



TraKine™ Pro Live-cell Lyso-traker kit (Green Fluorescence)

Cat #: KTC4200

Size: 50 T/250 T

	Live-cell Lyso-traker kit (Green Fluorescence)		
REF	Cat #: KTC4200	LOT	Lot #: Refer to product label
	Maximum Ex/Em: 490/525 nm		Color: Green
	Applicable samples: Mammalian Live Cells (U-2 OS cell lines have been tested)		
	Storage: Store at -20°C for 12 months, protected from light		

Assay Principle

Lyso Green is a fluorescent probe with green fluorescence that can specifically label lysosomes in living mammalian cells. It has strong water solubility and pH stability; its maximum excitation wavelength is 490 nm, and its maximum emission wavelength is 525 nm (see Figure 1 for details and excitation/emission spectra). The fluorescence performance of Lyso Green-labeled Lyso compared with the commercially available Lyso tracker-Green: The comparison of imaging results of HiS-SIM super-resolution microscope and Spinning disk confocal microscope with the same shooting parameters shows that Lyso Green has excellent labeling effect and resists bleaching ability.

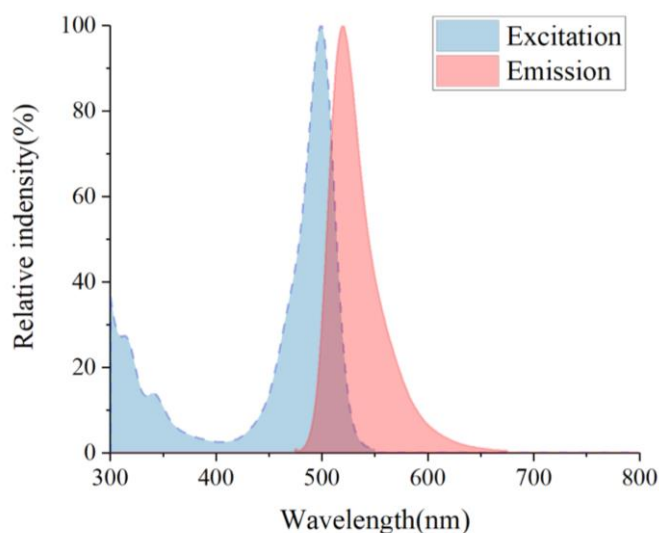


Figure 1. Lyso Green Maximum Ex/Em.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	250 T	

Lyso Green (200 µM)	140 µL	650 µL	-20°C, protected from light
Buffer A (200 µM)	30 µL	130 µL	-20°C

Materials Required but Not Supplied

- Super-resolution microscopy, Laser Scanning Confocal Microscopy
- PCR tubes, Precision pipettes, disposable pipette tips
- Glass bottom dishes or transparent flat bottom orifice plate
- Cell culture media with 10% and 0% FBS
- Phosphate-buffered saline (PBS), PH 7.4

Reagent Preparation

Lyso Green: Ready to use as supplied. Stored at -20°C and protected from light after aliquoting.

Buffer A: Ready to use as supplied. Stored at -20°C after aliquoting.

Assay Procedure

A Lyso Green Staining Solution:

For 24 or 48 well plates, the amount of Lyso Green incubation solution required per well is 200 µL and 100 µL, respectively; The amount of Lyso Green incubation solution required for 15 mm and 20 mm confocal imaging dishes was 100 µL and 200 µL, respectively. The preparation method of 100 µL incubation solution is as follows: add 2.5 µL Lyso Green (5 µM), 1 µL Buffer A (2 µM) in 96.5 µL Cell culture media with 0% FBS in PCR tube, pipet up and down to mix thoroughly. (Other volumes of incubation solution can be prepared in accordance with this ratio)

Note: Cell culture media with 0% FBS is required for incubation solution preparation; Cells should be completely covered by incubation solution when staining. In addition, Lyso Green working concentration is 5-10 µM; The working concentration of Buffer A is 1-2 µM; Users can adjust the amount of incubation solution according to the specific situation.

