

## TECHNICAL PRODUCT INFORMATION

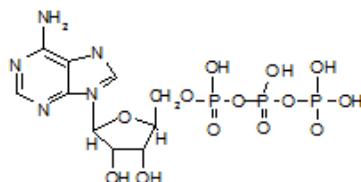
# AccuPoint<sup>®</sup> 2



## Rapid Sanitation Monitoring with ATP

### A Review: AccuPoint<sup>®</sup> 2 Sanitation Monitoring System

#### Why is ATP measurement important for rapid sanitation monitoring?



Adenosine triphosphate (ATP) is used as an energy source by all living organisms in nature, including bacteria, fungi, algae, animals and plants. For this reason, ATP is a good target for sanitation verification.

Human skin constantly sheds cells with a large amount of ATP, making ATP easily detectable on surfaces that have been touched. It can also be found on various surfaces and in materials where contamination with living organisms occur.

Food residues remaining on poorly cleaned surfaces will act as a source of nutrients for bacteria and a biofilm made of microbial layers may protect them from the action of sanitizers. For this reason, monitoring of working surfaces and their surrounding environments for the presence of ATP is vital for maintaining a high degree of sanitation.

The phosphate bonds in ATP are high-energy phosphate bonds that deliver 31 kilojoule/mole for the terminal phosphate bond. ATP is stable in heat, acidic pH and in biological fluid.

There are various methods for sanitation monitoring such as a wipe-test for bacterial contamination that requires growing organisms in a growth medium; a sampler test that involves detecting proteins and reducing sugars through a chemical reaction; and a sampler format that detects ATP using firefly luciferase bioluminescence. ATP monitoring is the fastest and most accurate evaluation of sanitation effectiveness.

#### ATP detection using bioluminescence

ATP-bioluminescence has been used for sanitation monitoring for many years and is offered by several manufacturers. AccuPoint 2 ATP monitoring devices are designed to monitor the presence of ATP on a surface or in a liquid using firefly luciferase, luciferin and magnesium in a reaction where ATP generates bioluminescence — or light — as shown at right.

Since a universal energy marker (ATP) is detected with the above reaction, this technique does not discriminate between microbial and non-microbial ATP. The purpose of AccuPoint 2 devices is to monitor for proper sanitation and ensure a surface or liquid is clean from both microbial and non-microbial sources of contamination.

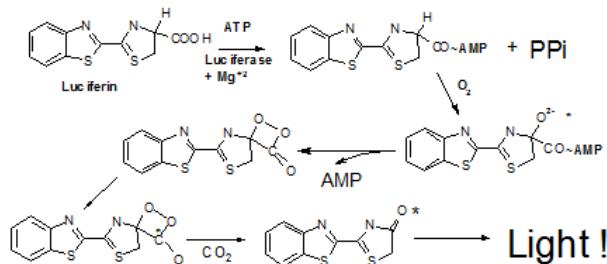


When ATP comes in contact with the mixture of luciferin-luciferase-magnesium, yellowish green light (~560 nm) is emitted within a few seconds. Depending on the composition of assay ingredients, the light emission lasts for a few seconds (flash) or for a longer time (glow). AccuPoint 2 is designed to detect ATP amounts as small amount as 5 femtomole of ATP in a sample. It can detect ATP from a surface,

narrow area such as inside a filler tube or in liquid in real time and record the level of relative light units (RLU). With this method, a contaminated work area can be identified immediately after cleaning and the data can be later reviewed for trends as a tool for sanitation management.

AccuPoint 2 is unique in design, detecting bioluminescence with a photo-diode that uses low voltage direct current. Other units use the conventional photo-multiplier tube (PMT) which uses a high bias voltage to accelerate electrons generated by the light. The optical, mechanical and electronic components of AccuPoint 2 are housed in a rugged case that resists damage from dropping and tends to require fewer repairs than PMT-based instruments.

#### Luciferin/luciferase reaction scheme



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### AccuPoint 2 Surface, Liquid and Access Samplers

As shown, the **AccuPoint 2 Surface or Liquid Sampler** is composed of two components: the cup and sampler handle. Specially treated luciferin-luciferase complex is immobilized on a polymer support (enzyme pad) at the bottom of the cup. The enzyme pad is kept dry in the lower chamber with a few milligrams of drying agent that does not interfere with the luciferase-luciferin-ATP reaction. The handle is made of a surface sampler which is optimally wet with an extractant solution for rapid extraction of ATP from the surface and microbes. Above the sampling sampler, there is a 200 µL-capacity capsule with a trigger agent (neutralizer) sealed with a polyolefin membrane. Above the compartment that houses the enzyme pad, there is a cutter and probe. Upon activation of AccuPoint 2 surface sampler, the cutter opens the plastic seal, coring the cup and pushing it downward away from the travelling sample sampler. The probe penetrates a seal of the sample sampler and enters into the capsule to release neutralizer.

