

Data Sheet

BioTracker™ Green Endocytosis Live Cell Probe

Live Cell Probe

SCT271**Pack Size: 1 vial of 40 µL****Store at -20 °C**FOR RESEARCH USE ONLY**Not for use in diagnostic procedures. Not for human or animal consumption.**

Background

Endocytosis is process by which macromolecules are taken up through the cell membrane or a plasma membrane-derived vesicle called an endosome. The endocytic pathway contributes to the maintenance of intracellular homeostasis by bringing in various nutrients to the cell and transporting unwanted components to the lysosome, which acts as a waste disposal system. Significant research has revealed that disruption of endocytosis is related to certain neurodegenerative disorders and immune pathologies. Consequently, investigation of the endocytic pathway attracts considerable interest in the scientific community.

Green Endocytosis Live Cell Probe (SCT271) is a pH-dependent fluorescence dye that localizes to the vesicle membrane. The visualization of endocytosis using SCT271 is a more direct method than fluorescent analogs and allows visualization of endocytosis from the early endosome stage.

Source

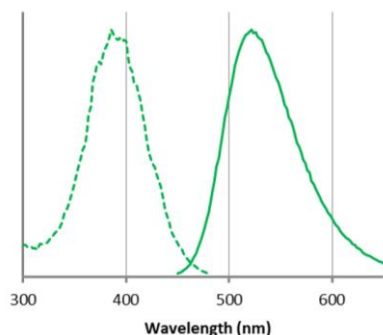
Green Endocytosis Live Cell Probe (SCT271) does not contain genetically modified organisms.

Spectral Properties

Green Endocytosis Live Cell Probe (SCT271)

Excitation peak: 386 nm

Emission peak: 522 nm



Storage and Handling

Store BioTracker™ Green Endocytosis Probe at $-20\text{ }^{\circ}\text{C}$, protected from light.

Presentation

Yellow-green liquid

Representative Data

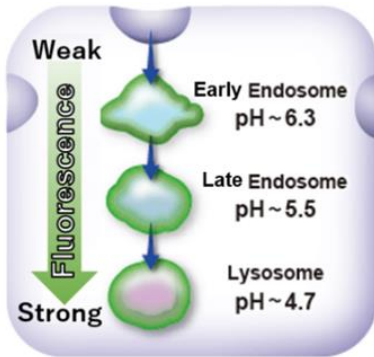


Figure 1. Fluorescence intensity of BioTracker™ Green Endocytosis Live Cell Probe (SCT271) by endocytosis stage. SCT271 is more sensitive to pH changes than fluorescently labeled dextrans. It is therefore appreciable for detection of early endosomes with high sensitivity.

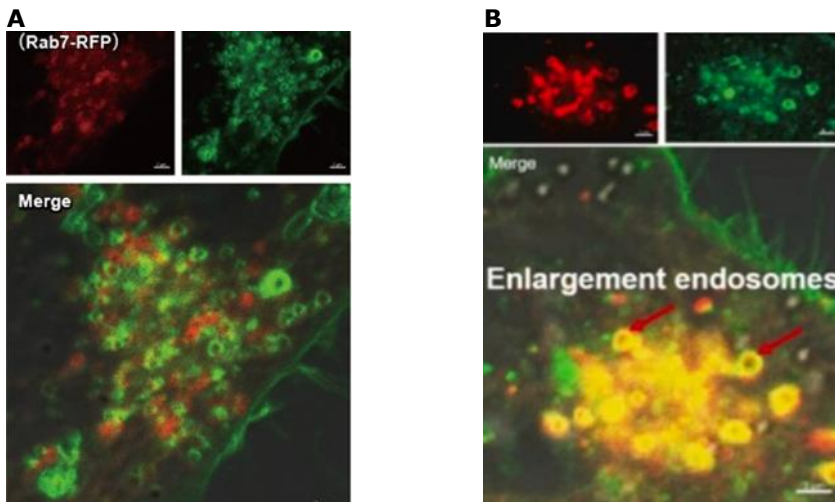


Figure 2. (A) BioTracker™ Green Endocytosis Live Cell Probe (SCT271) does not colocalize with late endosomes, supporting Wortmannin's effect in inhibiting endosomal recycling. (B) BioTracker™ Green Endocytosis Live Cell Probe (SCT271, green) colocalizes only with enlarged early endosomes and recycling endosomes, but not with late endosomes.

