DCFH-DA [2',7'-Dichlorodihydrofluorescein diacetate] *CAS 4091-99-0*

Catalog number: 15204
Unit size: 25 mg

Component	Storage	Amount
2',7'-Dichlorodihydrofluorescein diacetate [2',7'-Dichlorofluorescin diacetate]	Freeze (<-15 °C), Minimize light exposure	25 mg

OVERVIEW

DCFH-DA, 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 H2O2. 2',7'-dichlorodihydrofluorescein diacetate might be reactive toward a broad range of oxidizing reactions that may be increased during intracellular oxidant stress. This probe is widely used to monitoring cellular redox processes.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	FITC filter set
Emission:	FITC filter set
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC filter set

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

2',7'-Dichlorodihydrofluorescein diacetate stock solution:
Make working solution in the concentration range of 1-10 mM in DMSO.

Note The unused DMSO stock solution should be aliquoted into a single use vial and stored at -20 °C. Keep from light.

PREPARATION OF WORKING SOLUTION

2',7'-Dichlorodihydrofluorescein diacetate working solution:
Make working solution in the concentration range of 1-10 µM in a physiological buffer such as PBS, HBSS, HEPES buffers.

Note The optimal working concentration for your application must be empirically determined.

SAMPLE EXPERIMENTAL PROTOCOL

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

1. Remove cells from growth media, add the dye working solution to the cells, and incubate the cells at room temperature or 37°C for 5 to 60 minutes.

2. 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.

3. 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:

4. Examine unstained cells for autofluorescence in the green emission range.

5. 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. 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.

7. 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.

8. Positive controls may be stimulated with H2O2 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).

EXAMPLE DATA ANALYSIS AND FIGURES

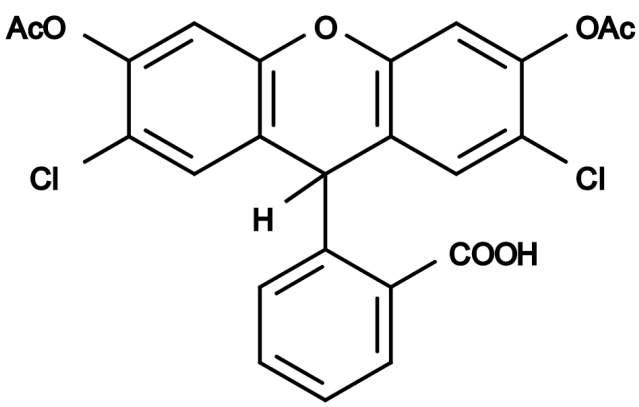

Figure 1. Chemical structure for DCFH-DA [2',7'-Dichlorodihydrofluorescein diacetate] *CAS 4091-99-0*

DISCLAIMER