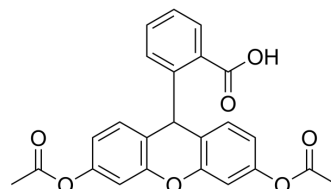


## Dihydrofluorescein diacetate

Cat. No.:	HY-101893
CAS No.:	35340-49-9
Molecular Formula:	C <sub>24</sub> H <sub>18</sub> O <sub>7</sub>
Molecular Weight:	418.4
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : ≥ 106.5 mg/mL (254.54 mM)  
\* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg
	1 mM		2.3901 mL	11.9503 mL	23.9006 mL
	5 mM		0.4780 mL	2.3901 mL	4.7801 mL
	10 mM		0.2390 mL	1.1950 mL	2.3901 mL

Please refer to the solubility information to select the appropriate solvent.

### BIOLOGICAL ACTIVITY

#### Description

Dihydrofluorescein diacetate is a fluorimetric probe mainly used for oxidative stress measurements, in both cell-free systems and cellular models.

#### In Vitro

Dihydrofluorescein diacetate may be a superior fluorescent probe for many cell-based studies. It is a better fluorescent probe for detecting intracellular oxidants because it is more reactive toward specific oxidizing species. Dihydrofluorescein diacetate demonstrates fluorescence of linear structures, consistent with mitochondria, in reoxygenated endothelium<sup>[1]</sup>. Dihydrofluorescein diacetate is able to detect the presence of ROS in mitochondria. Dihydrofluorescein diacetate fluorescence was sharp and delineated thin filaments which corresponded in all details to TMRM-stained mitochondria. It enters mitochondria and reacts with ROS released in the matrix<sup>[2]</sup>. Dihydrofluorescein diacetate could be an useful and quantitative method for measuring the oxidative potential of nanoparticle-treated cells<sup>[3]</sup>.  
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

### PROTOCOL

## Cell Assay

Dihydrofluorescein diacetate is used at a final concentration of 5  $\mu$ M for the cell-free studies. The excitation wavelength is 488 nm and the emission wavelength is 521 nm, the same as the excitation and emission wavelengths of the confocal microscope. All fluorescence measurements are performed on room temperature solutions. Human umbilical vein endothelium at first passage are cultured to confluence on 48 well plates. Cells are washed with PBS, then PBS with glucose, pH 7.4, is added to the cells. Dihydrofluorescein diacetate is added at a final concentration of 20  $\mu$ M. Wells without cells are also loaded with fluorescent probes to serve as controls. Fluorescence measurements are made on a fluorescent plate reader every 10 min at Ex $\lambda$  488 nm and Em $\lambda$  510 (bandpass 5 nm) and 538 nm (bandpass 10 nm). The Em $\lambda$  510 nm and Em $\lambda$  538 nm values are added together<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Food Hydrocolloid. 93 (2019) 261-269.

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## REFERENCES

- [1]. Hempel SL, et al. 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*. 1999 Jul;27(1-2):146-59.
- [2]. Diaz G, et al. Mitochondrial localization of reactive oxygen species by dihydrofluorescein probes. *Histochem Cell Biol*. 2003 Oct;120(4):319-25.
- [3]. Aranda A, et al. Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay: a quantitative method for oxidative stress assessment of nanoparticle-treated cells. *Toxicol In Vitro*. 2013 Mar;27(2):954-63.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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