pyroscience
sensor technology

Optical pH Sensors

USER MANUAL

OPERATING INSTRUCTIONS

Document Version 1.04

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1 Introduction

PyroScience offers a variety of fiber-based and contactless optical pH sensors. For an overview see our homepage www.pyroscience.com

These can be read-out with the fiber-optic, multi-analyte & multi-channel PC-operated ***FireSting pro.***

The required ***PyroWorkbench*** software is available as free download from the ***PyroScience*** website and must be installed on the Windows PC/laptop before connecting the ***FireSting pro*** for the first time. For details on the read-out device, the software and user interface, please see their respective manuals and handling guidelines.

This manual is intended to provide all necessary information on standard application of optical pH sensors from ***PyroScience***.

For more information concerning advanced applications, please contact us at info@pyroscience.com.

Your ***PyroScience*** Team

2 Quick Start

Step 1: Download the ***PyroWorkbench*** software from the download tabs of your purchased read-out device on the ***PyroScience*** website, unzip and start the installer, and follow the instructions.

Step 2: Connect the ***FireSting pro*** to the Windows PC/laptop with the micro-USB cable.

Step 3: Unpack the sensors and carefully remove the protective caps from the sensor tip, from the fiber plug and from the optical connector(s) at the ***FireSting pro***.

Step 4: Connect the ***PyroScience*** pH sensor(s) to the optical connector(s) of the device.

Step 5: **For automatic temperature compensation,** connect an appropriate Pt100 temperature sensor to the temperature port or, alternatively, an optical temperature sensor to one of the remaining channel connectors (multi-channel ***FireSting pro*** only).

Step 6: Prepare appropriate pH calibration buffers (see chapter 4.2).

Step 7: Start the ***PyroWorkbench*** software.

Step 8: Enter all required **Sensor Settings,** including the **Sensor Code**, the **Fiber Length (m)** for each sensor, as well as the **compensation of environmental parameters** (temperature and salinity).

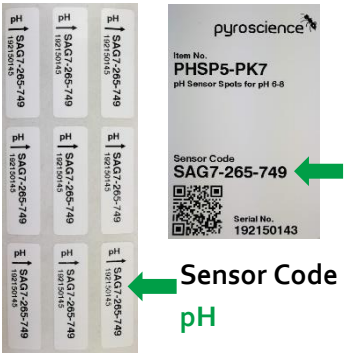
Step 9: Perform a 2-point **Calibration** of the pH sensor**.**

Step 10: Start Measurements and activate **Data Logging**.

3 Sensor Settings

Each optical pH sensor comes with an individual **Sensor Code,** containing important information on adjustments for optimal sensor performance and pre-calibration of the respective sensor type. Therefore, it is important to enter the Sensor Code of the connected sensor into the **Sensor Settings** of the ***Pyro Workbench*** software. For multi-channel ***FireSting pro***, the number of the channel tab must correspond with the channel number at the ***FireSting pro*** read-out device.

IMPORTANT: Enter the correct **Sensor Code** for sensors connected to a channel at the ***FireSting pro***. The sensor code can be found on the label attached to the cable (fiber-based sensors) or on the bag of contactless sensors.



3.1 Conditions in the Sample

When entering the sensor settings, the **Conditions in the Sample** during the measurements have to be determined. There are two important parameters to be taken into account and which can be automatically compensated:

- Temperature
- Salinity

3.1.1 Temperature

Several options for **Automatic Temperature Compensation** of optical pH sensors are available:

- External Temperature Sensor (Pt100, temperature port)
- Fixed Temperature (must be entered, kept constant and controlled)
- Optical Temperature sensor connected to a channel connector (its channel number must be selected) of a multi-channel ***FireSting pro***

If **External Temperature Sensor** or **Optical Temperature Channel** is selected, **automatic compensation** of **temperature changes** on the respective pH sensor readings is activated. The **Compensation Temperature** will be displayed in the corresponding channel row of the main window.

If an External or Optical Temperature Sensor was selected, the sensor has to be fixed in the sample/calibration standard in which pH measurements/calibration will be performed.

