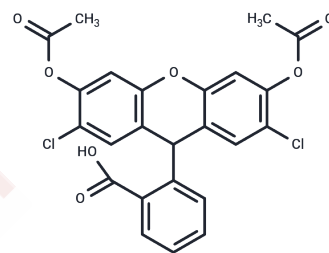


H2DCFDA

Chemical Properties

CAS No. :	4091-99-0
Formula:	C ₂₄ H ₁₆ Cl ₂ O ₇
Molecular Weight:	487.29
Appearance:	no data available
Storage:	keep away from direct sunlight, store at low temperature
	Powder: -20°C for 3 years In solvent: -80°C for 1 year



Biological Description

Description	H2DCFDA (DCFH-DA) belongs to the class of green fluorescent dyes and is a probe for the detection of intracellular reactive oxygen species (ROS) (Ex/Em=488/525 nm) with cell membrane permeability.
Targets(IC50)	Reactive Oxygen Species
In vitro	<p>METHODS: Flow cytometry was used to detect ROS levels:</p> <ol style="list-style-type: none"> 1, H2DCFDA was dissolved into 10 mM DMSO stock solution and further diluted with PBS before use. 2. Adherent cells are incubated with 5 μM H2DCFDA solution for 30 min at 37°C, protected from light, then harvested with 0.05% trypsin-EDTA solution, suspended in fresh medium and immediately analyzed by flow cytometry (488 nm). [1] <p>METHODS: Confocal microscopy was performed to detect ROS levels:</p> <ol style="list-style-type: none"> 1. H2DCFDA was dissolved into 10 mM DMSO stock solution and further diluted with PBS before use. 2. Coverslips containing cells were placed in 5 μM H2DCFDA staining solution and incubated for 60 min at 37°C, protected from light, then washed and imaged with a confocal laser scanning microscope Leica TCS SL equipped with an argon laser. [1]
In vivo	<p>METHODS: Fluorescence microscopy was used to analyze the oxidative activity of LPS induced peritonitis in mice:</p> <ol style="list-style-type: none"> 1, H2DCFDA was dissolved in 100 μL ethanol and further diluted with PBS before use. 2. C57BL/6J mice were injected intraperitoneally with LPS (0.1-1 mg/mL) to induce peritonitis. 3.5 h later, H2DCFDA (0.1-0.8 mg/ml) was injected intraperitoneally. 3. 30 min after H2DCFDA injection, the animals were killed by cervical dislocation, and the peritoneal cells were recovered by rinsing with 5 mL of ice-cold HBSS solution at pH 7.4. 4. Peritoneal cells were washed and resuspended in PBS. Macrophages were removed by adhesion method after incubation in a polystyrene dish at 37°C for 30 min. The supernatant was recovered and approximately 20,000-25,000 leukocytes were added to microscope slides using a cytocentrifuge. The slides were then analyzed using fluorescence microscopy. [2]
Cell Research	<p>Instructions:</p> <ol style="list-style-type: none"> I. Solution preparation <ol style="list-style-type: none"> 1. Prepare storage solution: Prepare 10 mM H2DCFDA stock solution with DMSO.

Note: It is recommended to store H2DCFDA storage solution at -20 °C or -80 °C in the dark after aliquoting.

2. Preparation of working solution: Dilute the storage solution with preheated serum-free cell culture medium or PBS to prepare 1-10 μ M H2DCFDA working solution.

Note: Please adjust the concentration of H2DCFDA working solution according to actual conditions and prepare it before use.

II. Cell staining (suspended cells)

1. Collect cells by centrifugation and wash twice with PBS for 5 minutes each. The cell density is 1×10^6 /mL.

2. Add 1 mL of dye working solution and incubate at room temperature for 5-30 minutes.

3. Centrifuge at 400 g for 3-4 minutes and discard the supernatant.

4. Add PBS to wash the cells twice, 5 minutes each time.

5. Resuspend the cells with 1 mL serum-free medium or PBS, and observe using a fluorescence microscope or flow cytometer.

III. Cell staining (adherent cells)

1. Culture the adherent cells on a sterile coverslip.

2. Remove the coverslip from the culture medium and remove the excess culture medium.

3. Add 100 μ L of dye working solution, gently shake to completely cover the cells, and incubate for 5-30 minutes.

4. Aspirate the dye working solution, wash 2-3 times with culture medium, 5 minutes each time, and observe using a fluorescence microscope or flow cytometer.

