

Test Kit Instructions

Deposit & Surface Analysis Test Kit

Product # DSA-25 / DSA-100

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 all the exciting opportunities that our technology can bring to your application.

Whereas traditional microbiological tests require days for feedback and measure only a fraction of the microorganisms, 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
- Where to find more information and how to contact us.

Choosing the Right Test Kit



DSA Test Kit (DSA-25C)

LuminUltra provides 6 core test kits for measuring total microbiological concentration via ATP, each tailored to specific applications:

- Quench-Gone Aqueous (**QGA™**):
For low-solids water-based samples, such as drinking, cooling and oilfield waters.
- Quench-Gone Organic Modified (**QGO-M™**):
For low-solids organic-based samples, such as fuel, oily brine, lubricants and latex polymers.
- Deposit & Surface Analysis (**DSA™**):
For measuring deposits and surfaces. Including corrosion products and slimes.
- QuenchGone21™ Industrial (**QG21I™**):
For high-solids process fluids, including paper process and other wash waters.
- QuenchGone21 Specialty (**QG21S™**):
For chemical product testing, such as slurries, adhesives and other coatings.
- QuenchGone21 Wastewater (**QG21W™**):
For wastewater and bioprocessing samples, whether influent, bioreactor or effluent.

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

Test Kit Instructions – Deposit & Surface Analysis (DSA)

Where to use the DSA Test Kit



The Deposit & Surface Analysis (DSA) test kit is designed for

measurement of microorganisms on surfaces, in deposits, and on biofilm collection devices (e.g. corrosion coupons). Using a single analysis, you will be able to quickly measure total microbiological concentration on any surface or deposit sample with a wide detection range. Use DSA to detect total microbiological activity in:

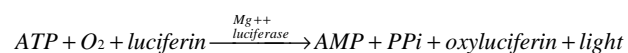
- ✓ Process Surfaces
- ✓ Solid Deposits
- ✓ Biofilm Collection Devices
- ✓ Slimes

...and more!

NOTE: The DSA test kit is unique in its ability to accurately quantify microbial content on surfaces. For guidance on how to get set up for surface hygiene monitoring, contact LuminUltra's support staff.

How Does ATP Testing Work?

LuminUltra's test kits are based on the measurement of ATP. ATP is a direct and interference-free indicator of total living biomass. ATP is measured using the firefly luciferase assay, where a sample containing ATP is introduced to a solution containing the enzyme Luciferase, which naturally occurs in the tails of fireflies, to produce light. The light is detected in a **luminometer** as Relative Light Units (RLU).



The DSA test kit utilizes a 10-minute dilution-based analysis to measure a parameter called Total ATP (tATP™). When measured on surfaces or in deposits, tATP represents the accumulation of **sessile** biomass on process equipment and therefore can indicate the presence of or potential for microbiological corrosion.

Getting Started

LuminUltra's test kits contain all of the consumable materials required to run their specified number of tests (Defined by the last 2 or 3 digits of the product code). To use these test kits, LuminUltra recommends one of the following equipment bundles:

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



Field Kit & Luminometer Package (EQP-PAC)

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

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

Getting started with DSA is easier with direct training. LuminUltra can supply you with on-site auditing and training services, web-based training, and one-on-one consultation to get your process improvement program off the ground. Contact us today to learn more!

Test Kit Contents and Storage

When you receive your DSA 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.

DSA Test Kit Contents & Storage Conditions

Component (LuminUltra P/N)	Store At	Shelf Life
Luminase™ Dropper (Lu-5mL) <i>Luciferase Enzyme Reagent, 5mL</i>	4°C*	4 mo*
UltraCheck™ 1 Dropper (UC1-2.5mL) <i>1 ng ATP/mL Standard, 2.5mL</i>	20°C	12 mo
UltraLyse™ 7 Bottle (UL7-125mL) <i>tATP Extraction Reagent, 125mL</i>	20°C	12 mo
UltraLute™ Bottle (ULu-250mL) <i>tATP Dilution Reagent, 250mL</i>	20°C	12 mo
LumiSolve™ Bottle (LS-30mL) <i>Swab Wetting Reagent, 30mL</i>	20°C	12 mo
Sterile Swabs, 25/pk (DIS-SWAB-25)	20°C	-
100 to 1000µL Blue Pipette Tips, 100/rack (DIS-PT1-100R) **	20°C	-
1 to 5mL Natural Pipette Tips, 50/pk (DIS-PT5-50) **	20°C	-
12x55mm Test Tubes, 50/pk (DIS-CT12-50)	20°C	-
17x100mm Test Tubes, 25/pk (DIS-CT17-25)	20°C	-
17mm Caps, 25/pk (DIS-C17-25)	20°C	-

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

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

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

Preparing to Test

- New to 2nd Generation ATP technology? Before getting started, consult the training center at www.luminultra.com for video demonstrations, use guidelines, validation guidelines, and more!

