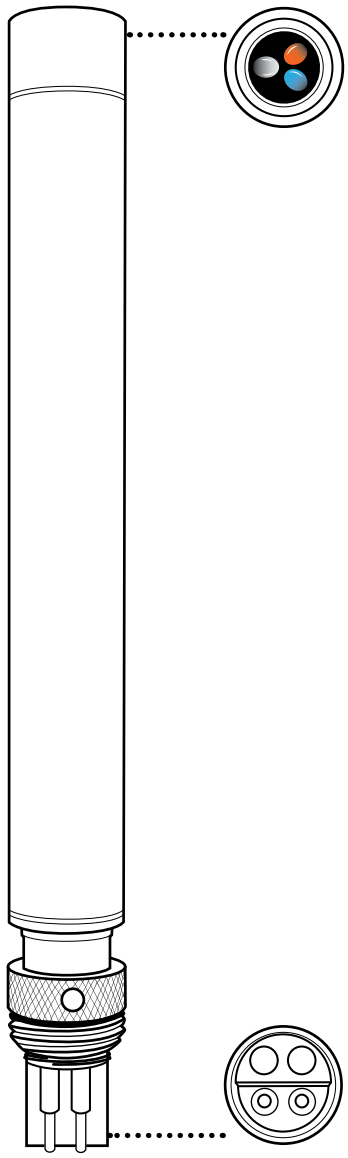
4.25 Total Algae
Sensor Overview

The Total Algae (TAL) sensors are dual-channel fluorescence sensors. The "channels" are for chlorophyll and phycocyanin (TAL-PC), or chlorophyll and phycoerythrin (TAL-PE), which are measured in the water. Each sensor thus yields two data sets: for TAL-PC, one results from a blue-emitting LED that excites the chlorophyll *a* (chl) molecule and the second results from an orange excitation beam that excites the phycocyanin (PC) accessory pigment. The TAL-PE sensor is similar, also having the chlorophyll channel, but rather than an orange-emitting LED there is a slightly blue-shifted beam that excites phycoerythrin (PE).

(continued)



599102-01 (TAL-PC)
599103-01 (TAL-PE)

Specifications

Units	
Chlorophyll	RFU, µg/L Chl
PC	RFU, µg/L PC
PE	RFU, µg/L PE
Temperature	
Operating	-5 to +50°C
Storage	-20 to +80°C
Range	*Chl:* 0-100 RFU, 0-400 µg/L Chl*; *PC:* 0-100 RFU, 0-100 µg/L*; *PE:* 0-100 RFU, 0-280 µg/L*
Response	T63<2 sec
Resolution	*Chl:* 0.01 RFU, 0.01 µg/L Chl; *PC:* 0.01 RFU, 0.01 µg/L; *PE:* 0.01 RFU, 0.01 µg/L
Sensor Type	Optical, fluorescence
Linearity	*Chl:* R^2>0.999 for serial dilution of Rhodamine WT solution from 0-400 µg/L Chl equivalents *PC:* R^2>0.999 for serial dilution of Rhodamine WT solution from 0-100 µg/L PC equivalents; *PE:* R^2>0.999 for serial dilution of Rhodamine WT solution from 0-280 µg/L PE equivalents
Optics: Chl Excitation	470±15 nm
PC Excitation	590±15 nm
PE Excitation	525±15 nm
Emission	685±20 nm

*Pigment concentration ranges of algae sensors were determined in monocultures of specific algae species. This range will vary depending on algae assemblage and environmental conditions. The best accuracy of pigment measurements can be obtained by user-built correlations between RFU and pigment concentrations measured by an independent method, and using samples from the site or sites of interest with representative algal populations.

Total Algae Sensor Units

The TAL sensors generate data in RFU or µg/L of pigment (chl, PC or PE) units, with RFU as the default. When using either RFU or µg/L, the sensor's response is highly linear: a reading of 50 of either unit represents twice as much fluorescence detected as a reading of 25, for example, if the temperature is constant.

However, users are advised to use default RFU, which stands for Relative Fluorescence Units. RFU is used to set sensor output relative to a stable secondary standard, rhodamine WT dye, which normalizes the sensor's output on a 0-100% scale. RFU calibration allows for the best comparisons of data from sensor to sensor, and also enables users to monitor for sensor drift and edaphic factors such as biofouling or declining sensor optical performance over time as the LEDs age. Another reason to use RFU is the excellent linearity once the channels are calibrated with Rhodamine WT, which translates to optimized accuracy of measurements.

