Protonex™ Red 600-Latex Bead Conjugate

Ordering Information	*Storage Conditions*
Product Number: 21209 (1 mL)	Keep at 4 °C and avoid light

Introduction

Phagocytosis is one of the first lines of defense against invading microorganisms. It is also important for the processes of tissue remodeling and removal of senescent cells. While most cells have some capability for phagocytosis, i.e. the need to phagocytose apoptotic cells, the "professional" phagocytes are the phagocytic leukocytes (granulocytes, monocytes and macrophages). 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 non-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.

Chemical and Physical Properties

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

Sample Assay Protocol

Note: The following is the recommended protocol for granulocytes. The protocol only provides a guideline, should be modified according to the specific needs.

1. Prepare cells as desired. For example, prepare the granulocytes at 10^7 cells/mL Hanks and 20 mM Hepes buffer (HHBS), add 100μL to a polypropylene tube.
   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.
2. Add 1-10μL 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 as control.
4. At the end of the 30 minute incubation, stop the phagocytosis by adding 2mL of ice cold HHBS. Mix and then wash the cells 2 times with cold HBSS.
5. Resuspend the cells in 500μL of cold HBSS, keep the samples at 4˚C and analyze as soon as possible at Ex/Em 570/600 nM (Texas Red® filter set).

References

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2. Schreiner L, Huber-Lang M, Weiss ME, Hohmann H, Schmolz M, Schneider EM. (2011) Phagocytosis and digestion of pH-sensitive fluorescent dye (Eos-FP) transfected E. coli in whole blood assays from patients with severe sepsis and septic shock. J Cell Commun Signal, 5, 135.
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5. Steinberg BE, Grinstein S. (2009) Analysis of macrophage phagocytosis: quantitative assays of phagosome formation and maturation using high-throughput fluorescence microscopy. Methods Mol Biol, 531, 45.