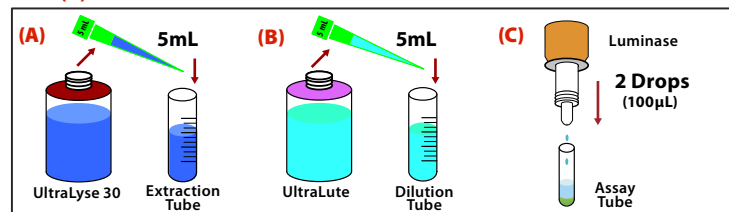


# TCB QUICK - REFERENCE GUIDE

## Total ATP (tATP™) Analysis

### 1. PREPARATION

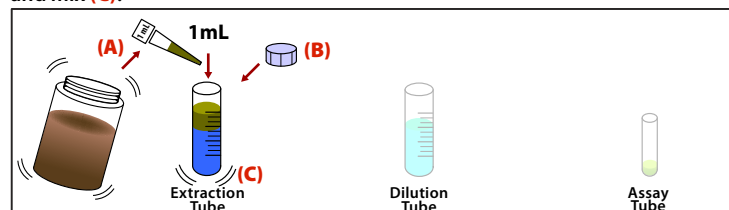
Prepare the following: 5mL of UltraLyse 30 into a 17x100mm tube (A), 5mL UltraLute into a 17x100mm tube (B), and 2 drops Luminase into a 12x55mm tube (C).



NOTE: Remove Luminase from fridge ~1 hour prior to measurement.

### 2. EXTRACTION

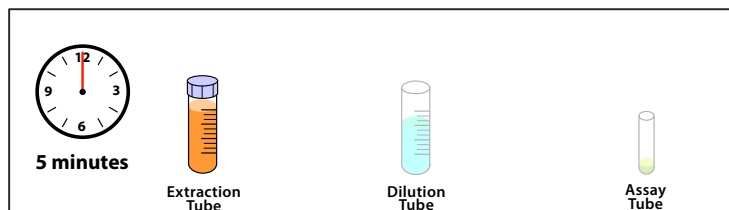
Mix the sample, then add 1mL of sample to the Extraction Tube (A). Cap (B) and mix (C).



NOTE: Always use a new pipette tip for each pipetting step!

### 3. INCUBATION

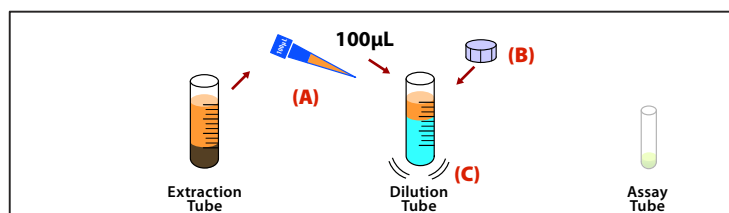
Allow 5 minutes for solids to settle in Extraction Tube.



NOTE: Wait time can be eliminated by centrifuging the extract.  
NOTE: Extract is stable for up to 1 week if stored between 2–8 °C.

### 4. DILUTION

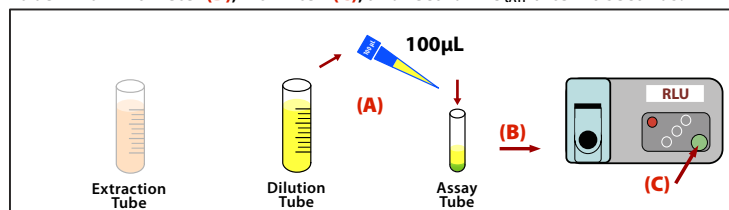
After incubation, transfer 100µL of extract supernatant to the Dilution Tube (A). Cap (B) and mix (C).



NOTE: Proceed to assay step immediately upon dilution.

