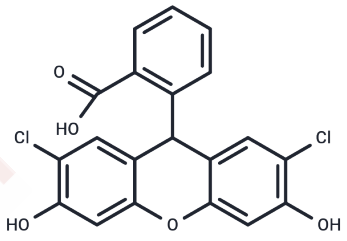
Data Sheet (Cat.No.T85404)

TargetMol

2,7-Dichlorodihydrofluorescein

Chemical Properties



CAS No. : 106070-31-9

Formula: C20H12Cl2O5

Molecular Weight: 403.21

Storage: Keep away from direct sunlight
Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	2,7-Dichlorodihydrofluorescein (DCFH) is a non-fluorescent probe that is oxidized by intracellular reactive oxygen species (ROS) to produce the highly fluorescent 2',7'-Dichlorofluorescein (DCF).DCFH is commonly used to assay the intracellular level of ROS, to detect ROS production and to analyze oxidative stress.
Targets(IC50)	Others
In vitro	Instructions I. Solution preparation 1. Preparation of stock solution: Dissolve 4.85 mg 2,7-Dichlorodihydrofluorescein in 1 mL dimethyl sulfoxide (DMSO) to make a 10 mM stock solution. 2. Preparation of working solution: Dilute the stock solution to a 10 μM working solution with pre-warmed DMEM before adding it to the wells. II. Operation steps 1. Cell seeding Inoculate 2 × 10^5 HCT116 colorectal cancer cells per well in a 24-well plate and maintain the cells in Dulbecco's modified Eagle's medium (DMEM) at 37 °C overnight. 2. 2,7-Dichlorodihydrofluorescein staining 1) Remove the medium containing the drug and wash once with DMEM. 2) Add 500 μL of 2,7-Dichlorodihydrofluorescein working solution to each well and incubate at 37 °C for 30 minutes. 3) Remove the 2,7-Dichlorodihydrofluorescein working solution, wash once with DMEM and twice with 1× phosphate-buffered saline (PBS). 4) Add 500 μL of 1× PBS to each well. 3. Imaging acquisition and intensity measurement 1) Take representative fluorescence images for each well using the green fluorescent protein (GFP) channel on a fluorescence microscope. 2) After taking the image, remove the PBS and add 200 μL of radioimmunoprecipitation assay (RIPA) buffer to each well. 3) Incubate on ice for 5 minutes, then collect the cell lysate into a 1.5 mL tube. 4) Centrifuge at 21,130 × g for 10 minutes at 4 °C. 5) Transfer 100 μL of supernatant to a black 96-well plate and measure the fluorescence intensity using a fluorescence microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. 6) Transfer 1 μL of supernatant to a clear 96-well plate containing 100 μL of 1× protein

In vitro	assay solution to measure protein concentration using Bradford assay. 7) Normalize the fluorescence intensity with the protein concentration. The above information is based on published literature. Experimental procedures should be appropriately modified to meet specific research demands.

Solubility Information

Solubility	DMSO: 250 mg/mL (620.02 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.4801 mL	12.4005 mL	24.801 mL
5 mM	0.496 mL	2.4801 mL	4.9602 mL
10 mM	0.248 mL	1.240 mL	2.4801 mL
50 mM	0.0496 mL	0.248 mL	0.496 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Reiniers MJ, et al. Preparation and Practical Applications of 2',7'-Dichlorodihydrofluorescein in Redox Assays. Anal Chem. 2017 Apr 4;89(7):3853-3857.
Chen X, et al. 2',7'-Dichlorodihydrofluorescein as a fluorescent probe for reactive oxygen species measurement: Forty years of application and controversy. Free Radic Res. 2010 Jun;44(6):587-604.
Kim H, et al. Detection of Total Reactive Oxygen Species in Adherent Cells by 2',7'-Dichlorodihydrofluorescein Diacetate Staining. J Vis Exp. 2020 Jun 23;(160):10.3791/60682.