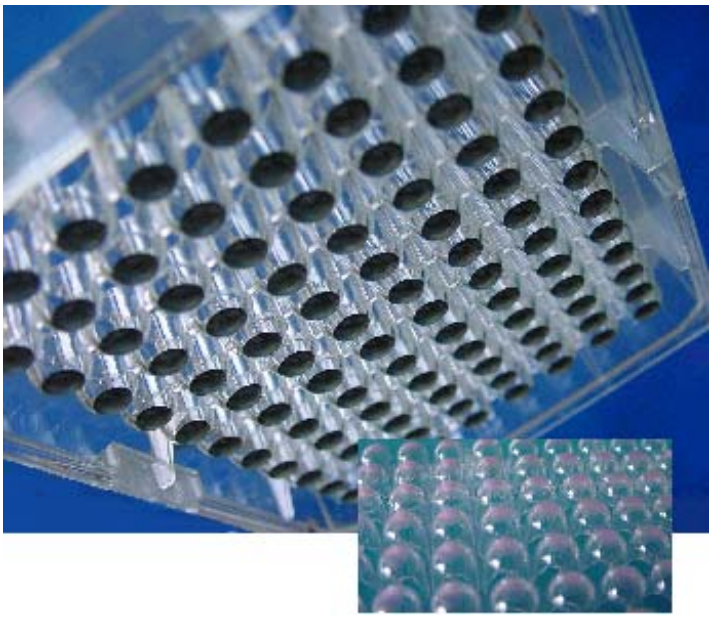
PreSens

PreSens Precision Sensing

Manual

# HydroPlate® HP96U

# HydroPlate® HP96C



Aug/17/2004

Manual HydroPlate

# TABLE OF CONTENTS

# 1 PREFACE

This manual will explain the use of the following types of HydroPlates®:

1. HydroPlate® HP96U
2. HydroPlate® HP96C

For most standard applications HydroPlate® HP96U is the best choice.

The HydroPlates® HP96U are round bottom microplates whereas HydroPlate® HP96C is a flat bottom microplate.

# 2 MEASURING PRINCIPLE OF THE HYDROPLATE®

## 2.1 Design of the HydroPlate®

A HydroPlate® is a sterile microplate in the common 96 well format. An optical pH sensor is fixed to the bottom of each well (figure 1), which can be read out through the bottom of the plate. The measuring can be made with most commercially available fluorescence readers.

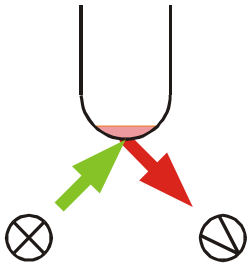


*Figure 1* *Measuring principle of a HydroPlate®*
*On the bottom of each well is a sensor which can be read out through the bottom with a commercially available fluorescence reader*

## 2.2 Design of the sensor

The sensor contains two different dyes. Both of them are covalently connected with a polymeric. One dye is the pH indicator, its fluorescence $I_{indicator}$ depends on the pH of the sample. The other dye serves as reference, its fluorescence $I_{reference}$ is independent of the pH. The ratio $I_R$ is calculated from these two fluorescences.

$$I_R = \frac{I_{indicator}}{I_{reference}} \qquad \textit{equation 1}$$

The signal $I_R$ corresponds to the pH.

The reader calibration (chapter 4, R*eader calibration,* page 6) determines four reader-constants by measuring the fluorescence of six different pH buffers. Subsequently used HydroPlates® are **calibration-free** on this reader.

A re-calibration of the reader can become necessary from time to time (see chapter 4, *Reader Calibration*, page 6).

# 3 INSTRUMENTATION

## 3.1 Fluorescence Reader

The HydroPlate® can be used with any commercially available fluorescence reader (fluorescence intensity reader) that can read out through the bottom. As the HydroPlate® contains two spectrally different dyes, the reader should be capable of measuring in the dual kinetic mode *(e.g. Labsystems Fluoroskan Ascent, BMG Fluostar, Wallac Victor 2, Tecan Genios*[1] and others*)*. If you have any questions regarding your reader, please contact our PreSens service team (info@presens.de).

## 3.2 Filter Pairs

Two filter pairs (4 filters) are required to measure the pH in the HydroPlate®.

| Filter combination | Measured fluorescence |
|---|---|
| 485 / 538 nm | $I_{indicator}$ |
| 485 / 620 nm | $I_{reference}$ |

If you have any questions regarding the choice of filters, please contact our PreSens service team (info@presens.de).

