TraKine™ Pro Live-cell Tubulin-traker kit (Red Fluorescence)

Cat #: KTC4110 Size: 50 T/250 T

	Live-cell Tubulin-traker kit (Red Fluorescence)		
REF	**Cat #:** KTC4110	LOT	**Lot #:** Refer to product label
	Maximum Ex/Em: 652/674 nm		**Color:** Red
	Applicable samples: Mammalian Live Cells (U-2 OS, Hela and MCF-7 cell lines have been tested)		
	Storage: Store at -20℃ for 12 months, protected from light		

Assay Principle

Tubulin Red is a fluorescent probe with deep Red fluorescence that can specifically label microtube in living mammalian cells. It has strong water solubility and pH stability; its maximum excitation wavelength is 652 nm, and its maximum emission wavelength is 674 nm (see Figure 1 for details and excitation/emission spectra). The fluorescence performance of Tubulin Red-labeled microtube compared with the commercially available Tubulin SIR: The comparison of imaging results of HiS-SIM super-resolution microscope and Spining disk confocal microscope with the same shooting parameters shows that Tubulin Red has excellent labeling effect and resists bleaching ability.

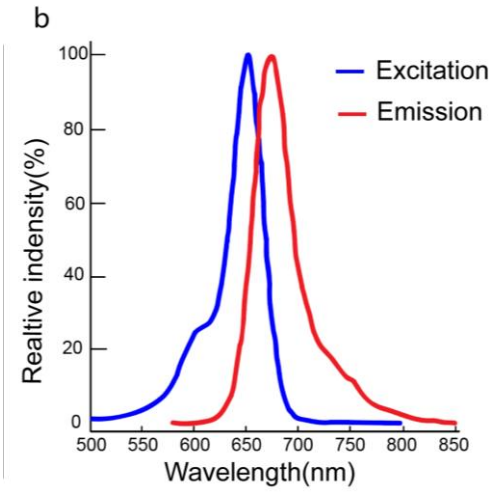

Figure 1. Tubulin Red Maximum Ex/Em

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	250 T	
Tubulin Red (200 μM)	140 μL	650 μL	-20℃, protected from light

Buffer A (200 μM)	30 μL	130 μL	-20℃
Buffer B (1 mM)	110 μL	520 μL	-20℃

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

Tubulin Red: Ready to use as supplied. Stored at -20℃ and protected from light after aliquoting.

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

Buffer B: Ready to use as supplied. Stored at -20℃ after aliquoting.

Assay Procedure

A Tubulin Red Staining Solution:

For 24 or 48 well plates, the amount of Tubulin Red incubation solution required per well is 200 μL and 100 μL, respectively; The amount of Tubulin Red 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 5 μL Tubulin Red (10 μM), 1 μL Buffer A (2 μM) and 3 μL Buffer B (30 μM) in 91 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, Tubulin deep Red working concentration is 10 μM; The working concentration of Buffer A is 1-2 μM; The working concentration of Buffer B is 30-40 μM; Users can adjust the amount of incubation solution according to the specific situation.

B Tubulin deep Red 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 Tubulin deep Red 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 Tubulin Red Staining Solution onto the glass bottom, Incubate the cells in a 5% CO_2 atmosphere at 37℃ 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% CO_2 atmosphere at 37℃ 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% CO_2 atmosphere at 37℃ 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