B Lyso Green incubation conditions and time

1. Cells were seeded on glass bottom dishes at a density of 8×10^4 cells per dish in growth medium. After 30-48 h incubation, the cells were 70-90% confluent.
2. Prepare the Lyso Green Staining Solution required in PCR tubes Refer to step A.
3. Discard the culture media, wash your dishes with PBS once, and then wash with Cell culture media with 0% FBS once.
4. Discard Cell culture media with 0% FBS, quickly dropwise Lyso Green Staining Solution onto the glass bottom, Incubate the cells in a 5% CO₂ atmosphere at 37°C for 1 h.
5. Remove the staining solution, PBS wash with 2-3 times, then add Cell culture media with 0% FBS, incubate the cells in a 5% CO₂ atmosphere at 37°C for 15 min.
6. Remove the Cell culture media, PBS wash with 2-3 times, then add Cell culture media with 10% FBS, incubate the cells in a 5% CO₂ atmosphere at 37°C for 15 min..
7. Remove the Cell culture media, PBS wash with 2-3 times, then add Cell culture media with 10% FBS, Finally, Image cells by microscope.

Precautions

1. To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions.
2. Make sure the pipette tips and PCR tubes were sterilized at high temperature and pressure. Do all experiments in a sterile environment and avoid light as much as possible.
3. Fluorescence quenching occurs in all fluorescent dyes. Please image as soon as possible after incubation and rinsing;
4. Incubation and rinsing time are the most suitable time after the test, in order to ensure the marking effect, do not change.

Typical Data

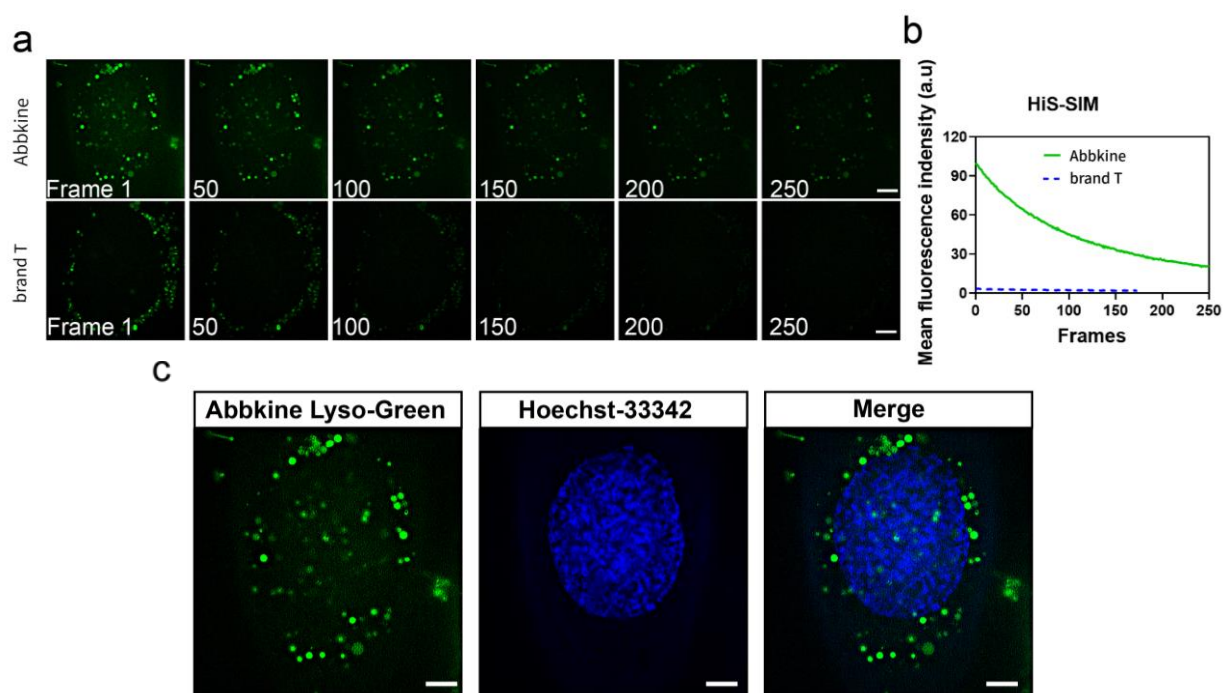


Figure 2. Long-term time Series images of Super Lyso-Green and commercially available Lyso Tracker-Green on U-2 OS cells (a), fluorescence bleaching curves (b), and Bicolor localization of nuclei (c). Scale bas: 5 μ M.

Recommended Products

Catalog No.	Product Name
KTC4100	TraKine™ Pro Live-cell Tubulin-traker kit (Green Fluorescence)
KTC4110	TraKine™ Pro Live-cell Tubulin-traker kit (Red Fluorescence)
KTC4210	TraKine™ Pro Live-cell Lyso-traker kit (Red Fluorescence)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.