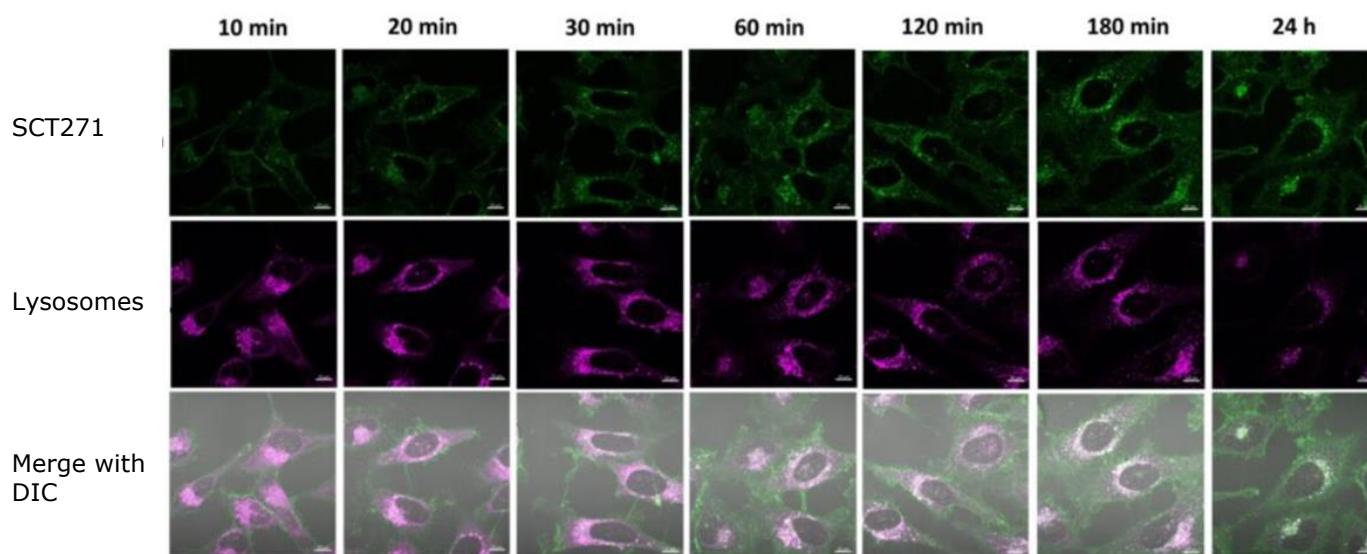


Figure 3. Endosomes are bound by BioTracker™ Green Endocytosis Live Cell Probe (SCT271) and increase in fluorescence intensity over time. Green, SCT271. Middle row, purple, lysosomes. Bottom row, lysosomes increasingly colocalize with endosomes over time (white, colocalization).

Protocols

General Protocol

1. Seed cells in a dish and culture them overnight at 37 °C in an incubator equilibrated with 95% air and 5% CO₂.
2. Discard the culture medium and wash the cells once with HBSS, PBS or growth medium.
3. Add the BioTracker™ Green Endocytosis Live Cell Probe (SCT271) working solution to the dish or plate containing the cells and incubate them for 10 minutes –24 hours at 37 °C in an incubator equilibrated with 95% air and 5% CO₂.
4. Discard the supernatant and wash the cells twice with HBSS, PBS or growth medium.
5. Add growth medium to the dish, then observe the cells under a fluorescence microscope.

Endosomal + Lysosomal Staining

1. Culture HeLa or other cells of interest overnight.
2. Wash the cells once with HBSS.
3. Add BioTracker™ Green Endocytosis Live Cell Probe (SCT271) diluted 1000-fold prepared in 10% FBS-containing MEM medium and lysosomal staining dye (final concentration: 100 nM).
4. Observe unwashed cells at each hour using a confocal or fluorescence microscope.

Wortmannin Endocytosis Inhibition Protocol

1. Prepare HeLa cells (or other cells of interest) in 8 wells of a microslide and incubate overnight.
2. After washing with HBSS, add 200 µL of Wortmannin solution (final concentration: 100 nmol/L) prepared in 10% FBS-containing MEM medium.
3. Incubate at 37 °C for 30 minutes.
4. 200 µL of BioTracker™ Green Endocytosis Live Cell Probe (diluted 1000-fold) prepared in 10% FBS-containing MEM medium without removing the supernatant.
5. Incubate at 37 °C for 30 minutes.
6. Wash the cells twice with HBSS and add MEM medium containing 10% FBS.
7. Observe with a confocal laser microscope.

References

1. Y. Miyakawa, M. Otsuka, K. Sekiba, K. Funato, K. Koike, "[Humanized virus-suppressing factor inhibits hepatitis B virus infection by targeting viral cell entry](#)", Heliyon, 2021, doi:10.1016/j.heliyon.2021.e07586.
2. A. O. Sato, Y. Fujioka, S. Kashiwagi, A. Yoshida, M. Fujioka, H. Sasajima, A. Nanbo, M. Amano, Y. Ohba, "[Interaction between PI3K and the VDAC2 channel tethers Ras-PI3K-positive endosomes to mitochondria and promotes endosome maturation](#)", Cell Reports, 2023, doi:10.1016/j.celrep.2023.112229.

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