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ROS Assay Kit
Catalog Number: A057

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
H_2DCFDA (Component A)	**200 μL**	**10 mM in DMSO**	• **-20 °C,** • **Protect from light**	**The product is stable for 1 year when stored as directed.**
TBHP (Component B)	**50 μL**	**70% solution**		

Number of assays: 1000 assays.
Approximate fluorescence excitation/emission maxima, in nm: 495/529 nm.

Introduction

Generation of reactive oxygen species (ROS) at a controlled level is a feature of live cell function. Under conditions of oxidative stress, ROS production is dramatically increased, resulting in damage of membrane lipids, proteins, and nucleic acids. Oxidative damage is associated with aging as well as many diseases. Probes for measuring ROS provide important tools for study oxidative stress related diseases.

The ROS assay kit is specifically designed to detect ROS in live cells. This kit uses a unique fluorescent probe H_2DCFDA to detect reactive oxygen species. H_2DCFDA is chemically reduced and acetylated form of 2',7'-dichloro- fluorescein (DCF) and is nonfluorescent and cell-permeant. In addition, the kit provides the common inducer of ROS production tert-butyl hydroperoxide (TBHP) as a positive control.

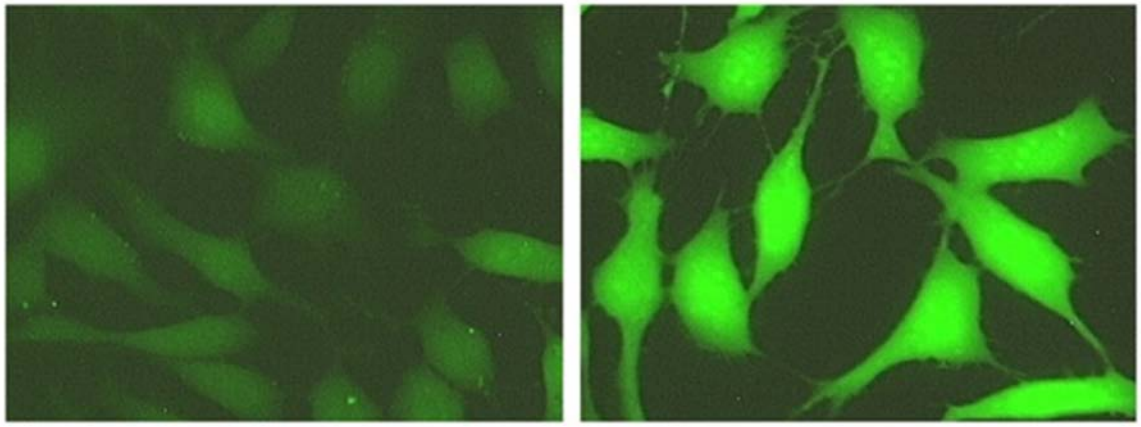
Once H_2DCFDA enters into cells, the acetate groups are removed by intracellular esterases to form H_2DCF. H_2DCF is well retained inside cells. Oxidation of H_2DCF by reactive oxygen species yields fluorescent DCF. Reactive oxygen species can be detected by monitoring the increase in fluorescence with a flow cytometer, fluorometer, microplate reader, or fluorescence microscope, using excitation sources and filters appropriate for fluorescein (FITC). Because the dyes are susceptible to photo-oxidation, low light conditions should be used for fluorescence microscopy applications whenever possible

Experimental Protocol

The following protocols provide general guidelines derived from various publications, and should be modified for the particular application and sensitivity required.

1. Harvest the cell samples. For suspension cell line, adjust the cell concentration of the samples to ~5×10^5 cells/ml in complete growth medium. For adherent cell line, ensure that the cells are sub-confluent. Staining of cells in phosphate buffered saline (PBS) is not recommended.
2. Induce ROS in cells using your interested chemicals. A negative control should be prepared by incubating cells in the absence of inducing agent.
3. Prepare positive control. Prepare a 50 mM stock solution of TBHP by adding 3.2 μL of 70% TBHP (Component B) to 496.8 μL of PBS. Then add 4 μL of 50 mM TBHP solution per mL cells to give a final 200 μM concentration of TBHP, and incubate for 30-60 minutes under normal growth conditions.
4. Briefly centrifuge the vial of H_2DCFDA (Component A) before opening the vial. Dilute H_2DCFDA stock solution (Component A) at 1:1000 in prewarmed buffer (HBSS, or HEPES) to provide a final working concentration of 10 μM dye. The optimal working concentration for your application must be empirically determined. Keep tightly sealed until ready to use.

5. Remove cells including negative and positive controls from growth media via centrifugation or pipetting. Resuspend cells in 1X H_2DCFDA staining solution (10 μM).
6. Incubate at 37°C for the cells. Generally, a loading time of 10-30 minutes is sufficient.
7. Remove the loading buffer, wash cells three times with prewarmed buffer (HBSS, or HEPES); return the cells to prewarmed growth medium and incubate at 37°C for 10-20 minutes for cellular esterases to hydrolyze the acetate groups and render the dye responsive to oxidation.
8. Observe immediately with a fluorescence microscope or a flow cytometer with 488 nm excitation using emission filter appropriate for fluorescein.



Control Induced

Reference:

MTI-101 (Cyclized HYD1) Binds a CD44 Containing Complex and Induces Necrotic Cell Death in Multiple Myeloma.

Gebhard AW, Jain P, Nair RR, Emmons MF, Argilagos RF, Koomen JM, McLaughlin ML, Hazlehurst LA, Mol Cancer Ther (2013) 12:2446-2458