NOTE: The Pt100 temperature sensor may have an offset which needs to be determined manually and calibrated first. To calibrate the Pt100 sensor please see the Appendix.

3.1.2 Salinity

The optical pH sensors show a minor cross sensitivity to the ionic strength of the medium. Ionic strength is a function of the concentration and charge of all the ions in a solution and is an important parameter for pH measurements. Theoretically, the ionic strength of a sample can be calculated, but from a practical point of view, other indicators can provide a rough estimate of the ionic

strength, such as using the concentration of all compounds or conductivity data.

To compensate for this effect, the salinity [g/l] must be entered in the sensor settings and during a pH calibration (please note that the ionic strength can differ between the calibration buffer and the medium of the measurements).

There are several general categories of salinity ranges to select in the ***Pyro Workbench*** software:

Category	*Salinity [g/l]*	*Conductivity [mS/cm]*	*Ionic Strength [mM]*
Freshwater	0.25 - 0.5	0.5 - 1	5 - 10
Wastewater	0.5 - 1.5	1 - 3	10 - 30
Intermediate	1.5 - 5	3 - 10	30 - 100
Physiological	5 - 15	10 - 30	100 - 300
Seawater	15 - 40	30 - 80	300 - 800

For more accurate measurements it is possible to enter a known salinity [g/l] of the sample in the software. This salinity value can be calculated by a simplified approach:

Salinity [g/l] = Conductivity [mS/cm] /2
Salinity [g/l] = Ionic Strength [mM] / 20

For experiments with strongly changing ionic strength it is recommended to update the settings and perform a new calibration in order to acquire accurate results.

4 Sensor Calibration

4.1.1 General Information

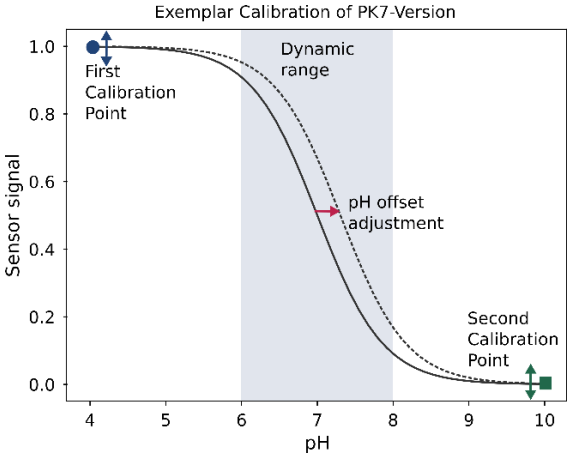
The pH calibration has to be **repeated periodically,** depending on the **individual application** (e.g. required accuracy, temperature of the sample, life-time of the sensor, substances in sample). It is advisable to perform a 2-point calibration before every measurement using freshly prepared buffer solutions.

A pH sensor calibration can be performed in three different ways:

- **1-point calibration (required):** Calibration of the completely protonated sensor at highly acidic conditions. Recommended before every measurement.

- **2-point calibration (highly recommended):** Additional calibration of the completely deprotonated sensor at highly basic conditions. Recommended before every measurement.

- **pH-offset adjustment (for complex medium):** Adjustment of the turning point of the sensor in highly complex medium (individual sample, see image below)



Do not use commercially available buffer solutions used for pH electrodes. These buffers (colored and uncolored) will affect the sensor performance irreversibly due to stabilizers in the solution. It is important to only use ***PyroScience*** buffer capsules or self-made buffers with known pH and ionic strength for calibration (with advice from ***PyroScience***).

