take reading within 10 seconds.



4A. FLOC-BULKING ATP ANALYSIS (RLU_{fbATP})

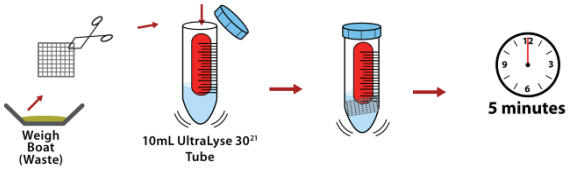
4.1a FILTRATION

- Using the forceps, place a new piece of the 2”x2” 250µm mesh over a new 1.5”x1.5” weigh boat.
- Pipet 1mL of the sample onto the mesh and collect the filtrate. Allow 1 minute for filtration.



4.2a EXTRACTION

- Carefully transfer the mesh into a new **10mL UltraLyse 30^{21} (Extraction) Tube.**
- Cap, mix and allow 5 minutes for extraction.

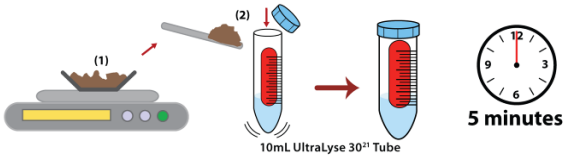


Continue on to step **4.3 Dilution**.

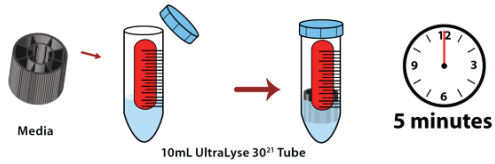
4B. ATTACHED GROWTH ATP ANALYSIS (RLU_{agATP})

4.1b EXTRACTION

- Measure 1g of media sample and add it to a new **10mL UltraLyse 30^{21} (Extraction) Tube.**
- Cap, mix and allow 5 minutes for extraction.



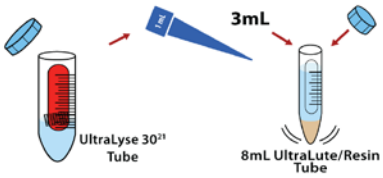
- **OR**, add 1-2 pieces of media discs to the **10mL UltraLyse 30^{21} (Extraction) Tube.**
- Cap, mix and allow 5 minutes for extraction.



Continue to step **4.3 Dilution**.

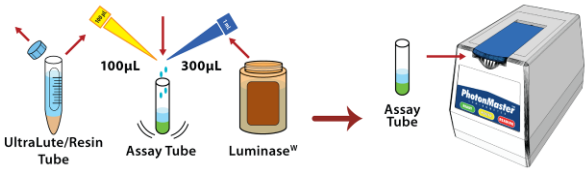
4.3 DILUTION

- 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, invert three times, and allow beads to settle.



4.4 ASSAY

- Add 100µL of the **UltraLute/Resin (Dilution) Tube** solution to a 12x55mm test tube.
- Use a new pipet tip to add 300µL of **Luminase^{W}**.
- Swirl the tube and take reading within 10 seconds.



Preliminary Calculations

For automatic calculations, utilize **LuminUltra Cloud**.

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

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

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

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

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

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

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

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

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 **LuminUltra Cloud**.

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

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

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. 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. 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.

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

Data 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.