

2nd Generation ATP How Does It Work?

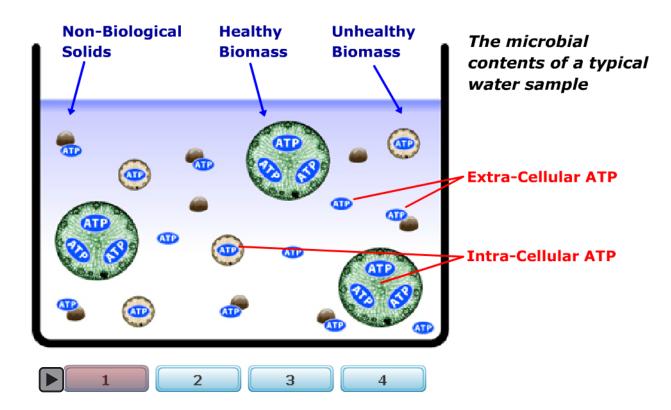
Within any sample containing microorganisms, there are two types of ATP:

- Intra-cellular ATP ATP contained within living biological cells.
- Extra-cellular ATP ATP located outside of biological cells that have been released from dead or stressed organisms.

Accurate measurement of these two types of ATP is critical to utilizing ATP-based measurements! Being able to accurately measure these different types of ATP offers the ability to assess biological health and activity, and subsequently control water-based processes!

Total ATP – tATP™

Total ATP is the measurement of all ATP contained in the sample. Hence, this measurement includes both intracellular and extra-cellular ATP. The series of figures below explains how the total quantity of ATP contained within the sample is analyzed.



*in order to see the whole diagram, please save this file to your local drive and re-open it in a PDF reader software.

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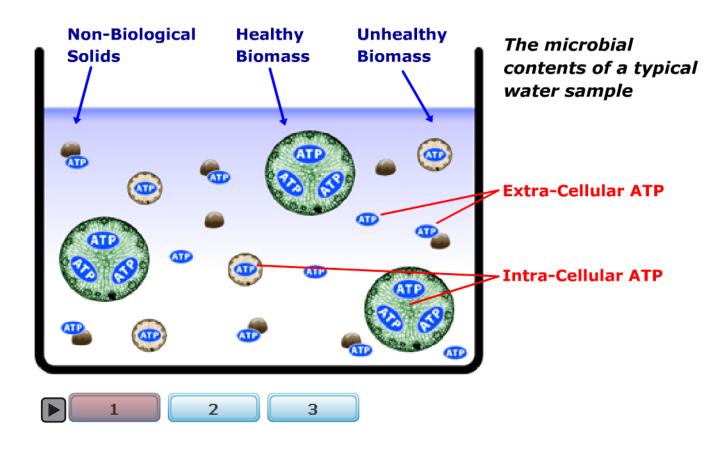




tATP[™] is one of two analyses involved in the LuminUltra Industrial, Specialty, and Wastewater test protocols (i.e. along with the dATP[™] measurement). In most competing ATP test kits, Total ATP is the only measurement offered. However, in many applications, correcting for the background of Dissolved ATP is extremely important.

Dissolved ATP – dATP™

Dissolved ATP is the measurement of only extra-cellular ATP. LuminUltra's dATP method is superior to competing methods, in that it measures the total amount of extra-cellular ATP, not just free or soluble dATP. Extracellular ATP is made up of free-floating ATP, bound ATP, complexed ATP, and easily extractable ATP. The series of figures below explains how the extra-cellular ATP component contained within the sample is analyzed:



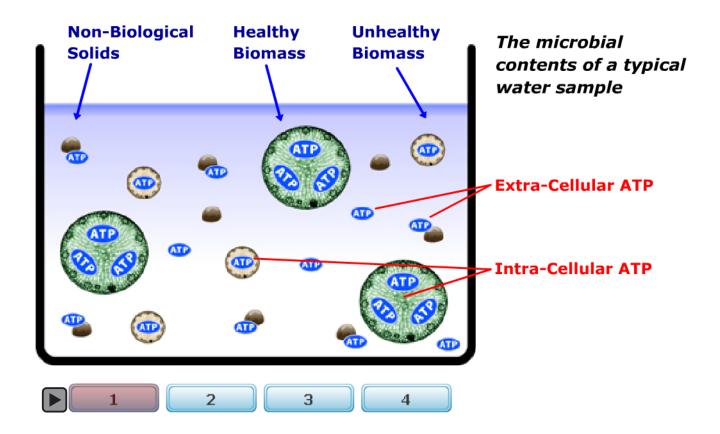
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dATP[™] is one of two analyses in the LuminUltra Industrial, Specialty, and Wastewater test protocols (i.e. along with the tATP[™] measurement). It provides information relating primarily to the relative health of the biomass. As biomass becomes unhealthy or dies, it releases ATP into the external environment. Therefore, higher dATP is a direct result of biomass mortality.



