

## 2',7'-Dichlorodihydrofluorescein diacetate

**Cat:** D6470

**Specification:** 25mg /100mg

**Storage:** Store at -20°C, avoid light, and it is valid for 1 year.

### Product Information

**CAS:** 4091-99-0

**English name:** 2',7'-Dichlorofluorescein diacetate

**Appearance (Character):** White to pink powder

**Molecular Formula:** C<sub>24</sub>H<sub>16</sub>Cl<sub>2</sub>O<sub>7</sub>

**Molecular Weight:** 487.28

**Purity:** ≥97%

**Solubility:** Soluble in DMSO

**Spectral characteristics:** excitation = 504 nm; Fluorescence = 529 nm

### Introduction

DCFH-DA, also known as 2',7'-dichlorofluorescein diacetate, can be hydrolyzed by cellular esterases to 2',7'-dichlorofluorescein, also known as 2',7'-dichlorofluorescein, and then mainly oxidized by H<sub>2</sub>O<sub>2</sub> to 2',7'-dichlorofluorescein. 2',7'-Dichlorodihydrofluorescein diacetate may be reactive to a wide range of oxidative reactions, which may increase during oxidative stress in cells. This probe is widely used to monitor the redox process of cells.

After cellular uptake, DC FH- DA It is deacetylated by cell esterases into a non-fluorescent compound, which is then oxidized by ROS to 2'-7' dichlorofluorescein (DCF). DC F is a fluorescent compound that can be detected by fluorometer, flow cytometer or fluorescence microscope.

### Protocols(for reference only):

#### Staining of cell samples:

1. Prepare a 1-10 mM stock solution of DMSO. Unused DMSO stock solution should be proportionally divided and stored at -20°C in the dark.
2. Prepare a 1-10μM dye working solution in physiological buffer such as PBS, HBSS, or HEPES. The optimal working concentration can be determined through preliminary experiments.
3. Remove the growth medium from the cells, add the dye working solution step 2, and then incubate the cells at RT or 37°C for 5 to 60 minutes.
4. Discard the staining working solution; Wash with preheated HBSS, then add preheated HBSS or growth medium and incubate at the optimal temperature. Due to the generally low esterase activity of certain cell types, the optimal recovery time may vary. Please refer to the actual situation.
5. Before exposing the cells to experimental inducers, determine the baseline fluorescence intensity of the loaded cell sample.
6. Negative control:

- 1) Examine the spontaneous fluorescence of undyed cells within the green emission range.
- 2) For flow cytometry, it should be confirmed that the forward and side scatter of cells remain unchanged after staining and processing. The change in cell size may be related to bleeding or contraction during the treatment process.
- 3) Test the fluorescence of the mixture of cell-free dye and buffer/medium with or without an inducer. In the absence of extracellular esterases and other oxidases, the gradual increase in fluorescence over time may be related to spontaneous hydrolysis, atmospheric oxidation, and/or light-induced oxidation.

7. Positive controls can be stimulated with H<sub>2</sub>O<sub>2</sub> or tert-butyl hydroperoxide TBHP, with a final concentration of ~100μM. The dose can be increased or decreased based on the sensitivity and response of the cells.

#### Note

1. Once dissolved, please store the solution in separate containers to avoid product degradation caused by repeated freezing and thawing.
2. The product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.
3. The products are all for scientific research use only. Do not use it for medical, clinical diagnosis or treatment, food and cosmetics, etc. Do not store them in ordinary residential areas.
4. For your safety and health, please wear laboratory clothes, disposable gloves and masks to operate.