Revised: September 1, 2015

Product Information

Light-On LysoView™ 555

Catalog Number: 70060-T, 70060

Unit Size:
70060-T: 10 uL
70060: 50 uL

Concentration: 1 mM in DMSO

Molecular Information:
MW: 470.64; see Figure 3 for structure.

Storage and Handling
Store at -20°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Spectral Properties
Abs/Em: 554/583 nm; see Fig. 1 for spectra.

Product Description

Light-On LysoView™ 555 is a red fluorogenic lysosome dye with pH-dependent fluorescence. Light-On LysoView™ dye is unique among commercially available lysosomotropic dyes in that its fluorescence in cells is activated by exposure to UV excitation. In solution, the dye shows pH-dependent fluorescence that does not require UV activation (Figure 1). In cells, the dye initially shows low fluorescence, but brief exposure to UV excitation from a mercury arc lamp through a DAPI filter activates bright red fluorescence localizing to lysosomes (Figure 2). We hypothesize that the dye assumes a non-fluorescent structure that can be switched to a fluorescent structure by UV excitation (Figure 3). Mercury arc lamp excitation of the dye using other filter sets (FITC, rhodamine) or dye excitation with a 405 nm laser does not activate fluorescence. Lysosomal fluorescence fades several minutes after UV exposure, but can be re-activated in the same cells multiple times by exposure to UV light (Figure 4). Therefore the dye provides a novel tool for UV-activated, reversible fluorescence imaging of lysosomes.

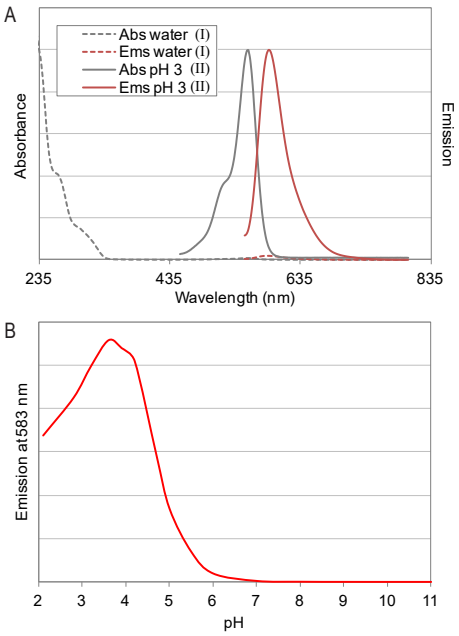

Figure 1. A. Normalized absorbance and emission of Light-On LysoView 555 in water (non-fluorescent form, structure I, dashed lines) or in pH 3 buffer (fluorescent form, structure II, solid lines). B. Emission of Light-On LysoView 555 at varying pH.

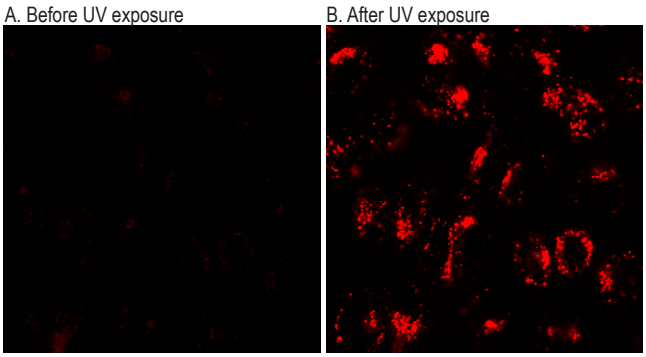

Figure 2. UV-activated lysosomal fluorescence with Light-On LysoView 555 dye. HeLa cells were stained with 1 uM Light-on LysoView 555 for 15 minutes at 37°C, then imaged using a Zeiss LSM 700 confocal microscope using a 40X objective and imaging settings for Cy®3. A. Before UV exposure, fluorescence was not detectable. B. After five seconds of exposure to UV light using an EXFO X-Cite® Series 120Q short arc lamp and DAPI filter set, bright red fluorescence localized to lysosomes was observed.

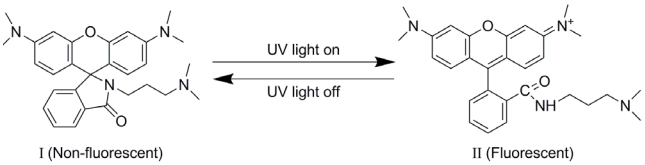

Figure 3. Proposed mechanism of UV activation of Light-on LysoView 555.

Staining Protocol

1. Dilute Light-on LysoView 555 in cell culture medium to a final concentration of 1 uM.

   Note: 1 uM is recommended as a initial concentration for testing. The final dye concentration that results in UV-activated staining may require optimization for different cell types. At higher dye concentrations, lysosomal staining may be observed without UV activation.