4.1.2 Temperature

It is crucial to determine exactly the temperature of the pH calibration buffer during the calibration process via one of the following possibilities:

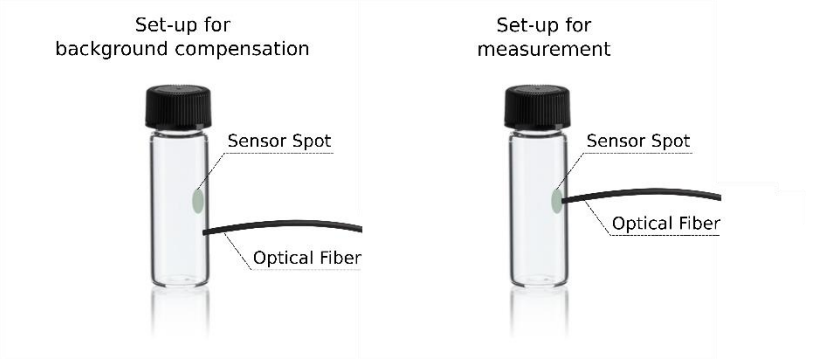
- Manual adjustment of a **Fixed Temperature** (needs to be determined and kept constant)
- Temperature Compensation with an **External** (Pt100) **Temperature Sensor** connected to the temperature port of a ***FireSting pro***, or
- **Optical Temperature Sensor** (calibrated) connected to a channel at a multi-channel ***FireSting pro*** (its respective channel number needs to be entered at **Optical Temp. Channel**).

4.1.3 Background Compensation

For pH Mini Probes, Sensor Vials, Flow-Through Cells and Sensor Spots the **fiber length** needs to be entered into the software for an automatic background compensation. This is recommended and sufficient for most applications.

Sensor Spots and Sensor Vials used in applications with low signal intensities (e.g. thick glass) and for measurements in colored media,

the option **MANUAL background compensation** should be used to determine the individual luminescence background of the sample. For more information please refer to the Workbench Software Manual Chapter 6.



4.2 Preparation of Calibration Standards

For the calibration it is strongly recommended to use ***PyroScience pH*** buffer capsules with pH 4.01 (item no. ***PHCAL4)*** and pH 10.0 (item no. ***PHCAL10***).

- pH 4.01 buffer is used for the first calibration point
- pH 10.00 is used for the second calibration point

For this, open the capsule by holding both ends and pulling them apart. Dissolve the powder of the capsule in 100 mL demineralized water. Mix well and insure, that the contents are completely dissolved.

Note: Always prepare a fresh buffer for calibration. Do not use the ***PHCAL4*** buffer for long-term storage of the sensor.

Alternatively, it is possible to perform a **Custom Calibration**. Prepare a buffer with known pH value and known ionic strength. For

the **first calibration point the pH must be <4** and for the **second calibration point the pH must be >10.** The respective pH value and salinity must be entered in the custom calibration menu in the software.

4.3 First Calibration Point (acidic pH)

Ensure that the correct **Sensor Code** has been entered into the settings (refer to chapter 3) and a calibration standard has been prepared correctly.

For the first calibration point, prepare a pH 4.01 buffer solution based using the ***PyroScience*** buffer capsules (item no. ***PHCAL4***) or a custom calibration buffer (chapter 4.2).

It is recommended to perform the calibration at the same **temperature** of the actual pH measurements.

It is strongly recommended to perform calibration at conditions close to the environmental conditions during measurements. Ensure constant conditions during calibration.

If the sensor was previously **stored under dry conditions,** wait for at least **30 min** to achieve wetting of the sensor membrane. This step is necessary to achieve high accuracy.

After completing the Settings Wizard for all channels, open the **Calibration Wizard** by clicking the "Cal." button in the respective row ("Ch. 1" to "Ch. 4") of the table in the main window. The software will guide you through the complete calibration procedure.

Insert the pH and temperature sensor into the stirred buffer, and ensure that the sensor tips are completely immersed into the water and free of air bubbles. Let the sensor equilibrate and perform the calibration. Wait for a **stable sensor signal** as this is substantial for correct pH measurements (this can take several minutes depending on the temperature and stirring rate).

With increasing stability of the sensor signal the color will change from red to orange to yellow to green. Although a calibration is possible in the orange state, we strongly recommend to wait until **Take Value** and the frame of the graph have turned to **green.**

After setting the first calibration point, wash the sensor with distilled water and submerse the sensor in the next calibration buffer without letting the sensor dry.

Note: If calibration is not possible due to a warning, please refer to chapter 7.4 on page 20 for more details.

4.4 Second Calibration Point (basic pH)

It is highly recommended to perform a 2-point calibration before every measurement.