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

- Perform ATP analyses on the same sample used for measuring other parameters for reliable interpretation.
- Waste reagent can be discarded as general waste in most cases. Consult MSDS for more information. Obtain MSDS and other product documentation from www.luminultra.com.

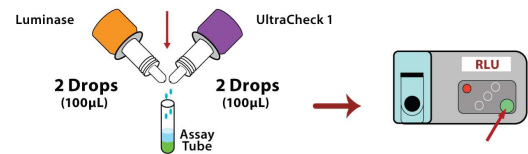
Diagnostics and Troubleshooting

- LuminUltra's test kits are the most robust and quantitative ATP test kits available. If you suspect you are encountering interferences in your application of these kits, first check to ensure you are using the correct kit type for your sample. If you are unsure or feel you have unique testing requirements, contact LuminUltra's support staff for assistance.
- When testing samples that yield low RLU values (i.e. $RLU_{iATP} \leq 50$ when using a Kikkoman Lumitester C-100 or C-110), it is recommended that you account for background noise in the test procedure prior to the final calculations.
 - To assess background noise, simply follow the procedure without adding any of the ATP-containing sample into the analysis.
 - Correct for background noise by subtracting the background RLU (RLU_{bg}) from the measured RLU (RLU_{iATP}) prior to executing calculations.
 - Typical RLU_{bg} when using a Kikkoman Lumitester C-100 or C-110 are ≤ 10 . If high RLU_{bg} are consistently observed, repeat assays in an area out of direct sunlight or intense lighting. If problems still occur, contact LuminUltra for assistance.
 - A single RLU_{bg} may be used for multiple analyses much like a single UltraCheck 1 RLU (RLU_{UC1}).
- If dropper bottles become plugged or you encounter difficulty dispensing drops, remove and discard the dropper tip and use a pipettor to measure and dispense the reagent.

Step 1 – UltraCheck 1 Calibration

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

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



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

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

Step 2 – Sample Preparation

The DSA test kit provides three options to collect and prepare samples:

A. Surface Swab – A measured area of the surface to be tested is swabbed to collect microbial particles. ATP is then extracted and measured from the swab.

B. Measured Deposit – A deposit is collected and a precise mass or volume is measured. ATP is then extracted and measured from the deposit.

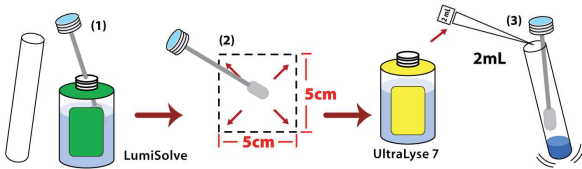
C. Biofilm Collector – A biofilm collection device (e.g. corrosion coupon) is directly immersed into UltraLyse 7 to extract and measure ATP.

Choose from any one of methods A, B or C, and then proceed to Step 3 (tATP Analysis). In general, option B is preferred as it is the most quantitative.

Test Kit Instructions – Deposit & Surface Analysis (DSA)

Option A – SURFACE SWAB

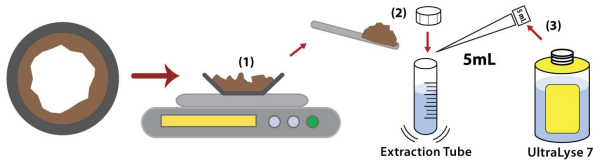
Obtain an unused Sterile Swab and wet with **LumiSolve**. Swab an approximately 5x5cm (2x2in) surface area. Insert swab into its container and use the micropipettor to add 2mL of **UltraLyse 7** to the container. Cap and mix the contents of the container.



TIP: To increase analysis sensitivity, increase the swabbed surface area.

Option B – MEASURED DEPOSIT

Obtain a portion of the deposit and weigh 1g of sample in a new 17x100mm test tube. Use a micropipettor to add 5mL of **UltraLyse 7** to the test tube. Cap and mix the contents of the test tube vigorously to disperse the deposit throughout the fluid. This becomes the Extraction Tube.

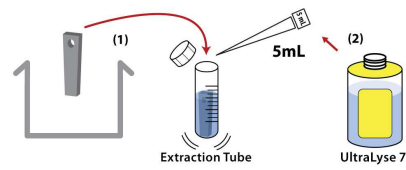


TIP: To increase analysis sensitivity, increase the amount of deposit added to the extraction tube.

