

New

LIFE SCIENCE



Intracellular Reactive Oxygen Species (ROS) Detection Assay Kit

Intracellular Reactive Oxygen Species (ROS) Detection Assay Kit

1kit [I1265]

Advantages

- Detects intracellular ROS sensitively and rapidly
- Allows for various imaging and detection methods
- Applicable to a wide variety of cells

Kit Components

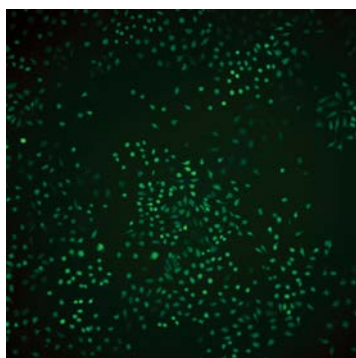
- 1000×Reaction Dye (DCFH-DA) : 10 μ L for 100 tests
- 10×Reaction Buffer : 25 mL×2 for 100 tests

† For microplate assays: 96 wells

† For flow cytometry: 1 mL/test for 1×10^6 cells/mL

† Optimal Reaction Dye working concentration may vary by cell lines.

Application: Fluorescence Microscopy Observation



ROS observed in HeLa cells

[Detection Conditions]

GFP filter

Excitation wavelength: 450 - 490 nm

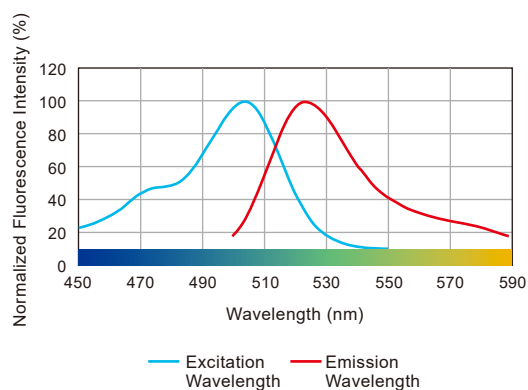
Emission wavelength: 500 - 550 nm

[1×Reaction Dye Preparation]

- 10×Reaction Buffer 100 μ L
- 1000×Reaction Dye 1 μ L
- Deionized water 900 μ L

When the confluency of preseeded HeLa cells reached 80%, the culture media was removed, replaced with 1×Reaction Dye, and allowed to incubate for 30 minutes in CO₂ incubator (37°C), intracellular ROS observation was carried out on a fluorescence microscopy.

Absorbance and Fluorescence Spectra



[Detection Conditions]

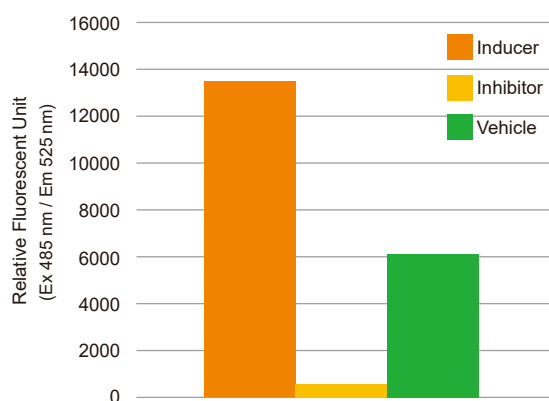
When the confluency of preseeded HeLa cells reached 80%, the culture media was removed, replaced with 1×Reaction Dye, and allowed to incubate for 30 minutes in CO₂ incubator (37°C). The excitation and emission spectra were then measured.

Maximum excitation wavelength: 503 nm

Maximum emission wavelength: 524 nm

Intracellular Reactive Oxygen Species (ROS) Detection Assay Kit

Application: Fluorescence Microplate Reader



ROS detected in NIH-3T3 cells

[Detection Conditions]

Excitation wavelength: 450 - 490 nm
Emission wavelength: 500 - 550 nm

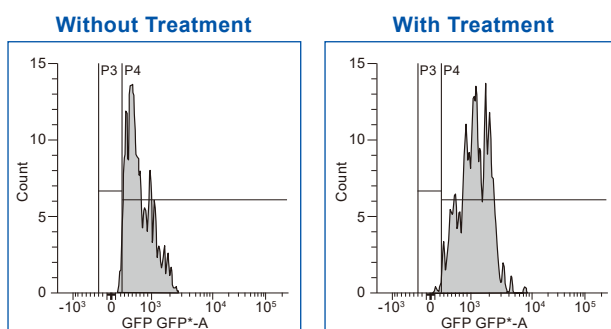
[1×Reaction Dye Preparation]

- 1000×Reaction Dye 1 μL
- 10×Reaction Buffer 100 μL
- Deionized water 900 μL

When the confluency of preseeded NIH-3T3 cells were reached 80%, the culture media was removed and replaced with fresh media either containing or without a ROS Inducer. After cells were allowed to incubate in a CO₂ incubator (37°C) overnight. A sample of cells was additionally treated with a ROS Inhibitor for 30 minutes. Finally, all cells were treated with 1×Reaction Dye in CO₂ incubator (37°C) for 30 minutes and tested intracellular ROS on a plate reader. The intracellular ROS production was confirmed to be induced by inducer and inhibited by inhibitor.

Reagent Concentration	Inducer	Inhibitor	Vehicle
ROS Inducer: 1mM Erastin [E1524]	+	+	-
ROS Inhibitor: 5mM N-Acetyl-L-cysteine [For ROS inhibition] [A3682]	-	+	-

Application: Flow Cytometry



ROS detected in NIH-3T3 cells

[Detection Conditions]

GFP filter
Excitation wavelength: 488 nm
Emission wavelength: 520 nm

[1×Reaction Dye Preparation]

- 1000×Reaction Dye 1 μL
- 10×Reaction Buffer 100 μL
- Deionized water 900 μL

After having reached 80% confluency, NIH-3T3 cells were treated with 1 mM Erastin [E1524] and incubated in a CO₂ incubator (37°C) overnight. Cells were collected and treated with 1×Reaction Dye for 30 minutes in a CO₂ incubator (37°C) and analyzed by flow cytometry. Intracellular ROS production was induced by Erastin [E1524].

Related Products

N-Acetyl-L-cysteine [For ROS inhibition]
Erastin

10mg / 50mg [A3682]
5mg [E1524]

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