

Causes of higher RLU values for AquaSnap Free Vs.Total

Introduction:

AquaSnap Free: Measures all free flowing ATP in a water sample. This product does not contain any surfactant and is essentially measuring all ATP in the sample from every source (except cellular sources).

AquaSnap Total: Contains a surfactant to lyse the cellular membrane and break into intracellular ATP sources, which are then mixed with the sources in the collection tube to get a “total” count of ATP from both intracellular and extracellular sources.

If you combine both samples and *subtract Free from Total*, you should get a theoretical value corresponding to the amount of ATP by cellular contamination in that water source.

Note - The RLU value is not equivalent to a CFU result but a measure of ATP contributed by microbial cells.

The AquaSnap Total and Free tests are designed to be used for industrial water samples eg. Cooling Tower water and where there is expected to be a high microbial count. Thus, the AquaSnap Total and Free should *only be used where there is expected to be greater CFU count than 10^4 to 10^5 microbial cells depending on organism type.*

Problem:

But what happens if you get a negative number? Or numbers that do not make sense It could be due to several issues.

- The water source contains a substance that is denaturing the luciferin/luciferase enzymatic mix and causing some enzymatic inhibition

in one or more samples. Thus, lowering the amount of ATP measured. This could skew the sample.

- The samples are not “mixed” properly. Total dissolved ATP is different in both samples. This can be fixed by stirring or vortexing the main water sample properly. For example, you have a 1 L sample from which you take your testing samples, the 1L sample should be properly vortexed to have equal parts dissolved ATP and/or bacteria in both testing samples.
- Ensure the instructions are followed carefully to avoid any user error. ATP based light production is produced on a curve. To test the samples properly, you cannot activate both samples at the same time and test one after another. The first reaction will test normal, the second reaction might test lower or higher. Each sample must be tested using the exact same procedures, on an individual basis.
- Highly contaminated samples can overload the test and instrument. If you are getting very high RLU values for your samples, what is happening is that the enzymatic mix is getting overloaded and may not give accurate values. To fix this, try diluting the main sample with serial dilutions and then quantifying the dilutions.
- Where samples are tested but have lower microbial viable counts than 10^4 to 10^5 microbial cells then a combination of test sensitivity and sample variation can show a Free RLU value greater than a Total RLU value.

Solutions and Examples:

Example 1: A 20 Gallon sample of water was taken from a freshwater stagnant lake. Testing determined the pH of the water was 7, with no other significant mineral or chemical abnormalities.

Round 1	Total	Free	Difference
John	2558	4784	-2226
Ken	1322	1222	100
Brian	817	1555	-738

It was not mixed. 3 samples were taken by 3 different individuals and arrived at 3 wildly different sample values, some were positive, some were negative. In this case, the most likely cause of the issue is that the sample was not properly stirred and while the values are high, they are not absurdly high. A serial dilution here would not be a bad idea either.

Example 2: A sewer line burst, and direct wastewater was mixed in with water from a swimming pool that contains muriatic acid (HCL). A 20-gallon sample was taken and tested.

Samples from the sewer line directly tested 20,000 RLU for Free and 20,000 RLU for total.

Samples from the pool were tested as follows.

Round 2	Total	Free	Difference
John	1223	1453	-230
Ken	1350	1156	194
Brian	1168	1250	-82

What you are seeing here is the direct sewer sample testing so high it is off the charts, the MAX RLU value that you can get from an EnsureTouch is 20,000 RLU. We know 100% the sample is contaminated.

The pool water is testing much lower. This is because the acidity in the water is causing enzymatic inhibition. The negative values could also be attributed to not stirring the sample properly. The HCL is not only killing the bacteria, but also inhibiting detection to the point that we do not know exactly how clean or dirty this sample really is.

Example 3: rainwater from an open-air collection tank is taken in for sample after organic plant material was found decaying in the tank.

Round 3	Total	Free	Difference
John	1223	900	323
Ken	1350	1050	300
Brian	12	1312	-1300

All 3 technicians tested samples. 2 of them came up with very similar numbers, but the third, Brian, activated both tubes and tested free before total. This caused a low value for total as he missed his testing window completely. This is due to user error. The total was tested incorrectly and came up with the difference as a negative value.

Product Selection instructions for EnSURE Touch

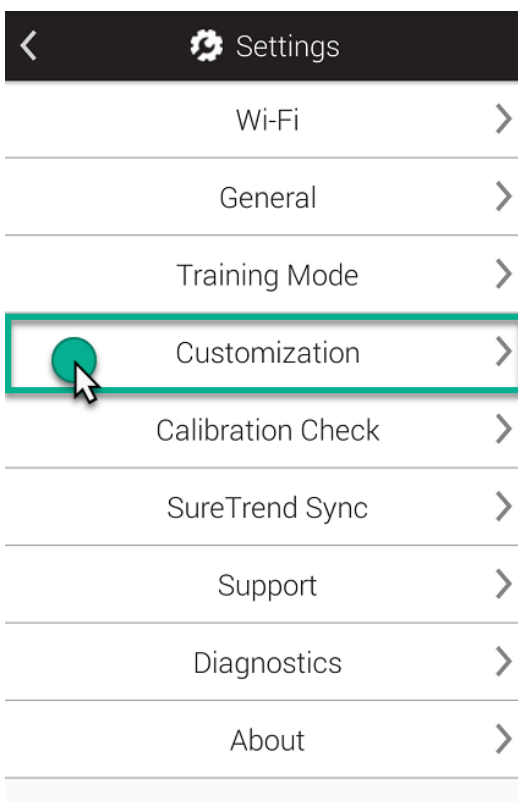
Introduction

Product Selections allows you to choose what apps and test devices are available on the EnSURE Touch™. In addition to testing ATP the EnSURE Touch also allows you test Micosnap and Enzymes. This article will show you how enable or disable products from EnSURE Touch and SureTrend Cloud.