Cellular ATP – cATP™

Cellular ATP is the measurement of only intra-cellular ATP. The series of figures below explains how the intracellular ATP component contained within the sample is determined:



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In the LuminUltra Industrial, Specialty, and Wastewater test protocols, cATP[™] is calculated as the difference of tATP[™] and dATP[™].

That is: **cATP = tATP - dATP**

In the LuminUltra Water and LuminUltra Organic test kit line, cATP is measured directly by physically separating the dATP contained in the sample (via filtration). cATP provides a direct indication of living biomass energy level, or in other words, living biomass concentration.

This parameter is extremely important for water applications, where the goal is to limit the amount of living biomass, and also in wastewater treatment applications, where the goal is to maintain a stable population of living biomass.



The following conversion can be used to convert ATP results into relatable parameters.

The average bacterial (E.Coli) cell contains 1 femtogram (fg) of ATP

This is a frequently cited conversion and has been observed by many researchers, including LuminUltra. There are a variety of ATP-to-cell number correlations that have been done by various researchers using pure cultures.

The reference citing the above conversion can be found in an October 2001 Application Note by **Lee and Deininger** entitled "Rapid quantification of viable bacteria in water using an ATP assay". The precise citation is as follows on the second page:

It is known that the average ATP content in one bacterial cell is about 1 fg.

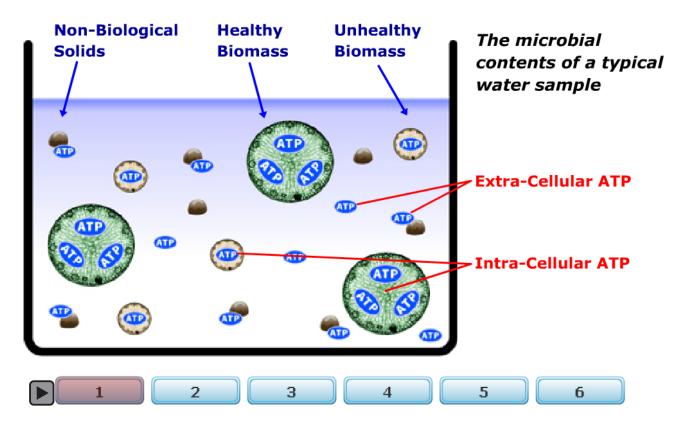
The reference cited as the source of this conversion: Crombrugge J, Waes G. ATP method. In: Heeschen W, ed. Methods for assessing the bacteriological quality of raw milk from the farm. Brussels: International Dairy Federation, **1991: pp. 53–60**.



Biomass Stress Index – BSI™

The Biomass Stress Index is a unique feature of the LuminUltra Industrial, Specialty, and Wastewater test protocols. Because of the precision and accuracy built into our test kits, we are able to measure the true tATP[™] and dATP[™], providing not only a precise measure of living biomass quantity via cATP[™], but also a measure of relative biomass population health through BSI[™].

There is no conventional equivalent available to measure biomass health – this measurable characteristic is available only through LuminUltra! The series of figures below explains how BSI is determined:



*in order to see the whole diagram, please save this file to your local drive and re-open it in a PDF reader software.

The BSI is calculated from tATP and dATP as a ratio.

That is: BSI (%) = dATP / tATP * 100%

Hence, the higher the dATP content of the sample relative to the tATP, the higher the BSI. High BSI values are related to stressful situations – for example, the addition of a biocide such as chlorine will impose a stress on the biomass and cause the BSI to rise. Other stresses, such as oxygen deprivation, toxicity, or nutrient deficiencies will also produce high BSI values. In general, BSI is a 'general alarm' of the water or wastewater process, where a change in BSI signals a change in biomass quality, for better or worse.