[1] with Xfluor V. 4.30 or higher

# 4 READER CALIBRATION

## 4.1 Principle of calibration

The ratio signal $I_R$ is correlated to the pH in the well. As each fluorescence reader has a slightly different performance, its characteristic has to be determined by a six-point calibration.

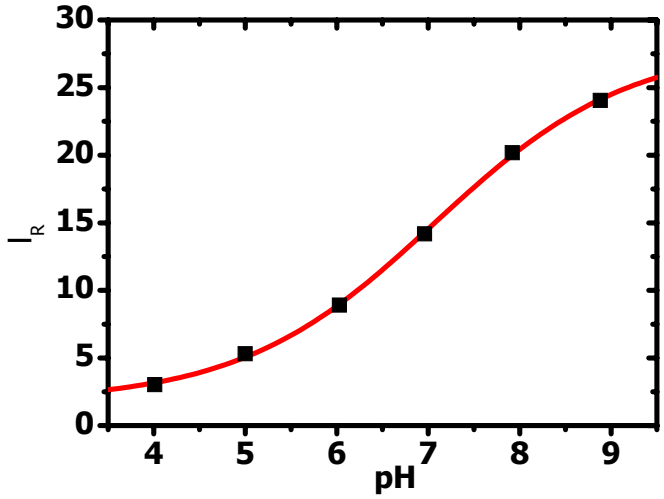



*Figure 2* *Calibration curve of HydroPlate HP96U. The dynamic range of the sensor is from pH 4.5 to 8.5 The referenced signal coresponds to the pH.*

The signal $I_R$ for the six buffer solutions has to be measured with the fluorescence reader. By using the PreSens pH-Calibration Tool (see chapter 5.3, *Calculation of the four reader constants*, page 9), the four reader constants $I_{min}$, $I_{max}$, dpH and $pH_0$ are calculated. This calibration having been made once, using twelve wells of one HydroPlate®, subsequent HydroPlates® can be measured (on the same fluorescence reader) without re-calibration.

The temperature for calibration and measuring has to be the same.

While HydroPlate® shows constant behavior from plate to plate, a re-calibration of the reader can be required from time to time due to drift phenomena in their optics. We recommend a weekly re-calibration.

## 4.2 Reader calibration procedure

1. Switch on your fluorescence reader (see instructions of your fluorescence reader).

2. Take six buffer solutions pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 (e.g. VWR No. 109435, 109436, 109437, 109439, 109460, 109461). Other buffers can also be used. However, those should evenly cover the range pH 4.0 to pH 9.0.

   Example: Buffers pH 4.0, 4.2, 4.5, 5.5, 8.0, 9.0 would produce bad results.

   **Note:** The HydroPlate's signal is depending on the ionic strength ***I***. In order to measure the absolute pH-value, the calibration buffers must have identical ionic strength. The above mentioned commercially available buffers work for ***I*** in the physiological range.
   For samples with deviating ***I,*** or if an exact pH has to be determined, self prepared buffers can lead to better results.
   Under http://www.bi.umist.ac.uk/users/mjfrbn/buffers/makebuf.asp you will find buffer recipes. For further information, please contact our service team at *info@presens.de*.

3. Fill 200 µl of the buffer into the wells of a Hydroplate® following scheme 1 below.

   *scheme 1: Possible Pipettier scheme for reader calibration*

| | 1 | 2 |
|---|---|---|
| A | pH 4.0 | pH 8.0 |
| B | pH 4.0 | pH 8.0 |
| C | pH 5.0 | pH 9.0 |
| D | pH 5.0 | pH 9.0 |
| E | pH 6.0 | |
| F | pH 6.0 | |
| G | pH 7.0 | |
| H | pH 7.0 | |

4. Close the HydroPlate® with its cover.

5. Place the HydroPlate® inside the reader (or any other tempered containment) at the desired temperature for at least 30 minutes to ensure constant temperature throughout the whole plate.
6. Measure the filled wells once with the recommended filter pairs (see chapter 3.2., *Filter Pairs*, page 5).

# 5 CALCULATION OF THE FOUR READER CONSTANTS WITH THE PRESENS PHCALIBRATION TOOL

The delivered PreSens pH calibration Tool **(pHSolver-v08.exe)** automatically calculates the calibration constants. It calculates a Boltzman curve fit for the measured values.

Note: It is also possible to use other mathematical software for this purpose.

