

## 2',7'-Dichlorodihydrofluorescein diacetate [2',7'-Dichlorofluorescin diacetate]

### **Ordering Information:**

Product Number: 15204 (25 mg)

### **Storage Conditions:**

Keep at -20 °C and desiccated

### **Chemical and Physical Properties**

Molecular Weight: 487.29

Solvent: DMSO

Spectral Properties: Excitation = 504 nm; Fluorescence = 529 nm

### **Biological Applications**

2',7'-Dichlorodihydrofluorescein diacetate (also called 2',7'-dichlorofluorescin diacetate) is hydrolyzed by cellular esterases to 2',7'-dichlorodihydrofluorescein (also called 2',7'-dichlorofluorescin) and is then oxidized to 2',7'-dichlorofluorescein primarily by H<sub>2</sub>O<sub>2</sub>. 2',7'-dichlorodihydrofluorescein diacetate might be reactive toward a broad range of oxidizing reactions during intracellular oxidant stress. This probe is widely used to monitor the cellular redox processes.

### **Sample Protocol for Staining Cells**

The following procedures provide a general guideline and should be modified for your particular application.

1. Make 1-10 mM DMSO stock solution. The unused DMSO stock solution should be aliquoted into a single use vial and stored at -20 °C. Keep from light.
2. Make the dye working concentration of 1–10 µM in a physiological buffer (such as PBS, HBSS, HEPES). The optimal working concentration for your application must be empirically determined.
3. Remove cells from growth media, add the dye working solution (from Step 2) to the cells, and incubate the cells at RT or 37 °C for 5 to 60 minutes.
4. Remove the dye working solution; wash with pre-warmed HBSS, and add pre-warmed HBSS or growth medium and incubate at the optimal temperature. The optimal recovery time can vary widely, as some cell types normally exhibit low levels of esterase activity.
5. Determine the baseline fluorescence intensity of a sample of the loaded cells prior to exposing the cells to experimental inducements.
6. Negative controls should be assessed as follows:

**6.1** Examine unstained cells for autofluorescence in the green emission range.

**6.2** For flow cytometry, ascertain that the forward and side scatter of cells is unchanged after dye-loading and treatment. Changes in cell dimensions may be related to blebbing or shrinkage resulting from handling or a toxic response.

**6.3** Examine the fluorescence of cell-free mixtures of dye and buffer/media with and without the inducer. In the absence of extracellular esterases and other oxidative enzymes, the gradual increase in fluorescence over time may be related to spontaneous hydrolysis, atmospheric oxidation, and/or light-induced oxidation.

**6.4** Examine the fluorescence of untreated (control) loaded cells that have been maintained in growth medium or simple buffer. In healthy cells, oxygen radicals are eliminated by cellular enzymes and/or natural antioxidants. Following the dye-loading recovery period, healthy cells should exhibit a low level of fluorescence that is relatively stable for the duration of the experiment; however, a gradual increase (due to auto-oxidation) or decrease (due to loss of dye from cells or photobleaching) in fluorescence may be observed. In the absence of any stimulus or inducement, a burst of fluorescence in healthy, untreated cells could indicate progress to cell death or some other oxidative event.

7. Positive controls may be stimulated with H<sub>2</sub>O<sub>2</sub> or *tert*-butyl hydroperoxide (TBHP) to a final concentration of ~100 µM (increase or decrease dose based on the sensitivity and response of the cells).

## References

1. Diaz G, Liu S, Isola R, Diana A, Falchi AM. (2003) Mitochondrial localization of reactive oxygen species by dihydrofluorescein probes. *Histochem Cell Biol*, 120, 319.
2. Saengkhae C, Loetchutinat C, Garnier-Suillerot A. (2003) Kinetic analysis of fluorescein and dihydrofluorescein effluxes in tumour cells expressing the multidrug resistance protein, MRP1. *Biochem Pharmacol*, 65, 969.
3. Hempel SL, Buettner GR, O'Malley YQ, Wessels DA, Flaherty DM. (1999) Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5(and 6)-carboxy-2',7'- dichlorodihydrofluorescein diacetate, and dihydrorhodamine 123. *Free Radic Biol Med*, 27, 146.
4. Schilling E, Dopke HH. (1978) A rapid diagnostic test for the viability of early cattle and rabbit embryos using diacetyl-fluorescin. *Naturwissenschaften*, 65, 658.
5. Robertson EJ, Patel JA. (1968) Determination of fluorescin sodium in ophthalmic solutions. *Am J Hosp Pharm*, 25, 598.

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