Protonex™ Red 600-Latex Bead Conjugate

Catalog number: 21209
Unit size: 1 mL

Component	Storage	Amount (Cat No. 21209)
Protonex™ Red 600-Latex Bead Conjugate	Refrigerated (2-8 °C), Minimize light exposure	1 vial (1 mL)

OVERVIEW

Protonex™ Red-latex bead conjugate demonstrated pH-dependent fluorescence. Unlike most of the existing fluorescent dyes that are more fluorescent at higher pH, acidic conditions enhance the fluorescence of Protonex™ Red-latex bead conjugate. The fluorescence of Protonex™ Red-latex bead conjugate dramatically increases as pH decreases from neutral to the acidic, making it a robust tool to study phagocytosis and its regulation by drugs and/or environmental factors. The lack of fluorescence outside the cell eliminates the wash steps. Protonex™ Red-latex bead conjugate provides a powerful tool to study phagocytosis. Protonex™ Red-latex bead conjugate is low fluorescent outside the cells, but fluoresce brightly red in acidic compartments (such as phagosomes, lysosomes and endosomes). This Protonex™ Red-latex bead conjugate can be also used for multiplexing cell functional analysis with green dyes such as GFP, Fluo-8, calcein, or FITC-labeled antibodies. Protonex™ Red has the spectral properties similar to those of Texas Red, making the common filter set of Texas Red readily available to the assays of Protonex™ Red.

AT A GLANCE

Chemical and Physical Properties

Solvent:	Water
Solids Content:	1% in PBS
Number of Microspheres per mL:	~4e+10
Ex/Em:	575/597 nm
Mean Diameter:	0.72 µm

KEY PARAMETERS

Fluorescence microscope

Emission	Texas Red filter set
Excitation	Texas Red filter set
Recommended plate	Black wall/clear bottom

SAMPLE EXPERIMENTAL PROTOCOL

Important

The following is a recommended protocol for granulocytes. This protocol only provides a guideline and should be modified according to your specific experimental conditions.

Protocol

1. Prepare cells as desired. For example, prepare the granulocytes at 10^7 cells/mL with Hanks and 20 mM Hepes buffer (HHBS), and add 100 µL to a polypropylene tube.

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

2. Add 1-10 µL of the Protonex™ Red 600-Latex Bead Conjugate to the tube and incubate with gentle shaking for 30 minutes at 37˚C.

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

3. Prepare an identical sample that is incubated at 4˚C and label it as a control.

4. At the end of the 30-minute incubation, stop phagocytosis by adding 2mL of ice-cold HHBS and mix well.

5. Wash the cells 2 times with cold HBSS.

6. Resuspend the cells in 500 µL of cold HBSS, keep the samples at 4˚C, and analyze immediately using a fluorescence microscope equipped with a Texas Red® filter set.

   Note: For fluorescence microplate readers, monitor the fluorescence intensity at Ex/Em = 570/600 nm (Cutoff = 585 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

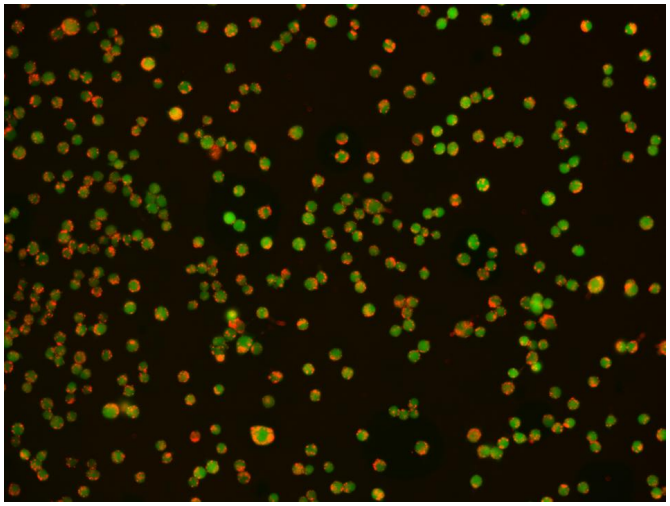

Figure 1. Phagocytosis was examined in RAW 264.7 cells by Protonex™ Red 600-Latex Bead Conjugate (Cat # 21209). The cells were incubated with Protonex™ 600 Latex Beads in a growth medium for 4 hours. CytoTrace™ Green CMFDA (Cat # 22017) was used to track live cells. The image (20X) was taken using Keyence Fluorescence Microscopy.

DISCLAIMER

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