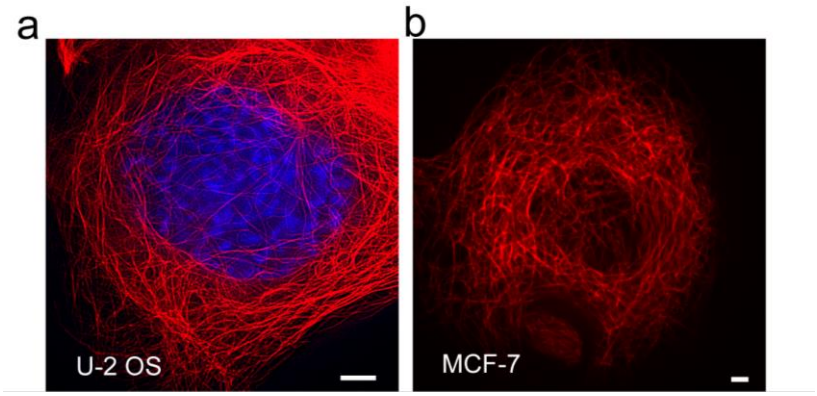

Figure 2. Markers of Tubulin Red in U-2 OS (a), MCF-7 (b) cell lines. Scale bars: 5 μM.

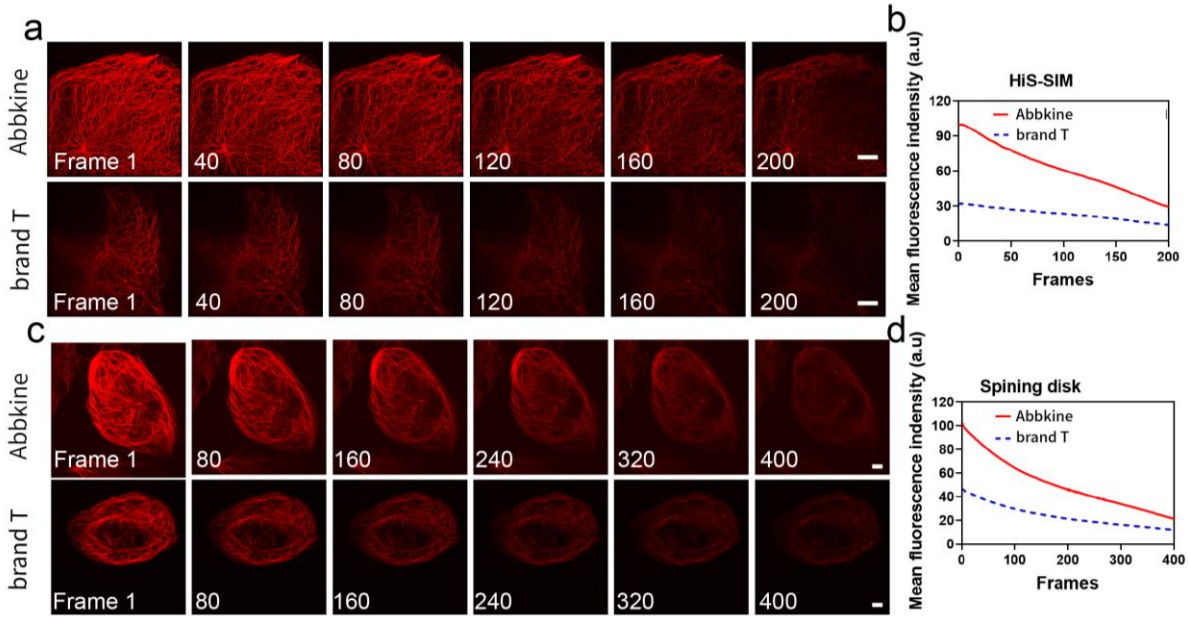

Figure 3. Long time series images (a, c) and corresponding fluorescence bleaching curves (b, d) were obtained by using HiS SIM super-resolution and Spining disk confocal microscopy of Tubulin Red and commercially available Tubulin SIR on U-2 OS cells. Scale bars: 5 μM.

Recommended Products

Catalog No.	Product Name
KTC4200	TraKine™ Pro Live-cell Lyso-traker kit (Green Fluorescence)
KTC4210	TraKine™ Pro Live-cell Lyso-traker kit (Red Fluorescence)
KTC4100	TraKine™ Pro Live-cell Tubulin-traker kit (Green Fluorescence)

Disclaimer

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