

Biomass ATP Kit

Part Number: BMK3M100

General Description / Intended Use

The Hygiena Biomass ATP kit is a high precision ATP (Adenosine Triphosphate) detection assay for liquid samples. The Kit is typically used to rapidly assess the efficiency of biocide treatments on process and waste water(s) and organic activity in water tanks and cooling towers. It can also be used to assess the standards of hygiene and sanitation procedures for equipment and the efficiency of Clean In Place (CIP) systems. The Biomass ATP kit has been designed to handle samples that are high quenching or contain particulates or fibers. If samples are not high quenching and free from particulates then AquaSnap (AQ100X) or WaterShot (W-SPXL133) all-in-one devices are an alternative test device option. The test is designed to be used in Hygiena luminometers or to replace the Cleantrace Multitrace Kit used in 3M/Biotrace luminometers.

Principle

The test is based on the measurement of total and free Adenosine TriPhosphate (ATP) in samples using bioluminescent reagents. Total ATP is the measurement of all ATP contained in the sample. This includes both intra-cellular and extra-cellular ATP. Free ATP, sometimes referred to as extra-cellular ATP, is ATP in the sample from dead or unhealthy biomass. Assaying free ATP provides information primarily, regarding the relative health of the biomass. As biomass becomes unhealthy or dies, it releases ATP into the external environment. Therefore, higher free ATP is a direct result of biomass mortality. The difference in total ATP and free ATP in a sample indicates the bioload or the amount of ATP coming from living organisms.

Materials Provided

The kit contains sufficient reagents for 100 tests with each component supplied in units of 10 tests.

- 10 bottles of liquid-stable Reagent; provided in plastic screw-cap bottle with a red cap (Bottle # 1).
- 10 bottles of Buffer; provided in plastic screw-cap bottle with a white cap, (Bottle # 2) to be mixed with Reagent to form a Working reagent.
- 10 bottles of Extractant to release ATP from any micro organisms in the sample; provided in plastic screw-cap bottle with a blue cap, Bottle # 3.
- 1 vial of ATP Positive Control
- 100 sample cuvettes.

Additional Materials Required (Not Included)

- Luminometer
- Hold-Rite Applicator (HR001)
- Pipette and ATP free tips for 100µL volume dispensing.

Storage and Shelf life

Store the kit at 2°–8°C (35°–46°F). DO NOT FREEZE.

The kit has 12 months shelf life at refrigerated temperatures.

Once reagents are mixed the Working Reagent has a shelf life of 7 days when stored at 2°–8°C (35°–46°F).

Preparation for Testing

Remove adequate number of bottles of each component, Reagent, Buffer and Extractant (1 bottle=10 tests) from the kit box for the number of tests to be performed. Return the remainder of the kit to the refrigerator. After testing is completed, cap the bottles and return unused reagents to the refrigerator.

Prepare a Working Reagent: Pour contents of Reagent bottle (red cap, Bottle #1) into the Buffer bottle (white cap, Bottle # 2). Swirl bottle to ensure mixing of the working reagent. Pour back into the Reagent bottle. Record the current date on Reagent bottle (now the Working Reagent).

Allow 10 minutes for mixed Working Reagent and Extractant to reach ambient temperature before use.

Testing

1. Before processing the test, it is important to ensure the luminometer is switched on and initialized. Refer to manual provided with the instrument for full details.
2. Pipette 100µL of sample into a cuvette using a sterile pipette tip.

- 3a. For testing Total ATP: Using a new pipette tip, pipette 100µL of Extractant (Blue cap, Bottle # 3) into sample cuvette. Mix gently for 3–5 seconds and let it stand for a minimum of 60 seconds.
- 3b. For testing Free ATP: Do not add Extractant. Proceed to step 4 directly.
4. Using a new pipette tip, pipette 100µL of Working Reagent and mix gently for 3–5 seconds.
5. Attach cuvette to Hold-Rite Applicator and insert into the instrument chamber and read immediately. The light emitted by sample will be measured and the results in RLU (Relative Light Units) will appear on the display.

Precautions

- Use a fresh, clean ATP-free pipette tip for each sample pipetting.
- Avoid touching solutions with bare hands and handling parts of cuvettes and solution vials that come into direct contact with sample and/or solutions to avoid contamination.
- Always write the date the Working Reagent was made on the Reagent bottle. Do not use Reagent without mixing the Buffer to make the Working Reagent. Unused portion of Working Reagent must be stored at 2°–8°C. Do not use Working Reagent past 7 days after mixing.

ATP Control

A negative control reading may be obtained using a sample of ATP-free water. In a cuvette pipette 100µL of ATP-free water or rinse water, pipette 100µL of Extractant and pipette 100µL of Working Reagent. Mix gently for 3–5 seconds and read in the luminometer.

A Positive ATP control test can be performed to check the storage/shipping conditions of the kit reagents. Pipette 100µL sterile water, pipette 100µL of Extractant, pipette 100µL of ATP Positive Control and pipette 100µL of Working Reagent into a cuvette. Mix gently for 3–5 seconds and read in the luminometer. The RLU value should be in excess of 20,000 RLU.

Interpretation of Results

1. The higher the RLU number, the more ATP is in the sample.
2. Occasionally free ATP number will be larger than the total ATP result. This occurs shortly after biocides or chemicals are added to water; killing organisms and exposing the cellular ATP to the environment, thereby increasing the free ATP.
3. It is recommended to set pass/fail levels so that action can be taken once the result is known. Determining pass/fail levels is sample specific. A common way to determine these levels is by running free and total ATP tests along with standard method micro plates. Once an acceptable level of correlation between the two methods is established; pass/fail levels can be set. Standard deviations can also be calculated from the correlated level to take variation into consideration. For more information on setting levels, see the Hygiena Hygiene Management Guide or call your local Hygiena representative or distributor.
4. For cooling or process water, establish a baseline RLU value over time by the same process as in No. 3. This baseline can then be used to identify abnormal readings, seasonal variations, and patterns of contamination that may occur with various treatment methods.

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