### 5. ASSAY

Transfer 100µL of diluted extract to the Assay Tube (A). Immediately place Assay Tube in Luminometer (B), hit 'Enter' (C), and record RLU<sub>tATP</sub> after 10 seconds.



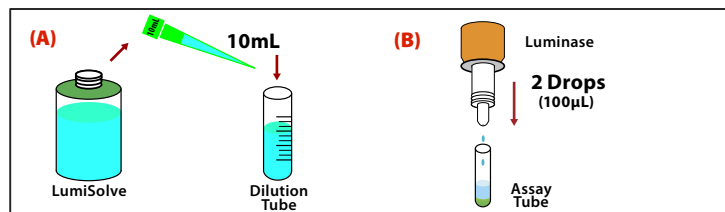
NOTE: If "SCALE OVER" is obtained, mix the dilution extract 1:1 in UltraBuff and repeat step 5.

Perform both analyses (tATP and dATP) on each sample!

## Dissolved ATP (dATP™) Analysis

### 1. PREPARATION

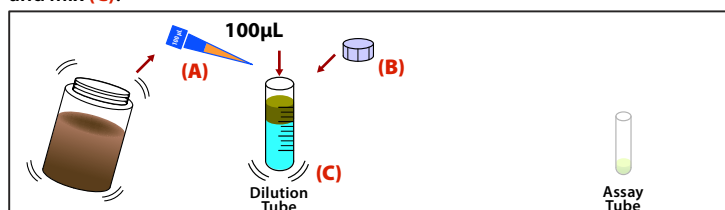
Prepare the following: 10mL of LumiSolve into a 17x100mm tube (A), and 2 drops Luminase into a 12x55mm tube (B).



NOTE: Remove Luminase from fridge ~1 hour prior to measurement.

### 2. DILUTION

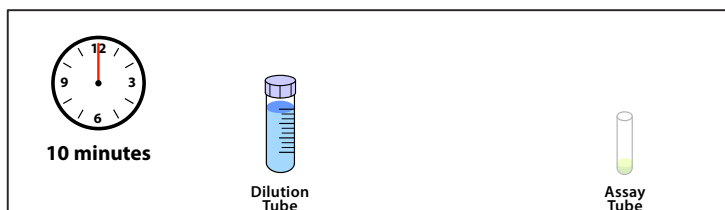
Mix the sample, then add 100µL of sample to the Dilution Tube (A). Cap (B) and mix (C).



NOTE: Always use a new pipette tip for each pipetting step!

### 3. INCUBATION

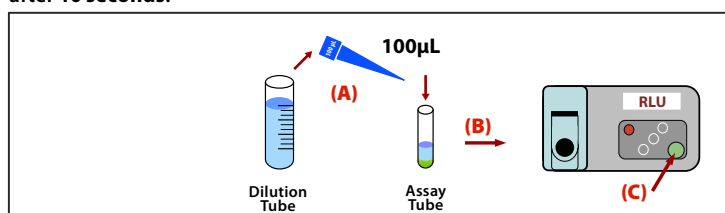
Allow 10 minutes of incubation for the Dilution Tube.



NOTE: The dATP dilution is not stable beyond 10 minutes. Proceed to assay step immediately.

### 4. ASSAY

Transfer 100µL of the diluted sample to the Assay Tube (A). Immediately place Assay Tube in the Luminometer (B), hit 'Enter' (C), and record RLU<sub>dATP</sub> after 10 seconds.



NOTE: If "SCALE OVER" is obtained, mix the dilution extract 1:1 in UltraBuff and repeat step 5.

➤ UltraCheck 1 calibration is required to convert RLU to ATP concentration.

➤ For a list of equipment requirements, consult the TCB Fact Sheet.

➤ See reverse for additional test kit use notes and tips.