For the second calibration point, prepare a pH 10.0 buffer solution based on the ***PyroScience*** buffer capsule (item no. ***PHCAL10)*** or a custom calibration buffer (chapter 4.2).

Insert the pH and temperature sensor into the stirred buffer, and ensure that the sensor tips are completely immersed in the water and free of air bubbles. Let the sensor equilibrate and perform the calibration. Wait for a **stable sensor signal** as this is substantial for correct pH measurements (this can take several minutes depending on the temperature and stirring rate).

4.5 pH Offset Adjustment (Advanced Applications)

This will perform a pH offset adjustment to a buffer with exactly known pH value. The pH of the solution has to be in the dynamic range of your sensor version (e.g. between pH 6.5 – 7.5 for PK7 sensors). This can be used for measurements in highly complex media (e.g. cell culture media). Please contact *PyroScience* for support.

To undo the pH offset, please click on **Delete calibrations** in the calibration wizard. This will delete all current calibration values and set the sensor back to factory calibration. A 2-point calibration has to be performed afterwards to start a measurement.

4.6 Available pH Sensors

4.6.1 Fiber-based Sensors

Sensor item	Sensor-specific application instructions
PHROB-…	Calibration: 2-point calibration with *PyroScience* buffer capsules *PHCAL4* and *PHCAL10* with optical isolation Remove air bubbles from sensor surface
PHFTCR-…	Calibration: 2-point calibration with *PyroScience* buffer capsules *PHCAL4* and *PHCAL10* Flow rate20-500 mL/min Remove air bubbles Clean regularly Ensure stable temperature conditions

4.6.2 Contactless Sensors

Sensor item	Sensor-specific application instructions
PHSP5-…	Calibration: 2-point calibration with *PyroScience* buffer capsules *PHCAL4* and *PHCAL10* with optical isolation Mind air bubbles. Glue carefully with silicone glue and let dry for 24h.
PHVIAL-…	Calibration: 2-point calibration with *PyroScience* buffer capsules *PHCAL4* and *PHCAL10* with optical isolation Remove air bubbles. Ensure stable temperature conditions

4.7 Combined Sensors

Sensor item	Sensor-specific application instructions	
PHTVIAL-…	pH & Temp	Calibration: 2-point calibration of pH sensor with *PyroScience* buffer capsules *PHCAL4* and *PHCAL10* 1-point calibration for temperature sensor
PHTOVIAL- …	pH & O2 & Temp	Calibration: 2-point calibration of pH sensor with *PyroScience* buffer capsules *PHCAL4* and *PHCAL10* 1- or 2-point calibration of O2 sensor with *OXCAL* calibration capsule 1-point calibration of temperature sensor

5 Sterilization, Cleaning and Storage

5.1 Sterilization

PyroScience optical pH sensors cannot be autoclaved or sterilized. Do not use bleach, ethanol, acetone or any solvent.

5.2 Cleaning and Storage

Do not use any organic solvents or surfactant solutions. The sensor may have cross-sensitivity to substances in your sample. Please contact us in doubt as irreversible damage of the sensor can occur with some compounds (e.g. surfactants).

After a measurement, the pH sensor should be rinsed carefully with deionized water, let dry and put on the protective cap. The sensor has to be stored in a dry and dark place at room temperature. For all sensors and fibers, put the black caps on the plug of the fiber to prevent that light is entering the fiber possibly causing photo-bleaching of the indicator.

Store the sensor in a dry, dark and secure place at room temperature.

6 Related Documents

Related documents for more detailed instructions on fiber-optic read-out devices, software and optical sensors are available:

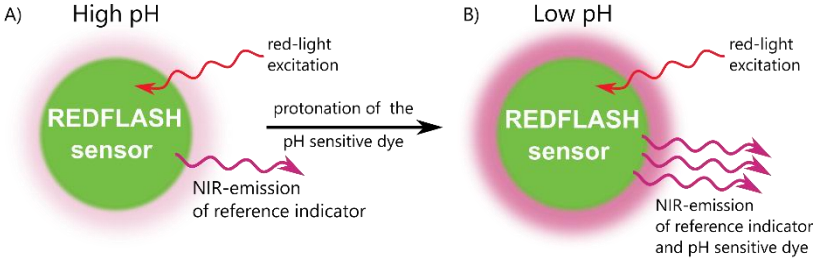
- manual for logger software "*Pyro Workbench*" (Windows)
- manual for multi-analyte meter *FireSting pro*
- manual for optical pH sensors
- manual for optical temperature sensors
- manual for optical oxygen sensors

