



# SZABO SCANDIC

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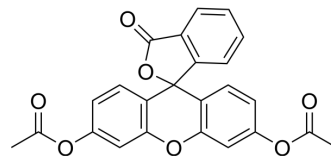
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## Fluorescein Diacetate

Cat. No.:	HY-D0719
CAS No.:	596-09-8
Molecular Formula:	C <sub>24</sub> H <sub>16</sub> O <sub>7</sub>
Molecular Weight:	416.38
Target:	Biochemical Assay Reagents
Pathway:	Others
Storage:	-20°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 26.67 mg/mL (64.05 mM; Need ultrasonic)						
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg	
				1 mM	2.4017 mL	12.0083 mL	24.0165 mL
				5 mM	0.4803 mL	2.4017 mL	4.8033 mL
				10 mM	0.2402 mL	1.2008 mL	2.4017 mL
Please refer to the solubility information to select the appropriate solvent.							
In Vivo	1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (5.00 mM); Clear solution						
	2. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.08 mg/mL (5.00 mM); Clear solution						

### BIOLOGICAL ACTIVITY

Description	Fluorescein diacetate is a cell permeable esterase-substrate. Fluorescein diacetate can be used as a fluorogenic substrate for hGSTP1-1.
In Vitro	Fluorescein diacetate (FDA) is an acetylated derivative of the green fluorescent dye fluorescein <sup>[1]</sup> . Fluorescein diacetate (FDA), a fluorescent probe used for vital staining, is a fluorescently activated by esterolytic activity of human Pi-class glutathione S-transferase (hGSTP1) selectively among various cytosolic GSTs. Fluorescence activation of Fluorescein diacetate susceptible to GST inhibitors is observed in MCF7 cells exogenously overexpressing hGSTP1, but not in cells overexpressing hGSTA1 or hGSTM1. Fluorescein diacetate can be used as a fluorogenic substrate for hGSTP1-1. To investigate whether the fluorescence activation is due to hGSTP1 activity, Fluorescein diacetate is incubated with recombinant hGSTP1-1 and GSH in vitro. Remarkable fluorescence activation is observed in the presence of both hGSTP1-1 and GSH, whereas only slight activation is observed in the absence of either of them or when the enzyme is heat inactivated.

This suggests that the fluorescence activation of Fluorescein diacetate depends on hGSTP1-1 activity. From the linear relationship between the rate of increase in fluorescence and the hGSTP1-1 concentration, the specific activity of the enzyme for 1  $\mu\text{M}$  Fluorescein diacetate is determined to be  $79 \pm 15$  nmol/min/mg protein. Fluorescein diacetate is applicable as a fluorogenic substrate for evaluating inhibitors of GSTP1-1 in vitro. For Fluorescein diacetate as a substrate, both Ethacrynic acid (EA) and NBDHEX suppress the hGSTP1-1-dependent fluorescent increase in a concentration-dependent manner, with  $\text{IC}_{50}\text{s}$  of  $3.3 \pm 0.5$   $\mu\text{M}$  and  $0.61 \pm 0.04$   $\mu\text{M}$ , respectively<sup>[2]</sup>.

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

## PROTOCOL

### Cell Assay <sup>[2]</sup>

MCF7 cells ( $2-3 \times 10^5$ ) are seeded in a 35-mm glass bottom dish before the experiment. Prior to imaging, cells are washed with 1 mL PBS, then incubated in 1 mL Hanks' Balanced Salt solution (HBSS(+)) without phenol red) containing 1  $\mu\text{M}$  Fluorescein diacetate (0.1% DMSO as a cosolvent) for 5 min at 37°C. Cells are washed twice with 1 mL PBS and 1 mL HBSS is added before imaging. Fluorescence images of fluorescein and DsRed-Express2 were acquired in the FITC channel (excitation at 473 nm) and the DsRed channel (excitation 559 nm). The 16-bit images obtained are analysed<sup>[2]</sup>.

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

## CUSTOMER VALIDATION

- ACS Appl Mater Interfaces. 2023 Jun 20.
- Cell Death Discov. 2021 Dec 4;7(1):374.

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## REFERENCES

[1]. Boyd V, et al. Limitations in the Use of Fluorescein Diacetate/Propidium Iodide (FDA/PI) and Cell Permeable Nucleic Acid Stains for Viability Measurements of Isolated Islets of Langerhans. *Curr Trends Biotechnol Pharm.* 2008 Mar;2(2):66-84.

[2]. Fujikawa Y, et al. Fluorescein diacetate (FDA) and its analogue as substrates for Pi-class glutathione S-transferase (GSTP1) and their biological application. *Talanta.* 2018 Mar 1;179:845-852.

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

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