With respect to **AccuPoint 2 Access**, the sampler is made of a thin foam that is wet optimally for swabbing a small orifice. The neutralizer solution is placed in a thin straw. By breaking the seal of the straw, neutralizer is released and ATP is eluted from the sampler surface. Activation of the sampler handle brings the neutralizer solution and the sampler to contact the enzyme pad to generate light.

#### ATP levels in food

A variety of foods have been tested to determine their ATP contents (see Table 1). Typically, orange juice and ketchup are common sources of high levels of ATP. ATP in ketchup is stored in acidic media at room temperature and ATP in this matrix is extremely stable.

In this test, the commodity was diluted with ATP-free water (e.g. MilliQ water) and 25 µL of solution or suspension was placed on the sampling pad and the device was activated.

**Table 1:** Results of AccuPoint 2 testing of a variety of commodities.

Commodity	ATP reading (in RLUs)
Ketchup (1:8)	9348.6
Cottage Cheese (1:8)	925
Salad Dressing (1:8)	349
Peanut extract 8.8 ppm (1:1000)	346
Peanut butter (1:100)	1521
Whole Milk (1:50)	467

**Table 2:** ATP recovery from pathogenic bacteria.

Pathogen	ATP reading (in RLUs)
E. coli (~10 <sup>9</sup> CFU/mL)	2442.6
Salmonella (~10 <sup>9</sup> CFU/mL)	2238.6
Listeria (~10 <sup>9</sup> CFU/mL)	570.8

The foodborne pathogens listed in Table 2 were cultures grown overnight and diluted 2x or 8x with ATP-free water and subject to AccuPoint 2 surface testing. Each commodity was tested in triplicate. The sampling pad contains an optimum amount of detergent to cause bacterial and fungal cell lysis.

**Table 3:** Results of AccuPoint 2 sample testing at various levels of dilution. All ATP levels reported in RLUs.

(mean of 10)	ATP Free	1:10	1:1000	1:2000	1:10000
Non-fat dry milk	0	30034	2326	760	214
Orange juice	0	6325	562	184	20

#### Effect of quaternary ammonium sanitizers on AccuPoint 2

Quaternary ammonium sanitizers are often used to sanitize the work surfaces and equipment in food processing facilities and restaurants. A diluted solution of quaternary ammonium sanitizer is used to wipe these surfaces, and sanitizer could impact ATP test results. A Neogen Technical Service Bulletin was issued in March 2006 that explains that no adverse effects were observed with any of the sanitizers tested. None of the sanitizer solutions caused elevated background levels nor did they inhibit the luciferin-luciferase enzyme systems' ability to detect ATP present. All tested sanitizers were found to be fully compatible at working concentrations with the AccuPoint 2 ATP monitoring system. Evaluation of sanitizers was carried out as described below.

#### Methodology

**The following method was utilized with AccuPoint 2 surface samplers:**

25 µL of sanitizer (as diluted according to the manufacturer's instruction where applicable) was pipetted directly onto a sampler sponge in blank data. ATP standards were also evaluated with and without sanitizer. With sanitizer was a combination of 25 µL of ATP standard + 25 µL of sanitizer. Without sanitizer was a combination of 25 µL of ATP standard + 25 µL of sterile water.

**Table 4:** Results of AccuPoint 2 surface sampler testing using sanitizer alone, ATP-standard with sanitizer and ATP-standard without sanitizer. All results reported in RLUs.

#### AccuPoint 2 Surface Sampler

Sanitizer	Sanitizer without ATP-Standard	ATP-Standard with Sanitizer	ATP-Standard without Sanitizer
Matrixx®	3	430	797
	8	544	899
Dibac®	3	443	367
	2	685	308
Demand®	1	415	351
	4	371	302
Chlorine	1	305	709
	1	343	584
Mandate®	13	409	375
	9	329	383

**The following method was utilized for AccuPoint 2 liquid samplers:**

100 µL of sanitizer was pipetted directly onto a sampler sponge in blank data. ATP standards were also evaluated with and without sanitizer. With sanitizer was a combination of 50 µL of ATP standard + 50 µL of sanitizer. Without sanitizer was a combination of 50 µL of ATP standard + 50 µL of sterile water. Volumes were increased due to the dry sponge on the water sampler.

