RatioWorks™ PH165, AM

Catalog number: 21212
Unit size: 10x50 ug

Component	Storage	Amount
RatioWorks™ PH165, AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

RatioWorks™ PH165 AM is a novel fluorescent probe that can be used to measure intracellular pH in live cells. It shows pH-dependent fluorescence spectra. Its dual excitation and dual emission make it a unique ratiometric pH probe for analyzing live cells. At low pH, RatioWorks™ PH165 is weakly fluorescent in far red spectrum region and highly fluorescent in red spectrum region. As pH increases, it shows higher fluorescence in far red spectrum and lower fluorescence in red spectrum. This probe can quantify cytosolic pH in the range from 4 to 9 with dual excitation/emission at 497/594 nm and 578/654 nm respectively. The pKa of PH165 is ~7.1. The red fluorescence spectrum of RatioWorks™ PH165 makes it suitable for multiplexing tests with green fluorophores such as GFP. RatioWorks™ PH165 AM is cell-permeable, and compatible with various platforms such as fluorescence microscopy and microplate reader.

AT A GLANCE

1. Prepare cells in growth medium
2. Stain cells with RatioWorks™ PH165, AM for 30 to 90 minutes at 37 °C
3. Wash and replace with HHBS
4. Analyze under fluorescence microscope with Cy3/TRITC and Cy5 filter set

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3-Cy5 filter set
Emission	Cy3-Cy5 filter set
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy3-Cy5 filter set

Fluorescence microplate reader

Excitation	497-578 nm
Emission	594-654 nm
Cutoff	570-630 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

RatioWorks™ PH165, AM stock solution
Prepare a 1-5 mM stock solution of RatioWorks™ PH165, AM in high-quality anhydrous DMSO.

Note Unused RatioWorks™ PH165, AM stock solution can be aliquoted and stored at ≤ -15 ˚ C with smaller aliquots. Protect from light and avoid repeated freeze-thaw cycles. The stock solution should be used promptly.

PREPARATION OF WORKING SOLUTION

RatioWorks™ PH165, AM working solution
Prepare a 20-50 µM RatioWorks™ PH165, AM working solution in Hanks and 20 mM Hepes buffer (HHBS).

Note The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells in growth medium and treat cells as desired.
2. Remove cell culture medium and wash twice.
3. Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of RatioWorks™ PH165, AM working solution in the cell plate.

   Note The optimal concentration of the cell membrane probe varies depending on the specific application.
4. Incubate the cells at 37 °C for 30-90 minutes, protected from light.
5. Remove working solution in each well. Wash cells and replace with HHBS buffer.
6. Observe the fluorescence signal in cells using fluorescence microscope with a Cy3/TRITC filter set for the acidic pH and Cy5 filter for the basic pH. Alternatively, For the ratiometric evaluation, measure signal intensity at Ex/Em= 497/594 nm (Cutoff=570 nm) and Ex/Em= 578/654 nm (Cutoff=630 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

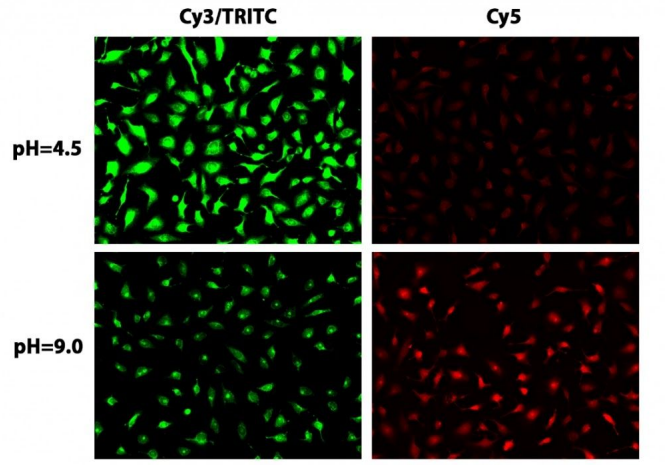

Figure 1. Ratiometric analysis of HeLa cells using RatioWorks™ PH165, AM with fluorescence microscopy. HeLa cells were stained with RatioWorks™ PH165, AM for 90 minutes at 37°C and shifted to solutions with different pH. Images were acquired using Cy3/TRITC (green, pseudo color) and Cy5 filter sets (red color).

DISCLAIMER

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