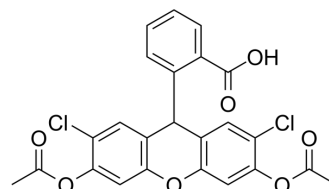


## H2DCFDA

<b>Cat. No.:</b>	HY-D0940
<b>CAS No.:</b>	4091-99-0
<b>Molecular Formula:</b>	C <sub>24</sub> H <sub>16</sub> Cl <sub>2</sub> O <sub>7</sub>
<b>Molecular Weight:</b>	487.29
<b>Target:</b>	Reactive Oxygen Species
<b>Pathway:</b>	Immunology/Inflammation; Metabolic Enzyme/Protease; NF-κB
<b>Storage:</b>	-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 Concentration	Mass		
		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

- 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
- 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
- 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)<sup>[1]</sup>.

#### In Vitro

- Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs).
- H2DCFDA is dissolved in DMSO to obtain a 10 mM stock solutions and further diluted before use.
  - 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<sup>[1]</sup>.  
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## PROTOCOL

### Kinase Assay

#### ROS Measurements<sup>[1]</sup>

For the detection of intracellular ROS level, ROS-sensitive probe H2DCFDA is used. Adherent cells (ESCs, difESCs, eMSCs, HeLa, U118) are incubated with 5  $\mu$ M 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  $\mu$ M 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<sup>[1]</sup>.

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.
- Adv Sci (Weinh). 2022 Mar 10;e2103317.
- ACS Nano. 2021 Oct 26;15(10):16286-16297.
- Signal Transduct Target Ther. 2022 Aug 15;7(1):288.
- Small. 2022 Jul 1;e2202002.

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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.

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

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