7 Appendix

7.1 pH Measuring Principle

The optical pH sensor is based on the successful ***PyroScience REDFLASH technology***. The sensor consists of a pH insensitive reference indicator and a pH sensitive luminescent dye. Both are excited with red-light (more precisely: orange-red at a wavelength of 610-630 nm) and show bright luminescence in the near infrared (NIR, 760-790 nm).

If the pH indicator is de-pronated at high pH, its fluorescence is quenched and only the NIR emission of the reference indicator is measured. If the pH gets more acidic, the pH indicator is protonated and emits bright NIR luminescence and the emission of both indicators is detected.



Principle: red light excited REDFLASH sensor shows luminescence in the near infrared (NIR). The REDFLASH sensor consists of a pH insensitive reference indicator and a pH sensitive dye.**A)** low NIR emission from just the reference indicator and **B)** high NIR emission from the protonated dye and the reference indicator.

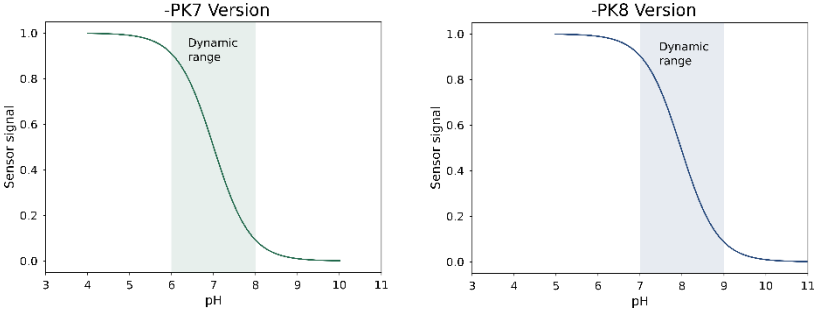
The ***REDFLASH technology*** impresses by its high accuracy, high reliability and low power consumption.

The red-light excitation of the ***REDFLASH indicators*** significantly reduces interferences caused by autofluorescence and reduces background fluorescence from biological samples / media.

The measuring principle is based on a sinusoidally modulated red excitation light. This results in a phase-shifted sinusoidally modulated emission in the NIR. The ***PyroScience*** read-out device measures this phase shift, which is then calculated into pH.

7.2 Explanation of the Different pH Ranges

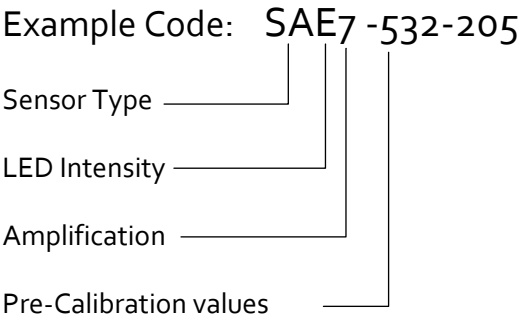
Optical pH sensors are sensitive to a pH range of 2-3 pH units. Sensors are offered for two different ranges for pH measurements between pH 6.0 to 8.0 (***-PK7*** version) and pH 7.0 to 9.0 (***-PK8*** version). Depending on the application it is important to choose the right version.



Calibration of both versions is performed at low pH values (completely protonated indicator dye) and at high pH values (completely de-protonated indicator dye). For this reason, it is possible to use the same pH buffer capsules (***PHCAL4*** and ***PHCAL10***) for both pH sensor versions.

7.3 Explanation of the Sensor Code

The pH sensors are delivered with an attached sensor code which must be entered in the Settings (refer to chapter 3). The following figure gives a short explanation about the information given in the sensor code.



7.4 Common Warnings during Calibration

During the calibration various errors can occur, resulting in different warnings.

