Live Cell Tracking Kit (Green Fluorescence)

Cat #: KTA1002 Size: 100 T/500 T/2000 T

	Live Cell Tracking Kit (Green Fluorescence)		
REF	**Cat #:** KTA1002	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Cell movement and location studies require specialized probes that are nontoxic to living cells. The Live Cell Tracking Kit provides a versatile and well-retained cell tracing reagent (Cell Tracker Green) for monitoring cell movement, location, proliferation, migration, chemotaxis, and invasion. Cell Tracker Green can passively diffuse into cells and remain colorless and nonfluorescent until its acetate groups are cleaved by intracellular esterases to yield highly fluorescent, amine-reactive carboxyfluorescein succinimidyl ester. The succinimidyl ester group reacts with intracellular amines, forming fluorescent conjugates (Ex/Em= 494/521nm) that are well-retained and can be fixed with aldehyde fixatives. Excess unconjugated reagent and by-products passively diffuse to the extracellular medium, where they can be washed away. After conversion to permeant versions, the Cell Tracker Green probes are well retained in living cells through several generations and can display fluorescence for at least a week. The probes are transferred to daughter cells, but are not transferred to adjacent cells in a population.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	100 T	500 T	2000 T	
Cell Tracker Green	50 μL	250 μL	1 mL	-20℃, protected from light
Assay Buffer (5×)	5 mL	10 mL	40 mL	4℃

Materials Required but Not Supplied

- Microcentrifuge
- 24 well plate, Precision Pipettes, Disposable Pipette Tips
- Fluorescence Microscopy or Flow Cytometer
- Deionized Water, PBS

Reagent Preparation

Cell Tracker Green: Keep on ice while using. Protect from light. Aliquot to avoid repeated freezing and thawing.

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1×Assay Buffer: Prepare 1×Assay Buffer by dilute 5× Assay Buffer with Deionized Water. Warm to 37℃ before use.

Staining Solution: Immediately before starting the assay, dilute Cell Tracker Green into 1×Assay Buffer at 1:1000 ratio. Scale up accordingly for larger numbers of assays. Protect from light and pre-warm to 37℃ before use.

Note: The final concentration of Cell Tracker Green should be empirically determined for different cell types and/or experimental conditions. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells will require 1: 1000 dilution to double the dye concentration. Dye at a lower concentration up to 1:5000 dilution may be needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts, the concentration of the dye should be kept as low as possible.

Assay Procedure

A. Quantification by Flow Cytometry

1. Treat cells with the desired method, and set up parallel control experiments.

Note: We recommend keeping unstained control cells (i.e. without Cell Tracker Green) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

2. For non-adherent cells, Collect 0.5-1×10^6 cells by centrifugation (4℃, 300 g, 5 min). Wash with ice-cold PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation.

3. Resuspend the cells pellet in 500 uL Staining Solution.

4. Incubate the cells at 37℃ for 15-30 min in the dark.

5. Centrifuge cells at 500 g and discard supernatant.

6. Resuspend in fresh prewarmed medium and incubate the cells for another 30 min to ensure complete modification of the probe and wash the cells again with PBS.

7. Resuspend cell pellet in pre-warmed PBS at a density of 5×10^5 to 1×10^6 cells/mL and analyze cells immediately by flow cytometry using the FL1 channel (usually FL1).

B. Detection by Fluorescence Microscopy

1. For non-adherent cells: Follow the protocol for flow cytometry from step A.1 to step A.7 and place the cell suspension from Step A.7 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible. If the cells are to be fixed and permeabilized, continue to Procedure B.3 Fixation and Permeabilization.

2. For adherent cells: the suggested protocol is as below.

(1) Grow cells directly on a coverslip in 24 well dish. Incubate in a CO_2 Incubator at 37℃ for at least 24 h before treatment.

(2) Treat cells with the desired method.

(3) Wash cells with PBS twice and discard the PBS.

(4) Add 0.5 mL of Staining solution to cells and incubate at 37℃ for 15-30 min in the dark.

(5) Discard the supernatant and replace with the fresh pre-warm growth medium and incubate the cultures for another 30 min at 37℃.

(6) Wash cells with PBS or an appropriate buffer twice. If the cells are to be fixed and permeabilized, continue to Procedure B.3.

(7) Invert coverslip on a glass slide and visualize cells fluorescence microscopy using the appropriate filters.

3.Fixation and Permeabilization

(1) Use aldehyde-containing fixatives for fixation. Typically, we fix the cells for 15 min at room temperature using 3.7% formaldehyde.

(2) After fixation, the cells should be rinsed in PBS for three times.

(3) After fixation, if the cells are to be subsequently labeled with an antibody, cells should be permeabilized by incubating them in ice-cold acetone for 10 min. Following permeabilization, the cells should be rinsed in PBS for three times.

Recommended Products

Catalog No.	Product Name

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KTA2020	Cell Cycle Staining Kit
KTA1001	Live and Dead Cell Double Staining Kit

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

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