DCFH-DA

Cat: ID3130

Storage: Powder: -20℃, 1 year; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year（protect from light）

Introduction

DCFH-DA is non-fluorescent and can freely pass through the cell membrane, and after entering the cell, it can be hydrolyzed by intracellular esterases to form DCFH, which is not permeable to the cell membrane, thus making it easy for the probe to be loaded into the cell. Intracellular reactive oxygen species can oxidize the non-fluorescent DCFH to produce fluorescent DCF, and the fluorescence of DCF can be detected to know the level of reactive oxygen species in the cell.

DCFH-DA is a cell-permeable probe for the detection of intracellular reactive oxygen species (ROS). DCFH-DA is not for use in sections or plant cells.

Parameter

CAS: 4091-99-0

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

Molecular Weight: 487.29

Solubility: Soluble in DMSO

Protocols (*only for reference*)

1. Preparation of storage solution

Prepare a 10 mM stock solution in DMSO. For example, 4.8729 mg of DCFH-DA powder was dissolved in 1 mL of DMSO.

Note:

a. Unused storage solution is recommended to be stored in portions at -20℃ to avoid repeated freezing and thawing.

b. Moisture-absorbing DMSO has a significant effect on the solubility of the product, use freshly opened DMSO.

2. Parameter setting

Using 488nm excitation wavelength and 525 nm emission wavelength, the intensity of fluorescence before and after stimulation can be detected in real time or time-point by time-point. The fluorescence spectrum of DCF is very similar to that of FITC, and DCF can be detected by using the parameter settings of FITC.

3. Loading probe

For cells with a short stimulation time (usually less than 2h), the probe is loaded first, followed by stimulation of the cells with an activated oxygen positive control or a drug of interest. For cells stimulated for a longer period of time (usually more than 6h), the cells are stimulated first with an activated oxygen positive control or a drug of interest, followed by loading of the probe. Usually the reactive oxygen positive control can significantly increase the reactive oxygen level after

stimulating the cells for 20-30 min.

In situ loading of probes: this method is only applicable to adherent wall culture cells

Dilute DCFH-DA with serum-free culture medium according to 1:1000 to make a final concentration of 10 μmol/L. Cells were collected and suspended in diluted DCFH-DA at a concentration of one to twenty million cells/mL, and incubated for 20 min at 37℃ in a cell culture incubator. Mixing was done upside down every 3-5 minutes to make full contact between the probe and the cells. Cells were washed three times with serum-free cell culture medium to adequately remove DCFH-DA that had not entered the cells. Cells were stimulated directly with a reactive oxygen positive control or a drug of your own interest, or after aliquoting the cells.

4. Detection

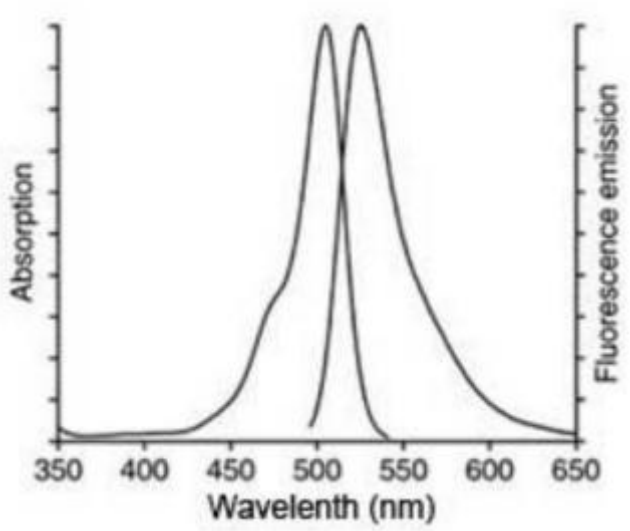
For samples loaded with probes in situ they can be observed directly with a laser confocal microscope or the cells can be collected and then detected with a fluorescence spectrophotometer, fluorescent enzyme marker or flow cytometer.

For samples loaded with probes after cell collection can be detected by fluorescence spectrophotometer, fluorescence zymography or flow cytometry, or can be observed directly by laser confocal microscopy.

Note

1. After probe loading, be sure to wash the residual probe that has not entered the cell, otherwise it will result in a higher background.
2. After the probe has been loaded and the residual probe has been washed, an excitation wavelength scan and an emission wavelength scan can be performed to confirm that the probe has been loaded properly. Please refer to the following figure for the excitation and emission spectra of DCF.
3. Minimize the time taken between the loading of the probe and the assay (except for the stimulation time) in order to reduce all possible errors.
4. For some cells, if it is found that the fluorescence of unstimulated negative control cells is also relatively strong, DCFH-DA can be diluted according to 1 : 2000 to 1 : 5000, so that the concentration of DCFH-DA at the time of loading the probe is 2 to 5 μmol/L. The time of probe loading can also be appropriately adjusted within 15 to 60 minutes, depending on the situation.
5. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
6. For your safety and health, please wear lab coat and disposable gloves.
7. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.

Spectrogram



Note: Excitation and emission spectra of DCF

Related Literature

[1]Tang Y, Wang K, Wu B, Yao K, Feng S, Zhou X, Xiang L. Photoelectrons Sequentially Regulate Antibacterial Activity and Osseointegration of Titanium Implants. Adv Mater. 2024 Jan;36(2):e2307756. doi: 10.1002/adma.202307756. Epub 2023 Nov 27. PMID: 37974525.（IF: 29.4）

[2]Wang Y, Zhang Y, Yang YP, Jin MY, Huang S, Zhuang ZM, Zhang T, Cao LL, Lin XY, Chen J, Du YZ, Chen J, Tan WQ. Versatile dopamine-functionalized hyaluronic acid-recombinant human collagen hydrogel promoting diabetic wound healing via inflammation control and vascularization tissue regeneration. Bioact Mater. 2024 Feb 14;35:330-345. doi: 10.1016/j.bioactmat.2024.02.010. PMID: 38379700（IF: 18.9）

Note: For more literature, please visit the Solarbio official website.