The µg/L output generates an estimate of pigment concentration that is based upon correlations we built between sensor outputs and extracted pigments from laboratory-grown blue-green algae. Synonymous with parts per billion (ppb), µg/L is still in common use by regulatory agencies, but has the drawback that it is very dependent upon the composition of the algal population, the time of day, the physiological health of the algae, and a number of other environmental factors. So if two populations of algae yield a reading of 50 µg/L of chlorophyll, it does not mean that those populations are equivalent in the number of cells, for instance. Further, since algal populations can regulate their intracellular pigment concentrations, the µg/L of pigment per cell changes with season, time of day, and population dynamics. Thus the challenge with the µg/L unit is user expectations: it should not be expected that µg/L will necessarily correlate well with pigment extractions that customers perform themselves, and it should not be expected that a doubling of µg/L necessarily represents a doubling of the algal population.

RFU is likewise affected by these dynamics: a doubling of RFU does not necessarily mean there has been an exact doubling of an algal population. But it is generally more clear to users that an RFU is detecting a change in relative fluorescence signal, which can occur for a number of reasons in situ.

In any case, many users are required for regulatory compliance to deliver data in µg/L, and in waters where the algal populations are fairly predictable or stable from year to year, with respect to species compositions, good correlations can be built. So users are advised to assess whether the pigment concentration delivered by the sensor is reasonable and acceptable for the algal populations and environment with which they work.

That assessment should start with calibration of both RFU and µg/L channels with rhodamine WT, as described in the next section. Next, with samples collected from the site of interest, measure both RFU and µg/L with the sensor(s). Observing careful handling and preservation of the samples, as soon as possible extract the pigments from the samples, using standardized or preferred methods to determine pigment µg/L in each sample. The extraction data may be used to assess how RFU and µg/L delivered by the sensor compare with the extracted µg/L of pigment that would be predicted by the sensor. Ideally this would be done with a dilution series of the original sample or at the very least multiple samples. The user's requirements for how well µg/L delivered by the sonde must correlate with their own extraction data will determine whether the µg/L output should be used for reporting.

Measuring cells/mL with EXO TAL Sensors

Similar to µg/L, some users have a requirement to report cell/mL data for blue-green alga monitoring, even though in reality these measurements vary widely from algal population to algal population in situ. Within KorEXO 2.0 and later software versions, there is the capability to have the sonde deliver this unit for the PC and PE channels, based upon user-applied correlations.

When selecting the TAL sensor in the Instruments and Sensors tab of the software, there is a "TAL-PC Phycocyanin Settings" window (or TAL-PE if that is the sensor in use). There are two radio buttons that appear when that window is opened:

- Use legacy cells/mL relationship
- Build my own cells/mL relationship

The first option was designed for users that were accustomed to this unit in our legacy 6-Series sondes, and who want their EXO data to tightly match the cells/mL data generated by these older sondes. The algorithm applied to "match" these outputs across sonde platforms is proprietary, and it is highly advisable that when using this unit at some point users actually test the validity of the outputs for their applications. This can be done by collecting grab samples and comparing actual cells/mL using microscopy or plating as appropriate.

A better method would be to use the second option of building one's own cells/mL relationship. This makes a module appear wherein users can enter an RFU measurement alongside a corresponding cells/mL measurement that has been made for the exact same sample, using microscopy or whatever method the user prefers. The software will derive the relationship between the columns entered by the user and will apply that equation to all subsequent measurements to deliver the cells/mL unit in the sonde's output.

From time to time and place to place, the validity of this correlation can be tested, verified, or validated by collecting grab samples and comparing in vitro measurements of cells/mL with the in situ values delivered by the sonde.

In all cases, proper calibration of the sensor with Rhodamine WT is necessary for the most reliable outputs, and for comparison of data from sensor to sensor.

4.26 Total Algae
Calibration

For best performance assure that the sensor face is clean prior to calibration. We advise that new sensors should be calibrated before use, and calibration checks and the user's own tolerance of drift should be used to determine when recalibration is necessary.

Users will prepare their own calibration standards. Rhodamine WT is a secondary standard (the actual pigments would be primary standards). It is used because of its stability and affordability. The units that the sensor delivers are in either RFU (recommended) or µg/L pigment equivalent units. We strongly recommend using RFU, but in either case Table A below must be used to derive the calibration values that the user will enter during the process outlined below. Use of this table requires a temperature measurement, and the best way to do this is to have an EXO conductivity/temperature sensor on the sonde bulkhead during calibration. In general fluorescence is inversely related with temperature, and this effect will be accounted for to optimize the accuracy of your calibration by using the following table.

