

Spexyte™ Micro pH Probe

Optimized for Nanodrop

Ordering Information	Storage Conditions	Instrument Platform
Cat#: 21230	<-15°C, avoid light	Nanodrop

Introduction

Biological samples are usually in a very narrow pH range from 4.5 to 8.5, and many of them need to be provided in micro-volume (e.g., microliter). It is difficult to measure the pH values with small volumes accurately using the conventional pH meters due to the large electrode sizes. Spexyte™ Micro pH Probe is optimized to measure μL samples in the range of pH 4.5 ~8.5 using a Naodrop or an equivalent device. The pH value is measured by reading the ratio of BCFL absorbances at 502 nm to 470 nm since the relationship between the pH value and the absorbance ratio (502/470nm) fits the Four-parameter logistic model well. The pH of a microvolume biological solution could be measured quickly and accurately in seconds with Spexyte™ Micro pH Probe. It provides a sensitive, easy and fast method to measure the pH values for micro-volume samples.

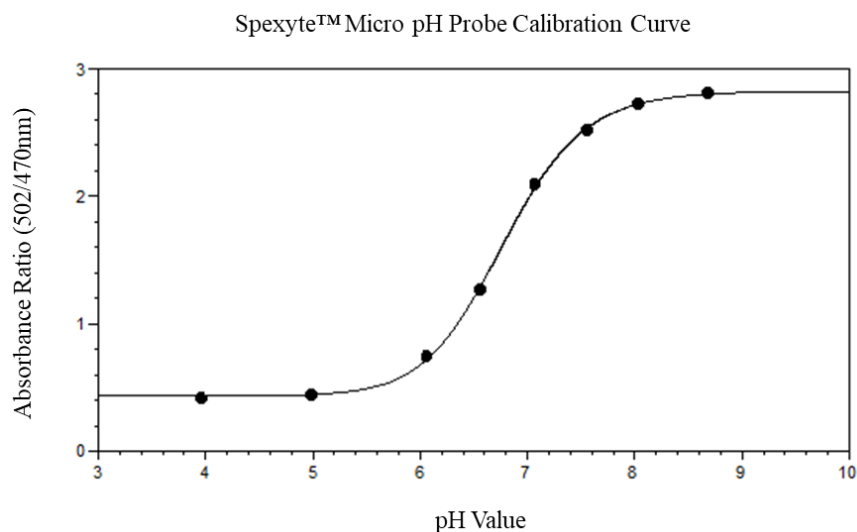
Experiment Protocol:

1. Mix equal volume of sample with Spexyte™ Micro pH Probe solution (for example: 3 μL sample with 3 μL Micro pH probe).
2. Add 2~3 μL to Nanodrop to read the OD values at 470 nm and 502 nm.
3. Calculate the ratio of OD (502 nm)/ OD (470nm).
4. Determine the pH from the following calibration table or read it from our website (<https://www.aatbio.com/products/spexyte-micro-ph-probe-optimized-for-nanodrop>).

Note: This product is designed to measure the samples having pH from 4.5~8.5. Contact us if your samples have pH out of this range.

pH Calibration Curve and Table:

Figure 1. pH Calibration Curve at room temperature:



Note: The pH value and the absorbance ratio (502/470 nm) fits the four-parameter logistic model and the fitting equation is $y = 0.426 + \frac{(2.828-0.426)}{1+(\frac{x}{6.781})^{-17.926}}$ ($y =$ absorbance ratio of 502/470 nm, $x =$ pH value)

Table 1. pH Calibration Table at room temperature:

pH	Ratio	pH	Ratio	pH	Ratio	pH	Ratio
4.5	0.427	5.5	0.481	6.5	1.192	7.5	2.489
4.6	0.428	5.6	0.501	6.6	1.341	7.6	2.553
4.7	0.429	5.7	0.528	6.7	1.498	7.7	2.605
4.8	0.431	5.8	0.564	6.8	1.657	7.8	2.647
4.9	0.433	5.9	0.609	6.9	1.813	7.9	2.682
5.0	0.436	6.0	0.667	7.0	1.960	8.0	2.710
5.1	0.440	6.1	0.739	7.1	2.096	8.1	2.733
5.2	0.446	6.2	0.828	7.2	2.217	8.2	2.751
5.3	0.454	6.3	0.933	7.3	2.322	8.3	2.766
5.4	0.466	6.4	1.055	7.4	2.413	8.4	2.777

Example Data Analysis And Figure:

Wavelength(nm)	Absorbance	Ratio	pH
470nm	1.428	2.744/1.428=1.922	7.0 (from table provided)
502nm	2.744		

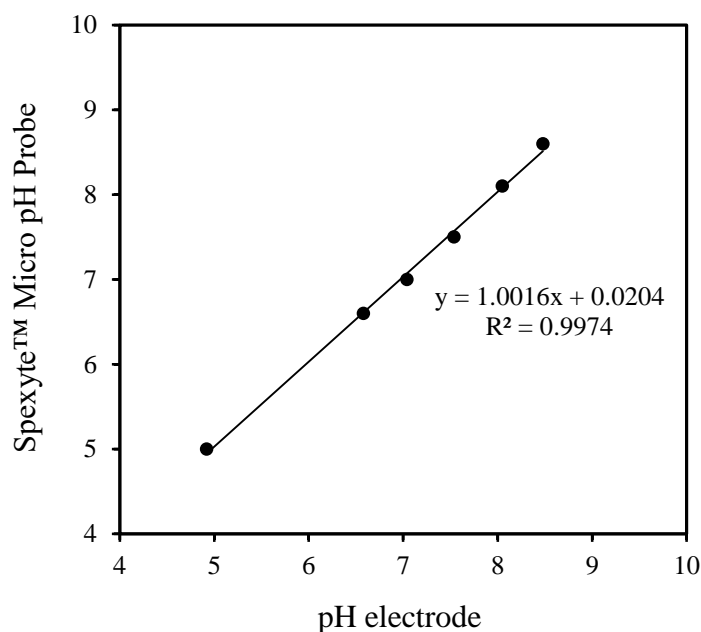


Figure 2. The relationship between the pH values measured by the electrode method and the AAT Spexyte™ Micro pH probe. 50mM Sodium Phosphate buffers with different pH values ranging from 4.5~8.5 were prepared. A glass membrane combination pH electrode using Ag/AgCl as a reference (Sartorius AG, Germany) was used to measure the pH values of each sample. Simultaneously, 3 μ L of Micro pH probe was mixed with 3 μ L of each buffer and then the OD values at 470 nm and 502 nm of the mixed solution were read by the Nanodrop. The pH values were determined by the calibration table (Table 1) based on the ratio of OD (502 nm)/ OD (470 nm).