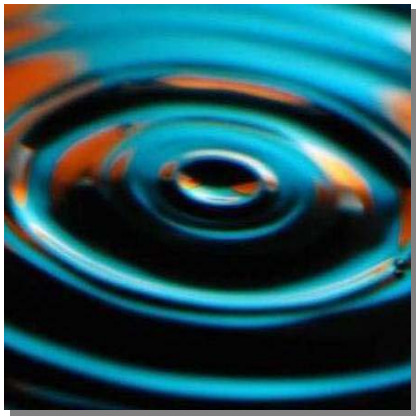
pH Monitoring



The monitoring of pH in microplates can be used for a variety of applications amongst others:

- Determination of enzyme activity (e.g. hydrolases)
- Monitoring of Cell Proliferation
- Starter Cultures

HydroPlate® can monitor enzymatically catalyzed reactions if a pH change is involved, for example during hydrolysis of an ester. From the pH-change the enzymatic activity can be calculated. Also different cell lines or dairy starter cultures can be compared using the HydroPlate®. Monitoring of cell growth can be done by detecting the pH-change as one of the metabolic key parameters.

For pH monitoring in microplates the **HydroPlate® (PreSens)** can be used and measured with the BERTHOLD TECHNOLOGIES multimode reader Mithras LB 940.

The sensors are thin, hydrophilic, and almost calibration-free: Only 16 wells of the first plate have to be calibrated by an easy six-point calibration. All other sensors of the same batch have the same calibration values. Thus, later comparison of 96 different compounds without losing wells by using standards in every plate can be done. 12 wells of one HydroPlate® per batch are needed for this calibration procedure. From time to time, re-calibration of the reader may be necessary.

The individual pH-sensor spots are located at the bottom of the wells for measurement of pH in the range between pH 5.0 to 8.0.

Instrument settings

As HydroPlate® contains two different dyes, the BERTHOLD TECHNOLOGIES microplate readers have to be measured in **dual kinetic mode** using two different filter pairs:

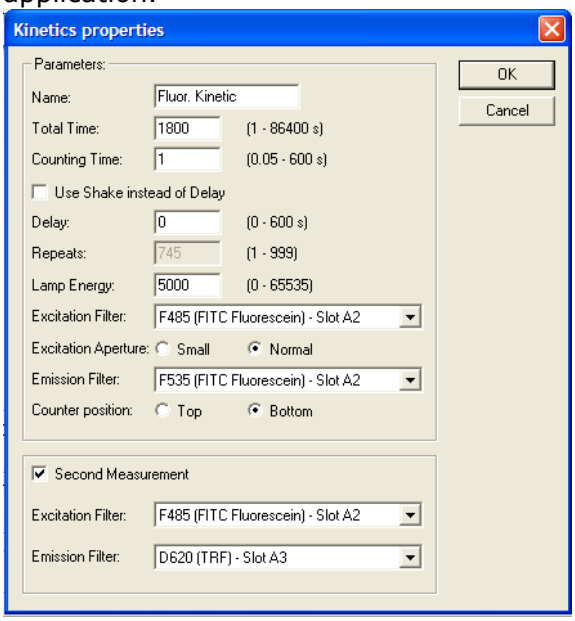
Filter pair 1 = Intensity indicator: Excitation 485 nm/Emission 535 nm
Filter pair 2 = Intensity reference: Excitation 485 nm/Emission 620 nm

For a better well to well reproducibility intensity of indicator and intensity of reference are measured and the ratio is calculated.

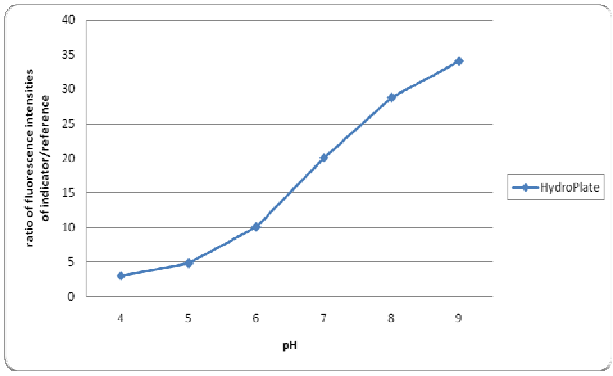
Filter order numbers:

Excitation 485nm:	40271	(Fluorescein Standard Filter in Mithras LB 940)
Emission 535nm:	40273	(Fluorescein Standard Filter in Mithras LB 940)
Emission 620nm:	47731	(TRF Standard Filter in Mithras LB940 TRF models)

Picture 1 shows the fluorescence kinetic in bottom reading with dual filter measurement set in MikroWin software as it can be done with the Mithras LB 940 multimode reader. The total reading time can be selected accordingly to the requirements of the performed application.



Picture 1: Dual kinetic mode settings in Mithras LB 940 with MikroWin software



Picture 2: Change of fluorescence intensity ratio signal with different pH values (pH 4 – pH 9), HydroPlate® measured with BERTHOLD Mithras LB 940 in top reading mode

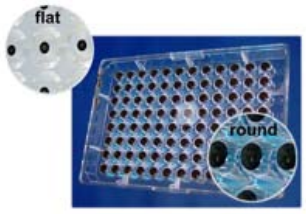
The change of fluorescence signal was measured with HydroPLate® in the Mithras LB 940. Fluorescence bottom reading mode is required as coloured, fluorescent or turbid ingredients of the sample will interfere with measurement from the top side. For transparent samples top reading mode can be selected.

Following settings in Mithras LB 940 were used:

Lamp energy: 5000 Measurement time: 1 second Counter position: top
Excitation: 485nm Emission: 535nm and 620nm

Picture 2 shows the change fluorescence intensity ratios between samples with pH 4, pH 5, pH 6, pH 7, pH 8 and pH 9. The wells were filled with 200 µL of respective pH standard buffer solutions. The reference signal Ir is correlated to the pH of the sample.
Imin, Imax, dpH and pH0 of the calibration curve can be calculated from the fluorescence raw data with programs with curve fitting capability (Berthold MikroWin Advanced II version) or the PreSens pH Calibration Tool.

Microplates from PreSens - Precision Sensing GmbH



HydroPlate® with 96-well round bottom microplate: HP96U

HydroPlate® with 96-well flat bottom microplate: HP96C

more information: www.presens.de

BERTHOLD TECHNOLOGIES' instruments:

Mithras LB 940 Multimode Reader