7.4.1 "Signal Intensity too low, refer to the manual"

The Sensor Signal Intensity can be too low for several reasons:

1. The sensor is connected to the wrong channel at the multi-channel ***FireSting pro***

2. The end of the sensor lifetime is reached and the sensor needs to be replaced.
3. In case of sensor spots: the positioning of the optical fiber on the sensor spot is not correct.
4. The vessel wall is too thick in the case of sensor spots.

In this case, please try to increase the LED intensity as maybe the vessel wall is too thick or the sensor is altered to a high extend.

To do this, go back to the **Settings** and increase the LED Intensity.

A higher LED intensity leads to a higher sensor signal. If the warning still shows up after setting the LED intensity up, please increase it further until the calibration is possible.

7.4.2 "Warning: Sensor signal out of range"

The reason for this can be:

1. The end of the sensor lifetime is reached and the sensor needs to be replaced.
2. The calibration buffer does not exhibit the correct pH value.
3. The pH of the sample is out of the dynamic range of the sensor.

7.4.3 "Warning: Wait for steady state"

The reason for this can be:

1. The sensor is not equilibrated yet. Wait for another 10 minutes.
2. The temperature of the sample is changing.

7.4.4 "Warning: Bad temperature measurement"

The reason for this can be:

1. No temperature sensor is connected
2. In case of optical temperature spots: The fiber is not located at the sensor spot anymore.

7.5 Advanced Calibration of Sensor Spots

If sensor calibration is not possible due to a too large vessel for the pH buffer to reach the spot, it is possible to use a smaller vessel with a second spot from the same product batch for calibration. Use the identical optical fiber and the same optical channel from the ***FireSting pro*** as your measurement will be performed. It is possible that the accuracy of the measurement will be influenced due to this calibration method.

7.6 Available sensors and read-out devices

Sensor Type	Compatible read-out devices	
	FireSting pro	*OEM-Module PICO-PH*
PHROB-…	✓	
PHSP5-…	✓	✓
PHFTCR-…	✓	
PHTOVIAL20-…	✓	✓
PHTVIAL20-…	✓	✓
PHVIAL20-…	✓	✓
PHVIAL4-…	✓	✓

7.7 Pt100 Temperature Sensor Calibration

For precise absolute temperature readings, an optional **1-point calibration of the external temperature sensor** is recommended.

For this, check the reading of the external temperature Pt100 probe periodically in stirred water/water bath/incubator of known temperature at steady state. It is also possible to prepare a water-ice-mixture giving 0°C, where at least 50 mm of the Pt100 temperature probe tip is submerged.

In the ***Pyro Workbench*** software please click on the calibrate button "Cal." to perform the Pt100 calibration.

IMPORTANT: After calibration of the Pt100, a new optical sensor calibration must be performed.

8 Warnings and Safety Guidelines

Before using *PyroScience* pH sensors, carefully read the instructions and user manuals for the respective *FireSting pro* read-out device. The manuals are available for download from our homepage www.pyroscience.com.

Prevent mechanical stress (e.g. scratching) to the sensing surface at the tip of the pH sensor. Avoid strong bending of the fiber-optic cables as they might break.

Ensure that the complete sensing surface at the tip is always covered by the sample and is free of air bubbles, and that the liquid sample is stirred.

Calibration and application of the pH sensors is on the user's authority, as well as data acquisition, treatment and publication.

***PyroScience* pH sensors and read-out devices are not intended for medical or military purposes or any other safety-critical applications. They must not be used for applications in humans; not for in vivo examination on humans, not for human-diagnostic or therapeutic purposes. The sensors must not be brought in direct contact with foods intended for consumption by humans.**

The sensors must be used in the laboratory by qualified personnel only, following the user instructions and the safety guidelines of the manual, as well as the appropriate laws and guidelines for safety in the laboratory. Safety data sheet for the buffer capsules (PHCAL4 and PHCAL10) are available on the *PyroScience* homepage.

Keep the *PyroScience* pH sensors and read-out devices out of reach of children. Store the pH sensors in a secure, dry and dark place at room temperature.