TIP: A measured volume of deposit (e.g. 1mL) can also be used instead of a weighed amount.

Option C – BIOFILM COLLECTOR

Obtain a biofilm collection device from the process and shake gently to remove residual fluid. Place the device into a new 17x100mm test tube. Use a micropipettor to add 5mL of **UltraLyse 7** to the test tube. Cap and mix the contents of the test tube vigorously to fully expose the device to the fluid. This becomes the Extraction Tube.



TIP: Attempt to test the biofilm collection device as quickly as possible following removal from process fluid. If the device is not to be tested immediately, it is preferred to store the device in a container containing process fluid until such time that it can be tested.

TIP: If the device is too large to fit into the supplied 17x100mm test tubes, obtain a larger vessel and ensure the device can be fully immersed in UltraLyse 7.

TIP: Because microorganisms and other materials collected on the device will be destroyed upon immersion in UltraLyse 7, it is preferred to have more than one device available for multiple analyses. If only one device is available, perform all other tests before performing ATP test.

Step 3 – DSA tATP™ Analysis

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

3.1 – INCUBATION

Allow at least 5 minutes for incubation of the Extraction Tube to proceeding to 3.2.



5 minutes



Extraction Tube

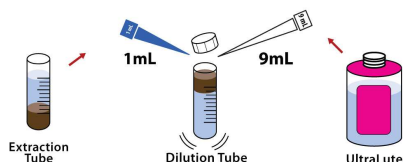
NOTE: At this point, the contents of the Extraction Tube can be capped and stored refrigerated between 2-8°C for up to 1 week prior to 3.2 and 3.3.

TIP: When using the biofilm collector method, place the Extraction Tube in an orientation to maximize immersion of the device in UltraLyse 7 during incubation.

Test Kit Instructions – Deposit & Surface Analysis (DSA)

3.2 – DILUTION

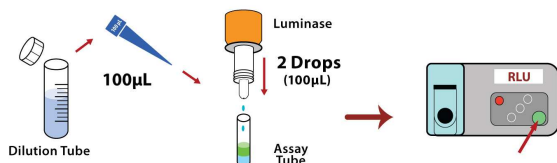
Use a micropipettor to transfer 1 mL from the Extraction Tube to a new 17x100mm test tube and add 9 mL of **UltraLute**. Cap and invert three times to mix. This new tube is called the Dilution Tube.



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

3.3 – ASSAY

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



NOTE: If $RLU_{iATP} \leq 10$ on a Kikkoman Lumitester C-100 or C-110, you are below the low-detection limit. Report $tATP$ ($pg\ ATP/mL$) = 0 in final calculations, or select a larger sample size in Step 2 and repeat the analysis.

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

TIP: If “Scale Over” is returned, repeat the analysis using a smaller sample in Step 2.

Final Calculations

Following completion of DSA analyses, RLU values must be converted to ATP concentrations using the following calculations. For easy calculations, utilize **LumiCapture™** software available on www.luminultra.com. For manual calculations, see below.

The Total ATP ($tATP$) analysis measures all ATP within the deposit, including ATP from living cells in addition to ATP that has been released from dead cells. Calculate $tATP$ according to the option selected in Step 2:

A – Surface Swab (Default $A_{Sample} = 25cm^2$):

$$tATP (pg\ ATP / cm^2) = \frac{RLU_{iATP}}{RLU_{UC1}} \times \frac{20,000 (pg\ ATP)}{A_{Sample} (cm^2)}$$

B – Measured Deposit (Default $m_{Sample} = 1g$):

$$tATP (pg\ ATP / g) = \frac{RLU_{iATP}}{RLU_{UC1}} \times \frac{50,000 (pg\ ATP)}{m_{Sample} (g)}$$

C – Biofilm Collector:

$$tATP (pg\ ATP / device) = \frac{RLU_{iATP}}{RLU_{UC1}} \times \frac{50,000 (pg\ ATP)}{1\ device}$$

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

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

A – Surface Swab:

$$tATP (ME / cm^2) = tATP (pg\ ATP / cm^2) \times \frac{1\ ME}{0.001\ pg\ ATP}$$

B – Measured Deposit:

$$tATP (ME / g) = tATP (pg\ ATP / g) \times \frac{1\ ME}{0.001\ pg\ ATP}$$

C – Biofilm Collector:

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

NOTE: For more discussion on the quantity of ATP per cell, visit www.luminultra.com.

Because many traditional culture-based methods report results in a similar fashion, it is sometimes convenient to report $tATP$ results in ME/quantity using Scientific Notation (i.e. **## x 10[#]**) or on a **Log₁₀** format for comparison purposes.