## 5.1 Installing the PreSens pHCalibration Tool

1. Ensure that decimal separation is set to “dot” and not to “comma” (US standard)
2. Copy the files
   a. Libraryfiles.exe and
   b. pHSolver-v08.exe to a folder named pH-solver
3. Start Libraryfiles.exe by double-click and follow the instructions on the screen. If the software asks you if an existing file should be overwritten click on *NO*, please.

## 5.2 Calculation of calibration constants

1. Start the software pHSolver-v08.exe by double-click.
2. There are three (3) possibilities to enter the measured calibration $I_R$ data
   a. Type in the measured $I_R$-values at the used pH concentration. The format is: First pH value of the buffer, then space, then $I_R$, enter, then next pair. Use all 6 measured buffers!

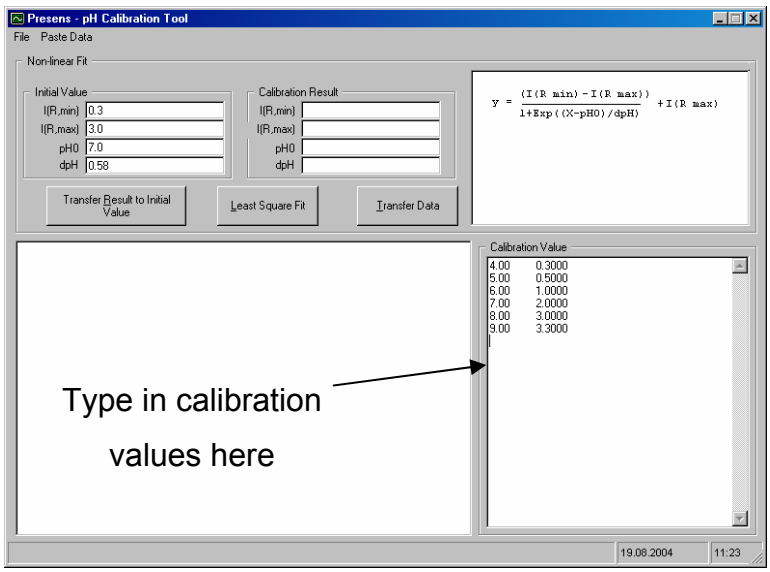



*Figure 3 Type in the measured values in the hown shown field*

b. Use the paste data function to transfer the data from an MS Excel™ sheet

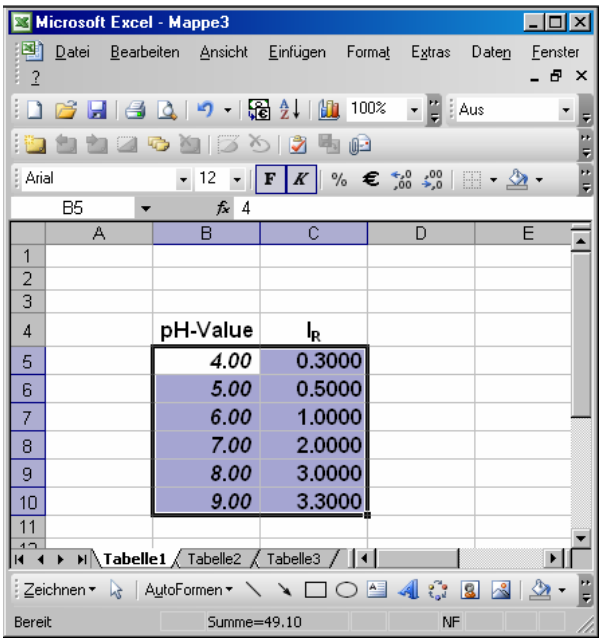



*Figure 4 You can copy the calibration values from an MS Excel™ sheet*

c. Open a data file containing the data in the format shown below. The values are separated by TAB

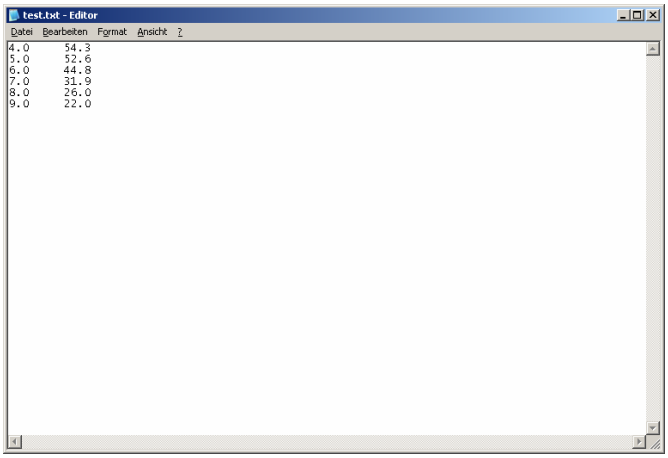



*Figure 5 The data can also be loaded in the format shown in this figure*

3. Click on "*Least Square Fit*". You will get a set of calibration data in the calibration result area. You can store these data by pressing "*Transfer data*".
4. Use the calibration data to calculate pH-values form the measured $I_R$-values.

