H2DCFDA

Cat. No.: HY-D0940

CAS No.: 4091-99-0

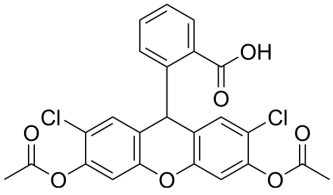
Molecular Formula: $C_{24}H_{16}Cl_2O_7$

Molecular Weight: 487.29

Target: Reactive Oxygen Species

Pathway: Immunology/Inflammation; Metabolic Enzyme/Protease; NF-κB

Storage: -20°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (256.52 mM; Need ultrasonic)
Ethanol : 20 mg/mL (41.04 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
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

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: 2.08 mg/mL (4.27 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (4.27 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (4.27 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

H2DCFDA (DCFH-DA) is a cell-permeable probe used to detect intracellular reactive oxygen species (ROS) (Ex/Em=488/525 nm)[1].

In Vitro

Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs).
1. H2DCFDA is dissolved in DMSO to obtain a 10 mM stock solutions and further diluted before use.
2. Cells are incubated with 5 μM H2DCFDA solution in PBS in the dark for 30 min at 37°C, then harvested with 0.05% trypsin-EDTA solution, suspended in a fresh medium, and immediately analyzed with flow cytometer.

3. Along with the H2DCFDA probe, if indicated, use ROS-insensitive modification of the fluorescein dye DCFDA as a positive control. The staining procedure is the same as for the H2DCFDA[1].

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

PROTOCOL

Kinase Assay

ROS Measurements[1]

For the detection of intracellular ROS level ,ROS-sensitive probe H2DCFDA is used. Adherent cells (ESCs, difESCs, eMSCs, HeLa, U118) are incubated with 5 μM staining solution in PBS in the dark for 30 min at 37°C, then harvested with 0.05% trypsin-EDTA solution, suspended in a fresh medium, and immediately analyzed with flow cytometer. Lymphocytes, both control and PHA-activated, are resuspended in PBS, incubated with 5 μM of H2DCFDA in the dark for 30 min at 37°C, and immediately analyzed. Along with the H2DCFDA probe, if indicated, ROS-insensitive modification of the fluorescent dye DCFDA is used. The staining procedure is the same as for the H2DCFDA[1].

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

CUSTOMER VALIDATION

- Cell. 2021 Jun 24;184(13):3528-3541.e12.
- Signal Transduct Target Ther. 2022 Aug 15;7(1):288.
- Adv Mater. 2023 Sep 5;e2306469.
- Drug Resist Updat. 2023 Jul;69:100974.
- Cell Mol Immunol. 2023 Aug 9.

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REFERENCES

[1]. Lyublinskaya OG, et al. Redox environment in stem and differentiated cells: A quantitative approach. Redox Biol. 2017 Aug;12:758-769.

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