

Product Data Sheet

Product Name: H2DCFDA (DCFH-DA)

Cat. No.: GC30006

Chemical Properties

Cas. No. 4091-99-0

SMILES O=C(O)C1=CC=CC=C1C2C3=C(OC4=C2C=C(Cl)C(OC(C)=O)=C4)C=C(OC(C)=O)C(Cl)=C3

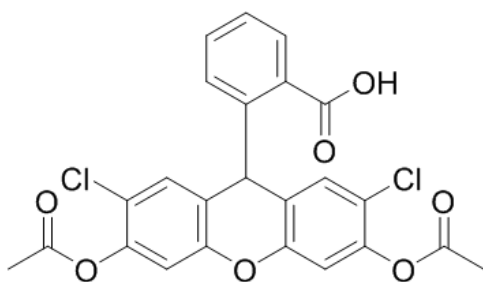
Formula $C_{24}H_{16}Cl_2O_7$ M.Wt 487.29

Solubility ≥ 150 mg/mL in DMSO(307.82 mM); Storage Store at $-20^{\circ}C$, protect from light
14.29 mg/mL in Ethanol(29.33 mM);
< 0.1 mg/mL in Water(insoluble)

General tips For obtaining a higher solubility , please warm the tube at $37^{\circ}C$ and shake it in the ultrasonic bath for a while.Stock solution can be stored below $-20^{\circ}C$ for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



Protocol

This plan for DCFH-DA only provides a guide, please modify it to meet your specific needs.

1. Preparation of staining solution

(1) Prepare stock solution: Dissolve H2DCFDA (DCFH-DA) in DMSO and prepare a stock solution with a concentration of 1-10mM.

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Note: Unused stock solution of DCFH-DA should be aliquoted and stored in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

(2) Prepare working solution: Dilute the DCFH-DA stock solution with a suitable buffer (such as serum-free medium or PBS) and prepare a working solution with a concentration of 1-10μM.

Note: Please adjust the concentration of the working fluid according to the actual situation and ensure the DCFH-DA is prepared fresh for immediate use.

2. Cell suspension staining

(1) Suspension cells: Centrifuge at 1000g for 3-5 minutes at 4°C, discard the supernatant and wash twice with PBS for 5 minutes each time.

(2) Adherent cells: Wash twice with PBS, add trypsin to digest the cells, and centrifuge at 1000 g for 3-5 minutes after digestion is completed.

(3) Add H2DCFDA (DCFH-DA) working solution to resuspend the cells, and incubate at 37 °C in the dark for 5-30 minutes. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(4) After the incubation, centrifuge at 1000 g for 5 minutes, remove the supernatant, add PBS, and wash 2-3 times, 5 minutes each time.

(5) Incubate cells with pre-warmed serum-free cell culture medium or PBS.

3. Cell adhesion staining

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100 μL of dye working solution from one corner of the coverslip and shake gently to evenly cover all cells with the dye.

(4) Incubate at 37 °C in the dark for 5-30 minutes. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(5) After the incubation, discard the dye working solution and use pre-warmed culture solution to wash the coverslip 2 to 3 times.

(6) Incubate cells with pre-warmed serum-free cell culture medium or PBS.

4. Microscope detection: The maximum excitation/emission wavelength of H2DCFDA (DCFH-DA) is 488/525nm.

Precautions:

① For diacetate derivatives, a short recovery time is required for intracellular esterase to hydrolyze them, allowing the dye to respond to oxidative stress. There is a wide range of optimal recovery

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times, as certain cell types often display extremely low levels of esterase activity;

- ② Before exposing cells to experimental stimuli, the background fluorescence intensity of cells needs to be measured;
- ③ For flow cytometry analysis, the forward angle scattering and side angle scattering of cells are unchanged before and after dye treatment, and changes in cell size may be caused by drug treatment or toxic reactions;
- ④ It is recommended to perform fluorescence detection on experimental systems that do not contain cells. In the absence of extracellular esterases and other oxidases, the gradual increase in fluorescence over time may be related to factors such as instantaneous hydrolysis, air oxidation, and light-induced oxidation;
- ⑤ For control group (unmedicated) cells, intracellular enzymes or natural antioxidants will scavenge oxygen free radicals. After the dye loading recovery time, healthy cells should show a low-level fluorescence signal and be relatively stable during the entire experiment;
- ⑥ A gradual increase (due to autoxidation) or a decrease (due to intracellular dye loss or photoquenching) of the fluorescence signal may be observed;
- ⑦ In a system that does not contain any irritants or inducers, strong fluorescence is suddenly found in healthy and untreated cells, indicating cell death or some other oxidative events;
- ⑧ Fluorescent dyes all have quenching problems. Please try to avoid light to slow down fluorescence quenching;
- ⑨ For your safety and health, please wear a lab coat and disposable gloves.

Background

H2DCFDA(DCFH-DA) is a redox-sensitive fluorescent probe, which could be used to measure intracellular reactive oxygen species levels.^[1] The most popular method used to measure the level of cellular ROS formation is the 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA(DCFH-DA)) assay.

The fluorogenic dye H2DCFDA(DCFH-DA) was used to detect ROS production. Usually, after diffusion into the cell, H2DCFDA(DCFH-DA) is deacetylated by cellular esterases into a non-fluorescent compound that is subsequently oxidized by ROS into 2',7'-dichlorofluorescein (DCF). The in vitro experiment to determine the ability of TBBPA alone to stimulate the conversion of H2DCFDA(DCFH-DA) to its fluorescent product DCF was conducted in a cell-free model. Dilution of 5 μ M H2DCFDA(DCFH-DA) and increasing concentrations of TBBPA (0.1–100 μ M) were added to 96-well plates containing PBS buffer without Ca²⁺ and Mg²⁺ or serum-free DMEM/F12 or

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DMEM/F12 supplemented with 5 % FBS in the final volume of 100 μ L. The fluorescence was measured 30 and 60 min after the addition of TBBPA. The deacetylated and oxidized version of H2DCFDA(DCFH-DA): DCF 's fluorescence was detected at 485 and 535 nm of maximum excitation and emission spectra, respectively. This in vitro study examined the impact of TBBPA on H2DCFDA(DCFH-DA) fluorescence without cells in PBS buffer, DMEM/F12, and DMEM/F12 with 5 % of FBS media. The obtained results showed that TBBPA in all tested concentrations interacted with H2DCFDA(DCFH-DA) in PBS buffer and caused a significant increase in fluorescence. H2DCFDA(DCFH-DA) assay cannot be used in cell culture experiments with TBBPA. Results suggested that the data regarding TBBPA-stimulated ROS production in cell culture models using the H2DCFDA(DCFH-DA) assay should be revised using a different method. [3]

References:

- [1]. Park JH, Moon S-H, Kang DH, et al. Diquafosol sodium inhibits apoptosis and inflammation of corneal epithelial cells via activation of Erk1/2 and RSK: in vitro and in vivo dry eye model. Invest Ophthalmol Vis Sci. 2018;59:5108–5115. doi.org/ 10.1167/iovs.17-22925.
- [2]. Szychowski KA, Rybczyńska-Tkaczyk K, et al. Tetrabromobisphenol A (TBBPA)-stimulated reactive oxygen species (ROS) production in cell-free model using the 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) assay-limitations of method. Environ Sci Pollut Res Int. 2016 Jun;23(12):12246-52.
- [3]. Gomes A, Fernandes E, et al. Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Methods JLFC (2005) 65:45–80

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