# 6 pH MEASUREMENT

***Performing the measurement***

Perform the measurement according to the instructions of your reader. Remember to measure both filter pairs.

***Evaluation of the measurement***

1. Calculate for each measuring point the signal $I_R$ by using equation 1.
2. Calculate pH according equation 2
   (this can be done with e. g. MS Excel™ )

$$pH = \ln\left(\frac{I_{\min} - I_{\max}}{I_R - I_{\max}} - 1\right) \cdot dpH + pH_0 \qquad \textit{equation 2}$$

# 7 TECHNICAL DATA

## 7.1 Performance Specification

| | | |
|---|---|---|
| **Measuring range** | pH 5.0 - pH 8.0 | |
| **Resolution** (at 37 °C)* | up to 0.01 pH* | |
| **Accuracy** (at 37°C)* | up to 0.05 pH* | |
| **Temperature range** | 15 - 45 °C | |
| **pH drift per hour*** | < 0.02 pH/h | |
| **Types of plates** | HydroPlate® HP96U | HydroPlate® HP96C |
| **Microplate** | Greiner, 96 well,<br>Round bottom<br>Microlon® 600 | Greiner, 96 well<br>Flat bottom<br>Microlon® 600 |
| **Response time ($t_{90}$)** | 30 sec. | 30 sec. |
| **Indicator filter** | 485 / 538 nm | 485 / 538 nm |
| **Reference filter** | 485 / 620 nm | 485 / 620 nm |
| **Special features** | Round bottom plate | Flat bottom plate |

* exact data dependent of used reader, provided constant salinity

## 7.2 Cross Sensitivity

### 7.2.1 Temperature

The influence of the temperature on the calibration curve is shown below. We recommend to calibrate and to measure at the same temperature.

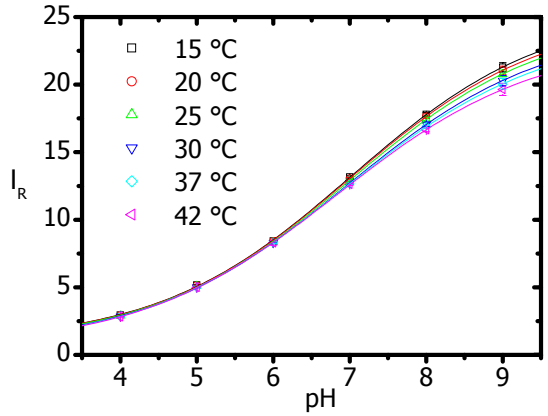



*Figure 56 Calibration curve of HydroPlate at different temperatures.*

### 7.2.2 Ionic strength

While pH electrodes are influenced by sulfide, electromagnetic fields or flow velocity, the optical pH measuring is interfered by changes in the ionic strength.
HydroPlate sensors display a cross sensitivity to ionic strength as depicted below. In physiological solutions, changes in the ionic strength during the measuring have only a very slight influence on the results.

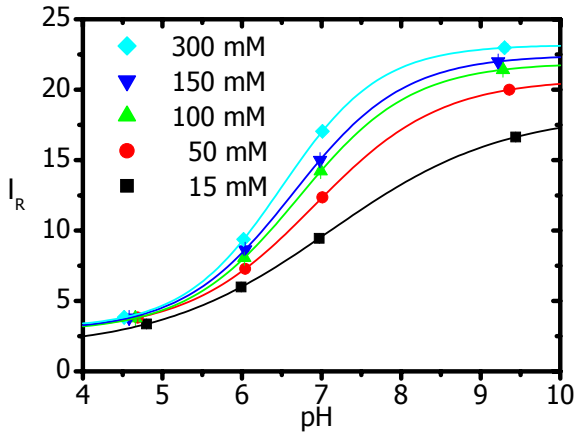



*Figure 7 Calibration curve of HydroPlate HP96 at different ionic strength*

## 7.3 Storage of the HydroPlate®

HydroPlates® should be kept dark and cool (under 25°C). To obtain best results open the package just before use.

# 8 CONTACT ADDRESS

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