Note: If flow cytometry is required, the cells need to be digested with trypsin and resuspended before staining.

Solubility Information

Solubility	Ethanol: 14.29 mg/mL (29.33 mM), Sonication is recommended. DMSO: 50 mg/mL (102.61 mM), Sonication is recommended. 10% DMSO+40% PEG300+5% Tween 80+45% Saline: 5 mg/mL (10.26 mM), Solution. (< 1 mg/mL refers to the product slightly soluble or insoluble)
------------	--

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.0522 mL	10.2608 mL	20.5217 mL
5 mM	0.4104 mL	2.0522 mL	4.1043 mL
10 mM	0.2052 mL	1.0261 mL	2.0522 mL
50 mM	0.041 mL	0.2052 mL	0.4104 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.

Reference

- Lyublinskaya OG, et al. Redox environment in stem and differentiated cells: A quantitative approach. *Redox Biol.* 2017 Aug;12:758-769.
- Zhang J, Liu P, Chen J, et al. Upgrade of chrysomycin A as a novel topoisomerase II inhibitor to curb KRAS-mutant lung adenocarcinoma progression. *Pharmacological Research.* 2022: 106565.
- Zhang Y, Cai Y, Jin X, et al. Persistent glucose consumption under antibiotic treatment protects bacterial community. *Nature Chemical Biology.* 2024: 1-9.
- Zhang Y, Cai Y, Zhang B, et al. Spatially structured exchange of metabolites enhances bacterial survival and resilience in biofilms. *Nature Communications.* 2024, 15(1): 7575.
- Liang W, Liu G, Zhou W, et al. Astaxanthin mediated repair of tBHP-Induced cellular injury in chondrocytes. *Redox Report.* 2024, 29(1): 2422271.
- Linda S, et al. Method for analysis of intracellular production of free radicals in vivo. SWEDISH DEFENCE RESEARCH AGENCY. 2001 Mar.
- Hu Y, Wen Q, Cai Y, et al. Alantolactone induces concurrent apoptosis and GSDME-dependent pyroptosis of anaplastic thyroid cancer through ROS mitochondria-dependent caspase pathway. *Phytomedicine.* 2022: 154528.
- Zhang Y, Cai Y, Wang T, et al. A common tolerance mechanism of bacterial biofilms to antibiotics. *bioRxiv.* 2023: 2023.01. 30.526163.
- Pan Y, Wang X, Tan Q, et al. Effects and Mechanisms of Prussian Blue Nanozymes with Multiple Enzyme Activities on Nasopharyngeal Carcinoma Cells. *Tissue and Cell.* 2024: 102316.
- Guo Y, Zhu L, Duan Y, et al. Ruxolitinib induces apoptosis and pyroptosis of anaplastic thyroid cancer via the transcriptional inhibition of DRP1-mediated mitochondrial fission. *Cell Death & Disease.* 2024, 15(2): 125.
- Zhou X, Qin M, He L, et al. Geraniin restricts inflammasome activation and macrophage pyroptosis by preventing the interaction between ASC and NLRP3 to exert anti-inflammatory effects. *International Immunopharmacology.* 2024, 129: 111656.
- Yong Y Y, Yan L, Wang B D, et al. *Penthorum chinense Pursh* inhibits ferroptosis in cellular and *Caenorhabditis elegans* models of Alzheimer's disease. *Phytomedicine.* 2024: 155463.
- Liu Z, Dang B, Li Z, et al. Baicalin attenuates acute skin damage induced by ultraviolet B via inhibiting pyroptosis. *Journal of Photochemistry and Photobiology B: Biology.* 2024: 112937.
- Li X, Mao C, Sun G, et al. Oleic and linoleic acids induce oxidative stress in chondrocytes by inhibiting autophagy-regulated ciliogenesis. *View.* 2024: 20240019.

Inhibitor · Natural Compounds · Compound Libraries · Recombinant Proteins

This product is for Research Use Only · Not for Human or Veterinary or Therapeutic Use

Tel: 781-999-4286 E_mail: info@targetmol.com Address: 36 Washington Street, Wellesley Hills, MA 02481