

# EXO Rhodamine Sensor Calibration Instructions

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.

YSI does not offer Rhodamine WT standards. Users will prepare their own calibration standards by diluting a 2.5% solution of Rhodamine WT. YSI recommends using Bright Dyes Fluorescent FWT Red 25 - Liquid (item # 106023) from Kingscote Chemicals.

The accuracy of the sensor will be directly influenced by the accuracy of the standard solutions used to calibrate the sensor. Preparation of the following solutions requires precise measurement equipment including graduated pipets and volumetric flasks.

Make sure all software and firmware is up to date. Earliest supported versions are:

- KorEXO Software v2.2.0.19
- EXO Sonde Firmware v1.0.83
- EXO Handheld Firmware v1.0.51

A 4-point calibration procedure is necessary to ensure proper performance over the entire 0-1,000  $\mu\text{g/L}$  range of the sensor. The sensor is calibrated to values in  $\mu\text{g/L}$ . The sensor can also report in units ppb and RFU.

An EXO Conductivity/Temperature sensor must be installed. The effect of temperature on the Rhodamine sensor electronics and the fluorescence of Rhodamine WT can be significant; however, the combination of these two factors is automatically taken into account by the sensor firmware providing temperature compensated readings. This means the "Standard Values" entered during the calibration procedure should match the concentration of the solutions prepared regardless of temperature variance.



Figure 1: EXO Rhodamine Sensor

## You Will Need:

- EXO Sonde
- EXO Conductivity/Temperature Sensor
- EXO Rhodamine Sensor
- EXO Handheld
- KorEXO Software (v.2.2.0.19 or later)
- Rhodamine WT Dye
- Graduated Pipet
- Volumetric Flask



## Preparation of Rhodamine WT Dye Solutions

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 liquid solution to a 2.5% final concentration, or adjust the dilutions below accordingly. Kingscote Chemicals (Miamisburg, OH, 1-800-394-0678) has historically offered 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.



**Figure 2:** Rhodamine WT dye and measurement equipment.

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### Prepare a 125 mg/L stock 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.

**NOTE:** If the solution is cooled in the refrigerator, make sure to let it warm to room temperature prior to using it for calibration.

Two concentrations of Rhodamine WT must be prepared from this stock solution:

1. a 25 µg/L standard
2. a standard equal or greater than 125 µg/L, but no more than 1,000 µg/L

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### Prepare a 25 µg/L stock solution\* of Rhodamine WT.

Depending on the amount (volume) of calibration solution you want to produce, use the following instructions:

For **1,000 mL** of 25 µg/L solution:

Transfer 0.2 mL or 200 µL of the 125 mg/L Rhodamine WT base solution into a 1,000 mL volumetric flask. Fill the flask to the 1,000 mL volumetric mark with deionized or distilled water and mix well to produce a standard solution that is 25 µg/L of Rhodamine WT.

For **500 mL** of 25 µg/L solution:

Transfer 0.1 mL or 100 µL of the 125 mg/L Rhodamine WT base solution into a 500 mL volumetric flask. Fill the flask to the 500 mL volumetric mark with deionized or distilled water and mix well to produce a standard solution that is 25 µg/L of Rhodamine WT.

\*This solution can be stored in the refrigerator (4°C). Its degradation is much more rapid than the base solution and should be used or discarded within 30 days.

**NOTE:** The second calibration standard solution\* must be at least 125 µg/L, but no more than 1,000 µg/L.

Kor Software will not allow for calibration point #4 outside of the 125-1,000 µg/L range. YSI recommends preparing a calibration solution closest to the expected measurement range within these limits.

Refer to the table below to determine the amount of 125 mg/L Rhodamine WT base solution to transfer to a volumetric flask. The columns represent the desired concentration of the standard solution, while the rows represent the desired amount (volume) of standard solution that users would like to produce

		Concentration			
		125 µg/L	250 µg/L	500 µg/L	1000 µg/L
Volume	500 mL	0.5 mL	1.0 mL	2.0 mL	4.0 mL
	1000 mL	1.0 mL	2.0 mL	4.0 mL	8.0 mL

**Table:** Amount (mL) of 125 mg/L Rhodamine WT base solution for mixing standard solution.

- **Concentration:** Users are encouraged to choose a concentration that is closest to the values they expect to measure.
- **Volume:** Users should select a volume appropriate for the amount of calibrations to be performed.
  - For multiple sensor calibrations, a larger volume (1,000 mL) may be preferred.
  - For a single calibration, a smaller volume (500 mL) may be preferred.

Transfer the appropriate amount of 125 mg/L Rhodamine WT base solution into the preferred volumetric flask based on the standard solution concentration desired. Fill the flask to the volumetric mark with deionized or distilled water and mix well to produce the standard solution.

\*This solution can be stored in the refrigerator (4°C). Its degradation is much more rapid than the base solution and should be used or discarded with 30 days.

## Calibration of the Rhodamine Sensor

Rhodamine is a dual channel sensor that requires a **4-point calibration**; 2 points for each channel. Calibration point #1 is simply a zero solution (typically DI water). Calibration points #2 and #3 are identical, using the same 25 µg/L Rhodamine WT solution. Calibration point #4 can be any concentration equal to or greater than 125 µg/L, but no more than 1,000 µg/L. For best practices, rinse and dry between standard solutions.

**NOTE:** Before proceeding with the calibration:

- make sure all sensors, guards, and calibration cups are clean
- make sure an EXO Conductivity/Temperature sensor is installed

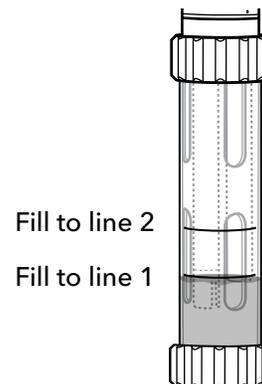


Figure 3: EXO Calibration cup.

**1 Calibration Point #1 at 0 µg/L.** Place the sonde into a clean calibration cup containing distilled or deionized water. The software or handheld will show a graph while the sensor is stabilizing. Make sure the "Standard Value" is equal to zero (0). When the Data Stability indicates "Stable", click "Apply" in the software or "Accept Calibration" on the handheld. In the software, select "Add Another Cal Point" and proceed to Step 2.

**2 Calibration Point #2 at 25 µg/L.** Place the sonde into a clean calibration cup containing the prepared 25 µg/L standard. Make sure the "Standard Value" is equal to 25. When the Data Stability indicates "Stable", click "Apply" in the software or "Accept Calibration" on the handheld. In the software, select "Add Another Cal Point" and proceed to Step 3.

**NOTE:** Keep the sensors submerged in the 25 µg/L standard solution as you proceed to calibration point #3.

**3 Calibration Point #3 at 25 µg/L.** With the sensors still in the 25 µg/L standard solution, make sure the "Standard Value" is still equal to 25. When the Data Stability indicates "Stable", click "Apply" in the software or "Accept Calibration" on the handheld. In the software, select "Add Another Cal Point" and proceed to Step 4.

**4 Calibration Point #4 at user preferred concentration (125-1,000 µg/L).** Place the sonde into a clean calibration cup containing the prepared µg/L standard solution. Make sure the "Standard Value" is equal to the concentration prepared. When the Data Stability indicates "Stable", click "Apply" in the software or "Accept Calibration" on the handheld. Finally, select "Complete Calibration" [software] or "Finish Calibration" [handheld] and proceed to the calibration review screen. From here users can view the Calibration Report or select "Exit" to return to the main calibration menu.

Note that throughout this process users have the option to "Redo" a calibration point without having to exit and start the process over.

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