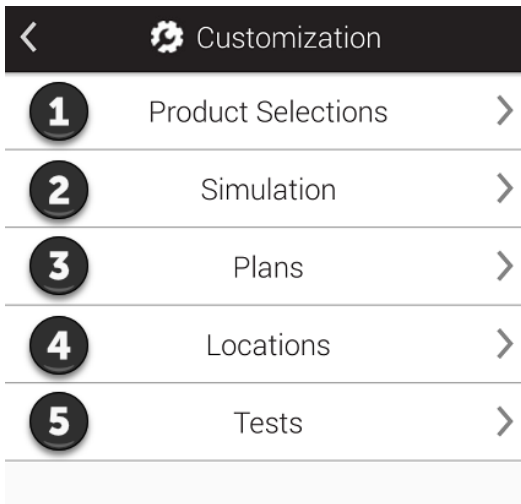
Solution

EnSURE Touch

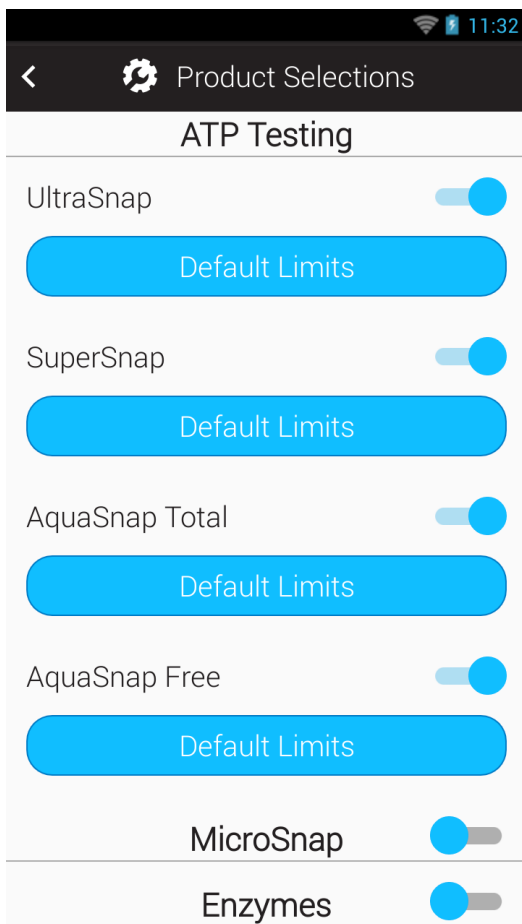
1. Touch the **Settings** App from the home screen. Next, touch **Customization**.



2. Select **Product Selections** □.



3. Select the product(s) you would like to enable/disable.



ATP Testing

Enable ATP products you would like to appear in the Device Type drop-down field when performing testing.

MicroSnap™

Toggle this on if you would like to run MicroSnap tests. Once turned on you'll be able to select products in the MicroSnap

product line.

Enzymes

Toggle this on if you would like to run Enzyme tests. Once turned on you'll be able to select Hygiena enzyme tests.

Note: Microsnap and Enzymes application will not appear unless enabled.

SureTrend Cloud

1. Login to [SureTrend Cloud](#).
2. Navigate to the **Instrument tab**.
3. Select the instrument you would like to manage products for.
4. Select **Settings** and then navigate to **Product Selections**.

The screenshot displays the SureTrend Cloud interface. At the top, the logo 'SureTrend by Hygiena' is visible. The navigation bar includes 'Dashboard', 'Results', 'Reports', and 'Instrument'. The 'Instrument' tab is active, showing a table of instruments. The selected instrument is 'Testing' with serial number 38019, site 'testing', and status 'Calibrated'. Below the instrument details, there are tabs for 'Locations/Samples', 'Plans', 'Users', 'Settings', and 'Calibration Checks'. The 'Settings' tab is selected, leading to the 'Product Selections' section. This section contains a table with columns for 'ATP', 'Lower', and 'Upper' values. The table lists four product types: UltraSnap, SuperSnap, AquaSnap Total, and AquaSnap Free, each with a checked checkbox and values of 20 for 'Lower' and 60 for 'Upper'. Below this, there are sections for 'Microorganisms By Sample' and 'Enzymes'.

SureTrend™ by Hygiena

Logout Settings Store Help

Dashboard Results Reports Instrument

Instruments

Serial No	Instrument Name	Site	Calibration Check	Model	Software	Last Sync
38019	Testing	testing	Calibrated	EnsureTouch	1.68.9	25-Sep-2020 10:47 AM

Instrument Name: Testing

Locations/Samples Plans Users **Settings** Calibration Checks

Product Selections

ATP	Lower	Upper
<input checked="" type="checkbox"/> UltraSnap	20	60
<input checked="" type="checkbox"/> SuperSnap	20	60
<input checked="" type="checkbox"/> AquaSnap Total	20	60
<input checked="" type="checkbox"/> AquaSnap Free	20	60

Microorganisms By Sample

Enzymes

5. Click the name of the product you would like to manage.

Product Selections

ATP

Microorganisms By Sample

Microorganisms enabled

MicroSnap Total

MicroSnap EB

MicroSnap Coliform

MicroSnap E. coli

Enzymes

6. If the product is disabled, check the box to enable it.

Product Selections

ATP

Microorganisms By Sample

Microorganisms enabled

MicroSnap Total

MicroSnap EB

MicroSnap Coliform

MicroSnap E. coli

Enzymes

7. Select the test device you would like to enable.

8. Sync the S/N you selected and the new product selections will be reflected on your instrument.

If you need assistance with product selection please contact Hygiena Technical Support by clicking [here](#).