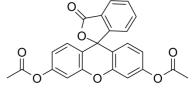


Data Sheet

Product Name: Fluorescein Diacetate

Cat. No.: CS-3540
CAS No.: 596-09-8
Molecular Formula: C24H16O7
Molecular Weight: 416.38
Target: Others
Pathway: Others

Solubility: DMSO: 26.67 mg/mL (64.05 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

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^[1]. 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 μM Fluorescein diacetate is determined to be 79±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 IC₅₀s of 3.3±0.5 μM and 0.61±0.04 μM, respectively^[2].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: $[^2]$ MCF7 cells (2-3×10⁵) 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 μ 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 $[^2]$.

References:

[1]. Boyd V, et al. Limitations in the Use of Fluorescein Diacetate/Propidium Iodide (FDA/PI) and Cell PermeableNucleic 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.

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