# TCB QUICK-REFERENCE GUIDE

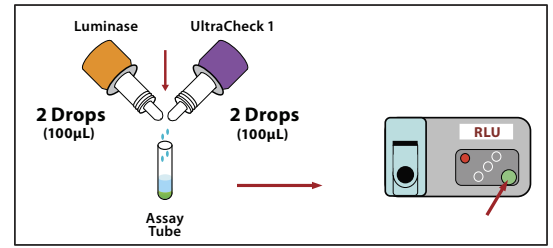
## UltraCheck™ 1 Calibration

Perform one calibration for each set of analyses!

### Procedure

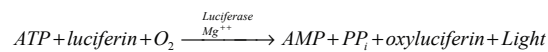
Add 2 drops of Luminase and 2 drops of UltraCheck 1 to an Assay Tube, mix gently, and insert into the Luminometer. Read and record  $RLU_{UC1}$

**NOTE:** If  $RLU_{UC1} < 5,000$  obtain a new bottle of Luminase for maximum sensitivity.



### ATP: The Basics

Total Control technology from LuminUltra™ is based on the measurement of Adenosine Triphosphate (ATP). ATP is a direct and interference-free indicator of total 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 fire flies, to produce light. The light is detected in a Luminometer.



### Kit Use and Interpretation

TCB is designed for measurement of biomass in biological wastewater treatment. **Measure** tATP and dATP on each sample, **convert** using UltraCheck results, and then **calculate** cATP, BSI and ABR. Trend these values to examine process performance.

For integration into control programs, LuminUltra recommends that testing be done at least daily! Although each process or application is different, use these guidelines to establish your control program.

Process	Parameter	Good Control	Preventive Action Required	Corrective Action Required
Process Influent	BSI (%)	< 50	50 to 75	> 75
Bioreactor	cATP (ng/mL)	<i>* Process Specific</i>		
	BSI (%)	< 30	30 to 50	> 50
	ABR (%)	> 25	10 to 25	< 10
Process Effluent	cATP (ng/mL)	< 50	50 to 250	> 250

*\* The optimum cATP control guidelines for your process will depend on reactor type and influent qualities. In general, deviation from previous values by ±25% should be considered a preventive guideline and ±50% should be considered corrective.*

For additional test kit use guidelines, consult the TCB fact sheet or visit [www.luminultra.com](http://www.luminultra.com).

### Calculations

The following formulae are used to calculate Total Control parameters. Note that  $RLU_{UC1}$  should be obtained on the same day and time as the sample  $RLU$ 's. A single  $RLU_{UC1}$  can be used for a series of samples processed together. For easy calculation, make use of LuminUltra's easy-to-use **LumiCapture™** software!

$$tATP (ng / mL) = \frac{RLU_{tATP}}{RLU_{UC1}} \times 306$$

$$dATP (ng / mL) = \frac{RLU_{dATP}}{RLU_{UC1}} \times 101$$

*cATP (Cellular ATP) is the concentration (quantity) of living organisms.*

$$cATP (ng / mL) = tATP - dATP$$

*BSI (Biomass Stress Index) is the stress level (quality) of the living organisms.*

$$BSI (\%) = \frac{dATP}{tATP}$$

*ABR (Active Biomass Ratio) is the percentage of solids that are alive.*

$$ABR (\%) = \frac{(cATP \times 0.5)}{TSS}$$

### Test Kit Handling

- Consult reagent labels for storage conditions.
- Download MSDS at [www.luminultra.com](http://www.luminultra.com).
- Waste reagent can be discarded as general waste in most cases. Consult MSDS for more information.
- When performing multiple tests, perform preparation steps all at once to save time.
- If assay interferences are suspected, contact LuminUltra to troubleshoot.

### Tips

- Avoid analysis and reagent contamination by always using a new pipette tip for each pipetting step!
- Because ATP and bacteria are present on skin, it is important not to touch the surface of pipette tips.
- Because TCB is a test for biological characteristics, and biological characteristics will begin to change immediately upon collection, **analyze samples within 2 hours of collection.**
- Wide-mouth pipette tips should be used for samples containing large particles.



ISO 9001:2000

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