**Table 5.** Results of AccuPoint 2 liquid sampler testing using sanitizer alone, ATP-standard with sanitizer and ATP-standard without sanitizer. All results reported in RLU.

**AccuPoint 2 Liquid Sampler**

Sanitizer	Sanitizer without ATP-Standard	ATP-Standard with Sanitizer	ATP-Standard without Sanitizer
Matrixx®	9	1589	3876
	5	1359	3321
Dibac®	5	4373	713
	7	1747	2090
Demand®	8	938	2462
	1	1404	2790
Chlorine	4	3606	2684
	4	2923	3078
Mandate®	8	3662	3193
	13	3165	3247

**Establishing ATP monitoring in sanitation management HACCP**

Using AccuPoint 2 in the sanitation monitoring of food processing or food service facilities relies on the users' environment and needs. Some facilities wished to use ATP monitoring system to replace sterility tests (culture method) but this is not practical due to the low ATP content of bacterial cells and requirement for detection of bacterial cells (e.g. 1 CFU/100 mL of drinking water). However, ATP monitoring system can be effectively used to monitor contamination of work surfaces and equipment since it gives a rapid results and time to remedy a contaminated area. A typical table for checking the surface ATP levels in the food processing facility is shown in the table to the right. It is recommended that each organization establish its own RLU pass and fail thresholds. Additionally, each test site can have its own unique thresholds. For instance, one can establish a cut-off limit for the blade of kitchen knife to be <250 RLU for pass and >500 RLU for fail.

AccuPoint 2 ATP monitoring system includes a data management program that can be used to download the data location, RLU reading and pass/fail criteria specific for each site. Using the AccuPoint 2 Data Manager, HACCP data can be compiled into a PC in compliance with GMP protocol so that all the data are tamper-proof and suitable for use in auditing. Detailed description of how to use AccuPoint 2 Data Manager is described in the AccuPoint 2 Data Manager User's Guide.

**A sample HACCP schedule for ATP monitoring**

**(XYZ Food Processing Factory)**

Date \_\_\_\_, Operator \_\_\_\_\_, AccuPoint 2 Sampler lot \_\_\_\_

AccuPoint 2 reader serial number \_\_\_, Room temperature \_\_\_°C

**Cut-Off levels:**

Pass: < 150 RLU; Caution: 151-299 RLU; Fail: > 300 RLU

Location	RLU	Pass/Caution/Fail	Recommendation
Meat slicer blade	350	Fail	Re-clean and test
Cutting board	250	Caution	Re-clean and test
Door knob	120	Pass	–
Computer keyboard	2367	Fail	Clean and re-test
Knife blade*	300	Fail	Re-clean
Refrigerator door handle	140	Pass	–
Sink	100	Pass	–

**Precautions to consider**

Due to the uneven distribution of ATP on a surface, there is no surface from which one can detect the same level of ATP twice. A larger number of replicates for each area will be important to assess the cleanliness of the work surface. A typical variation of ATP measurement is approximately 25% as with other ATP monitoring systems.

It is important to bring the temperature of the sampler to room temperature before initiating the assay since this is an enzymatic reaction.

Although ATP-bioluminescence method does not distinguish bacterial ATP from that of food products, ATP is a good index for surface cleanliness. Food matrices such as ketchup and milk contain endogenous ATP that is abundant and stable. Human skin sheds a large number of cells with ATP, therefore you can detect high levels of ATP on a surface where a human touches, (e.g., a keyboard, telephone receiver or door knob).

**Specifications of the AccuPoint 2 System**

AccuPoint samplers are carefully designed to provide the maximum recovery of ATP from a surface. The mechanical strength and the action of the extraction agent of the surface sampler allow better ATP recovery from bacterial biofilms.

The handheld AccuPoint 2 luminometer has been engineered to provide an accurate light reading through a third generation photodiode. The photodiode has a lower signal to noise ratio allowing clear detection of minute amounts of light coming from the bioluminescent reaction.

**For more information about AccuPoint 2, contact Neogen at 800/264-5333.**