TraKine™ Pro Live-cell Tubulin Staining kit (Green Fluorescence with Super Resolution)

Item NO.	Product Name
KTC4100	TraKine™ Pro Live-cell Tubulin Staining kit (Green Fluorescence with Super Resolution)

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Background & Principle

Tubulin is the major building block of microtubules. This intracellular cylindrical filamentous structure is present in almost all eukaryotic cells. Microtubules function as structural and mobile elements in mitosis, intracellular transport, flagellar movement, and in the cytoskeleton. Tubulin is a heterodimer, which consists of α-tubulin and β-tubulin; both subunits have a molecular weight of 55 kDa and share considerable homology. The most studied tubulins have been isolated from vertebrate brains. The microtubules can be viewed in immunofluorescent microscopy, which enables the observation of the intracellular organization of proteins that are in the form of a supramolecular structure.

TraKine™ Pro is series of long-term super-resolution cell staining imaging portfolio for labeling subcellular structures of live and fixed cells. TraKine™ Pro proprietary excellent fluorescent dyes span the full UV-visible and near IR spectrum. Customized products based on TraKine™ Pro technology are also available.

Abbkine TraKine™ Pro Live-cell Tubulin Staining kit (Green Fluorescence with Super Resolution) is a fluorescence imaging tool for staining of Tubulin in mammalian living cells with high specificity and low background. The proprietary probe in the kit consists of a microtubule recognition unit and a green fluorescent dye (Ex/Em=500/520 nm), the recognition unit can selectively recognize microtubules and binding with it. The whole probe is membrane impermeable, but after incubating with buffer T, it can enter cells through endocytosis pathway in vesicular compartments, and release into cytoplasm subsequently. It is especially suitable for Confocal and long-term super-resolution imaging (such as SIM, STED, TIRF, STORM and PALM).
Note: The product has been tested in U2OS, HeLa, COS-7 and ARPE cell lines to realize live-cell labeling. U2OS cell line is preferred.

Storage/Stability

Refer to list of materials supplied for storage conditions of individual components. Stable for at least 6 months at recommended temperature from date of shipment. Gel pack with blue ice.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	50T	250T	1000T	
TubGreen™ (200 μM)	100 μL	500 μL	2 mL	-20°C, Protect from light
Buffer T	40 μL	200 μL	0.8 mL	4°C for 1 month -20°C for 6 months (avoid freeze-thaw cycles)

Other supplies required, Not Supplied

- Pipettes and pipette tips
- Phosphate-buffered saline (PBS), PH 7.4
- Cell culture media without FBS (termed media (-))
- Cell culture media with 10% FBS (termed media (+))
- Glass Bottom Dishes or 96-well black wall/clear bottom plate
- PCR tubes
- Fluorescence Microscopy

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Make sure the pipette tips and PCR tubes were sterilized at high temperature and pressure. Make sure sterile environment and protect from light during the whole experiment.

ASSAY PROTOCOL

Reagent Preparation

media (-): Cell culture media without FBS
media (+): Cell culture media with 10% FBS

Staining Solution:
Make sure the working concentration of the TubGreen™ is 10 μM.
For example: For 100 μL staining solution (used in 15 mm diameter Glass Bottom Dishes, 100 μL/well), add 5 μL TubGreen™ and 1-3 μL Buffer T in 92-94 μL media (-) in PCR tube, pipet up and down to mix thoroughly.
Note:
1. The optimal concentration of TubGreen™ and Buffer T may vary depending on cell types and staining conditions. We have successfully used 5-10 μM TubGreen™ in U2OS cell. And when using Buffer T for the first time, it is recommended to try different concentrations. To avoid potential toxicity, please use the minimal concentration that can keep a good cell condition.
2. The volume of staining solution depend on cell culture plate you use, more solution is needed for larger diameter.
3. When prepare multiple wells of staining solution, first prepare the total amount, and then distribute it to each well respectively. Don't prepare each well separately. If the amount is too small, errors will easily occur between the wells.
4. This probe can only be used for labeling microtubules in living cells.

Recommended procedures

1. Cells were seeded in Glass Bottom Dishes or 96-well black wall/clear bottom plate in growth medium. After >24h incubation, the cells were 70-90% confluent.
2. Discard the culture media, wash your dish with PBS once, then wash with media (-) once.
Note: Make sure there are no culture media remain in dish, because the presence of FBS will affect the staining.
3. Discard media (-), quickly dropwise the staining solution onto the 15 mm diameter Glass Bottom Dishes (100 μL/well) or 96-well black wall/clear bottom plate (40 μL/well) and avoid outflow.
4. Incubate the cells in a 5% CO_2 atmosphere at 37°C for 1 h.
5. Remove the staining solution and wash with media (-) once, incubate the cells with media (-) in a 5% CO_2 atmosphere at 37°C for 15 mins.
6. Then remove media (-) and wash with media (+) once, incubate the cells with media (+) in a 5% CO_2 atmosphere at 37°C for 45 mins.
7. Image cells by microscope.