RatioWorks™ PDMPO Dextran

Catalog number: 21211
Unit size: 1 mg

Component	Storage	Amount
RatioWorks™ PDMPO Dextran	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

The existing pH probes are ill-adapted to study acidic organelles such as lysosomes, endosomes, phagosomes, spermatozoa and acrosomes because their fluorescence is significantly reduced at lower pH. The growing potential of ratio imaging is significantly limited by the lack of appropriate fluorescent probes for acidic organelles although ratio imaging has received intensive attention in the past few decades. RatioWorks™ PDMPO is characterized as acidotropic dual-excitation and dual-emission pH probe. It emits intense yellow fluorescence at lower pH and gives intense blue fluorescence at higher pH. This unique pH-dependent fluorescence makes RatioWorks™ PDMPO an ideal pH probe for acidic organelles with pKa = 4.47. Additionally, the very large Stokes shift and excellent photostability of RatioWorks™ PDMPO make it an excellent fluorescent acidotropic reagent for fluorescence imaing and flow cytometry applications. The unique fluorescence properties of RatioWorks™ PDMPO might give researchers a new tool with which to study endocytosis, phagocytosis and acidic organelles of live cells. RatioWorks™ PDMPO can be well excited by the violet laser at 405 nm for flow cytometric applications. This RatioWorks™ PDMPO SE can be readily used to make a variety of bioconjugates for imaging or flow applications, enabling the specific detection of phagocytosis and endocytosis with reduced signal variability and improved accuracy. These conjugates can be also used for multiplexing cell functional analysis with green dyes such as GFP, Fluo-8, calcein, or FITC-labeled antibodies. The short emission band of RatioWorks™ PDMPO is ~450 nm while the longer emission is ~550 nm, making the common filter sets of Pacific Blue and Pacific Orange readily available to the assays of RatioWorks™ PDMPO.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Replace the medium with RatioWorks™ PDMPO Dextran loading solution (100 µL/well for 96-well plate)
3. Incubate at 37 °C for 5-20 minutes
4. Wash and replace with HHBS
5. Read Fluorescence at Ex/Em= 360/540 and 360/450 nm

KEY PARAMETERS

Fluorescence microplate reader

Excitation	360 nm
Emission	450, 540 nm
Cutoff	420, 475 nm
Recommended plate	Black wall/Clear bottom
Instrument specification(s)	Bottom read mode

CELL PREPARATION

For example, plate adherent cells overnight in growth medium at 40,000 to 80,000 cells/well/100 µL for 96-well or 10,000 to 20,000 cells/well/25 µL for 384-well plates.

Note Each cell line should be evaluated on an individual basis to determine the optimal cell density.

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™ PDMPO Dextran stock solution
Prepare a 1 mg/mL stock solution of RatioWorks™ PDMPO Dextran in 1 mL of sterile water or Hanks and 20 mM Hepes buffer (HHBS).

Note The stock solution should be used promptly. Any unused solution need to be aliquoted and refrozen at < -20 °C.

Note Avoid repeated freeze-thaw cycles, and protect from light.

Note Some cells might need to make 50 mg/mL stock solution for further dilution.

PREPARATION OF WORKING SOLUTION

RatioWorks™ PDMPO Dextran working solution
Prepare a 20-100 µg/mL RatioWorks™ PDMPO Dextran loading solution in HHBS.

Note Some cells might need to have 1-5 mg/mL working solution for the experiment.

SAMPLE EXPERIMENTAL PROTOCOL

Endocytosis assay
The following is the recommended protocol for standard cell load. The protocol only provides a guideline, should be modified according to the specific needs.

1. Remove the medium, and add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) RatioWorks™ PDMPO Dextran loading solution into the cell plate.

   Note It is important to replace the growth medium with HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384- well plate before dye-loading) if your compounds interfere with the serum.

   Note Rapid trafficking of RatioWorks™ PDMPO dextran from early endosomes to late endosomes and subsequent fusion with lysosomes can occur. To aid the visualization of RatioWorks™ PDMPO dextran within the endosomes, we recommend increasing the labeling concentration and decreasing the loading time, and imaging immediately.

2. Incubate the dye-loading plate at cell incubator for 5 to 20 minutes.

   Note Some cells might require incubation time as long as 48 hours.

3. Wash and replace the dye-loading solution with HHBS or growth medium.

4. Run the endocytosis assay by monitoring the fluorescence at Ex = 360 nm, and Em = 450 and 540 nm for ratio measurements.

   Note The fluorescence signal from RatioWorks™ PDMPO dextran is stable for at least one hour after trafficking to lysosomes has occurred. Because lysosomes have a lower pH compared to endosomes, the signal from RatioWorks™ PDMPO dextran within the

lysosomes is brighter than the signal from RatioWorks™ PDMPO dextran within the endosomes. The lysosomal RatioWorks™ PDMPO dextran concentration is directly dependent on endocytotic uptake; therefore, the modulation of endocytosis can be inferred from the intensity of RatioWorks™ PDMPO dextran signal from the lysosomes.

EXAMPLE DATA ANALYSIS AND FIGURES

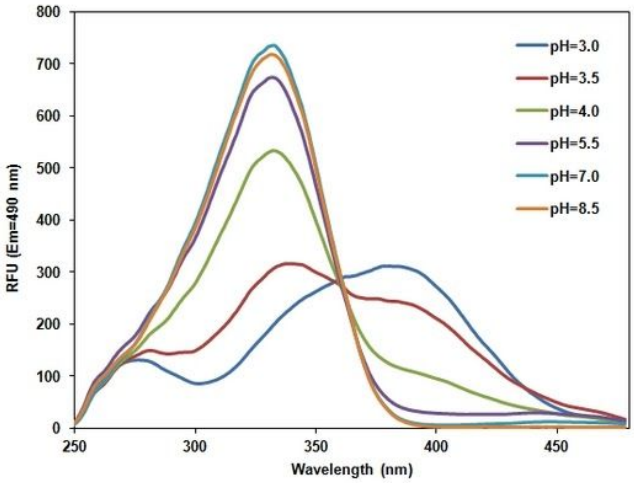

Figure 1. pH dependent Excitation spectra of PDMPO.

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