

Live Cell Tracking Kit (Green Fluorescence)

Item NO.	Product Name
KTA1002	Live Cell Tracking Kit (Green Fluorescence)



ATTENTION

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

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TABLE OF CONTENTS

INTRODUCTION

Background & Principle	1
Storage/Stability	1
Assay restrictions	1

PRODUCT INFORMATION

Materials supplied and Storage conditions	2
Other supplies required, Not Supplied.....	2
Precautions.....	2
Technical hints.....	2

ASSAY PROTOCOL

Reagent Preparation	3
Recommended procedures	3

INTRODUCTION

Background & 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 celltracing reagent (CellTracker Green) for monitoring cell movement, location, proliferation, migration, chemotaxis, and invasion. CellTracker 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 permanent versions, the CellTracker 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.

Storage/Stability

Refer to list of materials supplied for storage conditions of individual components. Stable for at least 12 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
	100T	500T	2000T	
CellTracker Green	50 μ L	250 μ L	1 mL	-20°C, Protect from light
Assay Buffer (5 \times)	5 mL	10 mL	40 mL	4°C

Other supplies required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescence Microscopy or Flow Cytometer
- 24-well plate for cell culture
- Phosphate-buffered saline (PBS)

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Reagent Preparation

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

Assay Buffer: Prepare 1×Assay Buffer by dilute 5× Assay Buffer with ddH₂O. Warm to 37°C before use.

Staining Solution: Immediately before starting the assay, dilute CellTracker 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°C before use.

Note: The final concentration of CellTracker 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.

Recommended procedures

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 CellTracker Green) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

2. For non-adherent cells, Collect 0.5-1×10⁶ cells by centrifugation (4°C, 300g, 5min). 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 500uL Staining Solution.

4. Incubate the cells at 37°C for 15-30 minutes 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 minutes 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 5×10⁵ to 1×10⁶ 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 1 to step 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 C Fixation and Permeabilization**.

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

2.1. Grow cells directly on a coverslip in 24 well dish. Incubate in a CO₂ Incubator at 37°C for at least 24 hours before treatment.

2.2. Treat cells with the desired method.

2.3. Wash cells with PBS twice and discard the PBS.

2.4. Add 0.5 mL of Staining solution to cells and incubate at 37°C for 15-30 minutes in the dark.

2.5. Discard the supernatant and replace with the fresh pre-warm growth medium and incubate the cultures for another 30 minutes at 37°C.

2.6. Wash cells with PBS or an appropriate buffer twice. If the cells are to be fixed and permeabilized, continue to **Procedure C Fixation and Permeabilization**.

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

C. Fixation and Permeabilization

1. Use aldehyde-containing fixatives for fixation. Typically, we fix the cells for 15 minutes 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 minutes. Following permeabilization, the cells should be rinsed in PBS for three times.