	Chlorophyll 0.625 mg/L Rhodamine		Phycocyanin 0.625 mg/L Rhodamine		Phycoerythrin 0.025 mg/L Rhodamine	
Solution Temperature (˚C)	Chl RFU	µg/L chlorophyll	PC RFU	µg/L phycocyanin	PE RFU	µg/L phycoerythrin
30	14.0	56.5	11.4	11.4	37.3	104.0
28	14.6	58.7	13.1	13.1	39.1	109.0
26	15.2	61.3	14.1	14.1	41.0	115.0
24	15.8	63.5	15.0	15.0	43.0	120.0
22	16.4	66	16.0	16.0	45.0	126.0
20	17.0	68.4	17.1	17.1	47.0	132.0
18	17.6	70.8	17.5	17.5	49.2	138.0
16	18.3	73.5	19.1	19.1	51.4	144.0
14	18.9	76	20.1	20.1	53.6	150.0
12	19.5	78.6	21.2	21.2	55.9	157.0
10	20.2	81.2	22.2	22.2	58.2	163.0
8	20.8	83.8	22.6	22.6	60.6	170.0

Table: Temperature-compensated standard solution values for TAL sensors.

Steps 1-3 below describe a standard two point calibration performed with Kor EXO 2.0 software. Calibration can also be performed using the EXO Handheld, the main differences simply being the references to windows. In some cases users may prefer to perform a re-zeroing of the sensor, sometimes referred to as a "one point calibration," and that is described later in this section.

Step 1: Prepare Rhodamine WT Dye Solution

Purchase Rhodamine WT as a 2.5% solution to follow the procedure below. Note that there are many types of Rhodamine—make sure Rhodamine WT is selected. If a 2.5% solution cannot be obtained commercially, prepare it from a solid or liquid solution to a 2.5% final concentration, or adjust the dilutions below accordingly. Kingscote Chemicals (Miamisburg, OH, 1-800-394-0678) has historically had a 2.5% solution (item #106023) that works well with this procedure. It should be stored in the refrigerator when not in use.

> ***NOTE:*** *Preparation of the following solutions requires precise measurement equipment including graduated pipets and volumetric flasks.*

1. **For any TAL sensor calibration, prepare a 125 mg/L solution of Rhodamine WT**. Transfer 5.0 mL of the 2.5% Rhodamine WT solution into a 1000 mL volumetric flask. Fill the flask to the volumetric mark with deionized or distilled water and mix well to produce a solution that is approximately 125 mg/L of Rhodamine WT. Transfer to a storage bottle and retain it for future use.

   *This solution can be stored in the refrigerator (4°C). Its degradation will depend upon light exposure and repeated warming cycles, but solutions used 1-2 times a year can be stored for up to two years. Users should implement their own procedures to safeguard against degradation.

2. **For calibration of any chlorophyll channel (on either the TAL-PC or the TAL-PE sensor) and the TAL-PC phycocyanin channel, prepare a 0.625 mg/L solution of Rhodamine WT.** Transfer 5.0 mL of the 125 mg/L solution prepared in step one into a 1000 mL volumetric flask. Fill the flask to the volumetric mark with deionized or distilled water. Mix well to obtain a solution that is 0.625 mg/L of Rhodamine WT. Use this solution within 24 hours of preparation and discard it after use.

3. **If using a TAL-PE sensor, additionally prepare a 0.025 mg/L solution of Rhodamine WT for calibration of the phycoerythrin channel.** Transfer 0.2 mL of the 125 mg/L solution prepared in step one into a 1000 mL volumetric flask. Fill the flask to the volumetric mark with deionized or distilled water. Mix well to obtain a solution that is 0.025 mg/L of Rhodamine WT. Use this solution within 24 hours of preparation and discard it after use.

Step 2: Select the pigment and channel to be calibrated.

In the Kor software or on the handheld, select the channel you want to calibrate (chl, PC, or PE) and the units you intend to use (RFU or µg/L).

Note that each channel of the sensor must be calibrated independently. Calibration of the chlorophyll channel does not set the calibration for the PC channel or the PE channel. Likewise, even just for the chlorophyll channel, calibration of RFU does not automatically calibrate the µg/L units. Calibration must be performed for each channel of interest, each unit of interest, and each calibration point (zero and the second point). It is thus possible that Step 3 below will be performed up to 8 times total, if one wants reading for all units from all channels. This is cut in half if only RFU are used, which is YSI's recommendation.

Step 3: Perform a two-point calibration.

Step 3a: Calibration at zero.

The zero point is always calibrated first. Place the sonde, loaded with a TAL and an EXO temperature sensor, into a clean calibration cup containing clean water. It is not required that this be deionized or even distilled water; it must be free of any particles that might fluoresce and interfere with the calibration process. Thus distilled water is typically what users prefer to have that assurance.

The software or handheld will show a graph while the sensor is stabilizing, and the temperature will also be shown. Temperature is not needed for the zero point; the user must enter a "Standard Value" of 0. When the Data Stability indicates "Stable", click to "Apply" the calibration. Next select "Add Another Cal Point" and proceed to Step 3b.