Interpretation Guidelines

Once DSA tATP 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 **tATP is minimized**.

LuminUltra’s ATP test kits can be used to audit microbial quantity to reveal differences at different process locations in an effort to quickly assess the ‘hot spots’ within a process that require more immediate attention.

For process control, daily monitoring using ATP test kits will give you true total microbial quantity parameters to trend over time against process characteristics and performance.

When utilizing ATP test kits it is important to remember that every process is different. During **audits**, relative comparisons from point to point are a reliable means to assess your process, while for **daily monitoring** it is important to establish a baseline trend before making control decisions. To get started, LuminUltra provides the following guidelines as the ratio of deposit tATP to bulk fluid cATP as measured by other LuminUltra test kits:

DSA tATP Interpretation Guidelines

Application	Good Control	Preventive Action	Corrective Action
Surfaces, Deposits & Coupons *	< 10x	10x to 100x	> 100x

* Guidelines are provided as a ratio of ATP on your surface/deposit/collector to bulk fluid ATP.

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

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

* Complete kits include LuminUltra-manufactured reagents plus all consumables (tips, tubes, filters, syringes) required to run analysis. If you supply your own consumables, reagent only kits are available.

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

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

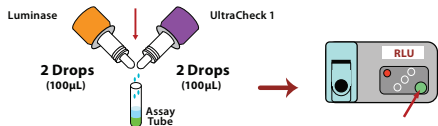


- Orders generally ship within 3 business days. You will receive order confirmation via Fax or Email.

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Step 1 – UltraCheck™ 1 Calibration

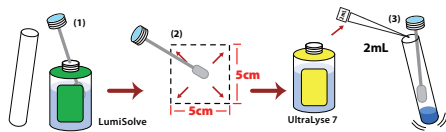
Perform one UltraCheck 1 calibration per day or per each set of samples analyzed.



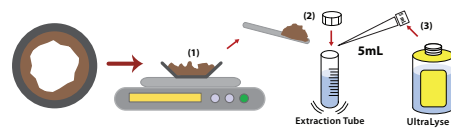
NOTE: If $RLU_{UC1} \leq 5000$ using a Kikkoman Lumitester™ C-100 or C-110, obtain a new bottle of Luminase.

Step 2 – Sample Preparation --> Select one of the following options:

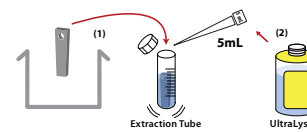
Option A - SURFACE SWAB



Option B - MEASURED DEPOSIT



Option C - BIOFILM COLLECTOR



Step 3 – DSA™ tATP™ Analysis --> Then perform the following steps:

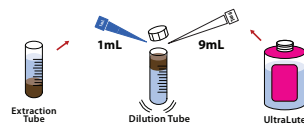
3.1 - INCUBATION

Allow time for complete extraction.



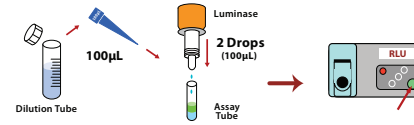
3.2 - DILUTION

Dilute out interferences.



3.3 - ASSAY

Measure ATP concentration.



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

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

Interpretation Guidelines

Location	Good Control	Preventative Action	Corrective Action
Surfaces, Deposits & Coupons	< 10x	10x to 100x	> 100x

*Guidelines are provided as a ratio of ATP on your surface/deposit/collector to bulk fluid ATP.

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

Final Calculations --> Carry out calculations that correspond to the selected preparation method:

A – Surface Swab (Default $A_{Sample} = 25cm^2$):

$$tATP (pg ATP / cm^2) = \frac{RLU_{tATP}}{RLU_{UC1}} \times \frac{20,000 (pg ATP)}{A_{Sample} (cm^2)}$$

OR

$$tATP (ME / cm^2) = tATP (pg ATP / cm^2) \times \frac{1ME}{0.001 pg ATP}$$

B – Measured Deposit (Default $m_{Sample} = 1g$):

$$tATP (pg ATP / g) = \frac{RLU_{tATP}}{RLU_{UC1}} \times \frac{50,000 (pg ATP)}{m_{Sample} (g)}$$

OR

$$tATP (ME / g) = tATP (pg ATP / g) \times \frac{1ME}{0.001 pg ATP}$$

C – Biofilm Collector:

$$tATP (pg ATP / device) = \frac{RLU_{tATP}}{RLU_{UC1}} \times \frac{50,000 (pg ATP)}{1 device}$$

$$tATP (ME / device) = tATP (pg ATP / device) \times \frac{1ME}{0.001 pg ATP}$$