2. Incubate live cells with medium containing Light-on LysoView 555 for 15-30 minutes at 37°C.

   Note: Staining time can be varied depending on cell type and application. HeLa cells incubated with the dye show no obvious signs of toxicity after overnight incubation with 1 uM dye, but toxicity may vary by cell type.

3. Image cells using excitation/emission settings for visible red dyes (such as Cy®3). No wash step is required before imaging. To activate dye fluorescence, expose cells to mercury arc lamp excitation through a DAPI filter cube for five seconds or longer. Fluorescence fades in 1-5 minutes after UV exposure, but can be re-activated in the same cells multiple times with no appreciable reduction in signal (see Fig. 4).

   Note: Light-on LysoView 555 staining is retained after subsequent fixation with formaldehyde. Formaldehyde fixation results in activation of dye fluorescence in lysosomes without UV exposure, although UV exposure may further increase fluorescence in fixed cells. Staining is not compatible with detergent or solvent permeabilization.

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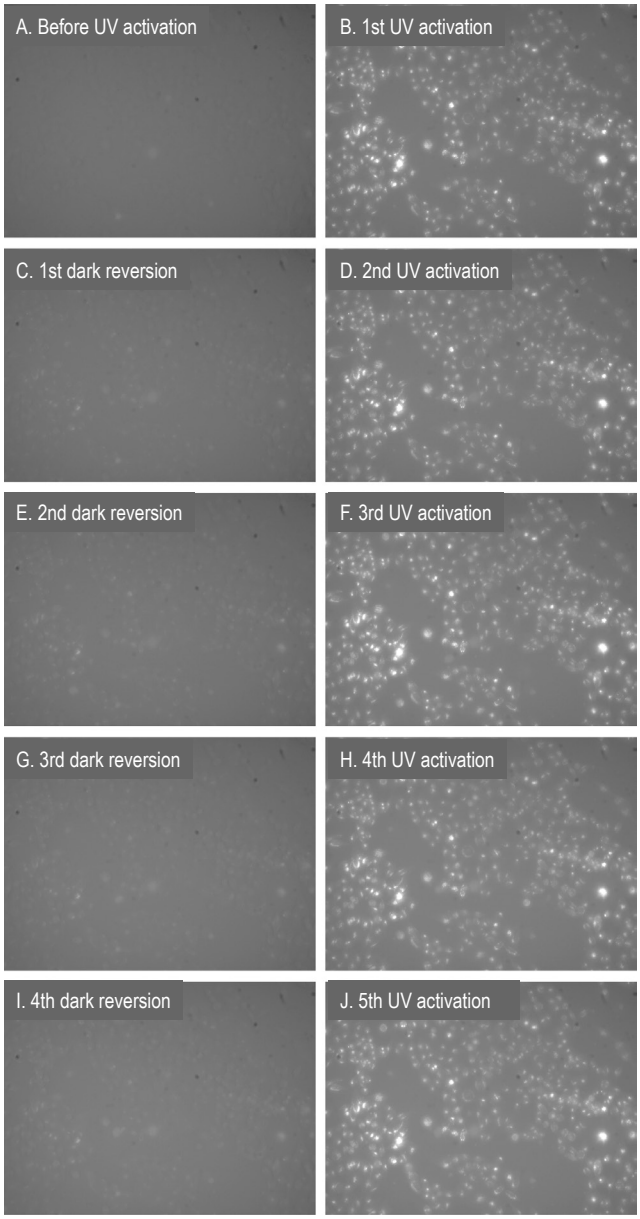

Figure 4. Repeated, reversible UV-activation of lysosomal fluorescence with Light-on LysoView 555 dye. HeLa cells were stained with 1 uM Light-On LysoView dye for 15 minutes at 37°C and imaged on an Olympus IX71 epifluorescence microscope using a Cy®3 filter cube. The same field of cells was imaged before exposure to UV excitation (A) and after a 5 second exposure to UV excitation with a mercury arc lamp through a DAPI filter cube to activate lysosomal fluorescence (B). Cells were allowed to rest in the dark for 2 minutes to allow the dye revert to a non-fluorescent state (C) before imaging of the same field before and after UV activation/dark reversion for five consecutive cycles (D-J). All images were captured with the same exposure settings.

Related Products

Catalog number	Product
70058	LysoView™633
70052	MitoView™ Blue
70054	MitoView™ Green
70055	MitoView™633
40060	RedDot™1 far-red nuclear stain, 200X in water
40061	RedDot™2 far-red nuclear stain, 200X in DMSO
30029	NucView™488 Caspase-3 Assay Kit for live cells

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF™dye conjugates of transferrin, cholera toxin subunit B, lectins, and Annexin V for cellular imaging, plus many more fluorescent probes and kits for cellular and molecular biology research.