Step 3b: Calibration with Rhodamine WT

The same basic procedure will be followed, but using either Kor software or the EXO handheld will require that users enter the temperature-compensated standard value for the calibration solution. In all cases, the reading from the EXO temperature sensor is the most reliable to use, and the value for the standard can be derived from the Table A provided above.

As an example, assume that you will calibrate the chlorophyll RFU channel, and that the temperature measured in the 0.625 mg/L rhodamine WT solution is 22°C. This temperature will show up on the calibration screens using the KorEXO software, and can be seen on the handheld's dashboard screen as well. The first standard value entered during calibration will be 0, since that standard will be water (see Step 3 below). The second standard value will be 16.4, as derived from Table A using a temperature of 22°C. Alternatively, if you intend to use the µg/L unit, the second standard value would be 66 for this example. Using the same 0.625 mg/L rhodamine WT solution to calibrate the PC channel will yield a second standard value of 16.0 RFU or 16 µg/L. You will enter these values when you perform the calibration.

Upon entering the Table A-derived value, wait for the sensor to show "Stable" and then click on "Apply". Now choose "Complete Calibration" and then "Exit."

Note that throughout this process users had options to "Redo a cal point" or to "View Calibration Worksheet." So for any channel and a given unit of interest, a point can be redone at any time without having to exit out to the beginning of the process.

However, to now calibrate other units for either the same or different pigment channels, this process must be started again at Step 2.

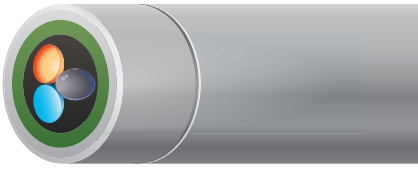
Re-zeroing the TAL Sensor.

Oftentimes users will perform a "cal check" in water to assess if the sensor has drifted beyond an acceptable limit defined by that user. When drift has occurred ideally a two-point calibration should be performed. However, when there isn't an opportunity to prepare the rhodamine solutions and perform a two-point calibration, or if users are mainly interested in accuracy at the lower end of the sensor's range, they may choose to re-zero the sensor.

Historically referred to as a "single-point calibration," doing a calibration with water only resets the zero value, called here "re-zeroing" the sensor. The main advantage of doing this is speed, and users should be aware that re-zeroing the sensor does not reset the second point entered during the most recent two-point calibration. The consequence is that drift error will be alleviated at and near zero, but more error can accumulate in the measurement the farther away from zero the measured value is. The amount of that error can be different from sensor to sensor, and use case to use case. It is dependent upon how much that second point may drift, which is not always equivalent to how much the zero point drifts.

For many users, especially those with sites where pigment is rarely detected and values are at or near zero most of the time, the far-from-zero accumulation of error is a non-issue. For others, a single point calibration may not be acceptable. A single-point calibration is an option in the software and is performed exactly the same way as the two- point calibration, using water as the standard and waiting for the value to stabilize before applying it. Rather than adding a second calibration point, the user would exit after the water calibration.

SmartQC for TAL Sensors



The SmartQC Score for any TAL sensor is based on an offset from 0 RFU, and a gain factor. Each individual channel (Chlorophyll, Phycocyanin, Phycoerythrin) has a unique offset and gain factor. It is possible to have a green SmartQC Score for calibration of one channel, but a yellow or red SmartQC Score for the second channel. In this case the TAL sensor SoftQC that is shown in Kor Software will appear as the worst QC Score (yellow or red), and one must look at the individual channels to investigate where the issue is. Thus the steps outlined here are for each channel, and for each unit calibrated within that channel.

Guidance on interpretation of the SmartQC Score for this sensor is as follows:

Green: Gain and offset are within acceptable limits. Calibration was performed successfully and results are within factory specified limits.

Yellow: The sensor gain or offset is near the threshold of calibration limits. If a user calibration results in a yellow QC Score, perform the following actions:

1. Perform a Factory Reset Calibration and complete a recalibration.
    a. If performing a 1-point calibration, use fresh, clear water.
    b. If performing a 2-point calibration, use fresh, clear water and freshly made Rhodamine WT solution.
2. Ensure that the standard value was entered correctly. Calibration of TAL channels is temperature-dependent; make sure the appropriate value from the table in Section 4.24 was entered during calibration for either RFU or µg/L.
3. Ensure that the sensor is free of debris. Refer to Section 5.6 for additional information on how to properly clean the sensor in order to avoid damage.

If the QC Score returns to yellow, the sensor is still able to be used, but the user should monitor this sensor during calibrations for any further drift.

Red: The sensor gain or offset are outside of factory specified limits. If a user calibration results in a red QC Score, follow the same steps described above for a yellow QC Score.

If the QC Score remains red, please contact YSI Technical Support for further assistance.