FT-467312

H_2DCFDA

Green reactive oxygen species (ROS) detection

Product Information

Product name cat.number	Structure	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em.\ max}$* (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Soluble in
H_2DCFDA 2',7'-Dichlorodihydrofluorescein diacetate FP-467312, 100 mg	$C_{24}H_{16}Cl_2O_7$	487.26	292/none	59 500	DMSO, DMF, EtOH
H_2DCFDA-SE 2',7'-Dichlorodihydrofluorescein diacetate, succinimidyl ester FP-59031A, 5 mg	$C_{28}H_{19}Cl_2NO_9$	584.36	301/none	59 500	DMSO, DMF, CH3CN and basic H2O
Carboxy-H_2DCFDA 5(6)-Carboxy-2',7'-Dichlorodihydrofluorescein diacetate FP-46634A, 25 mg	$C_{25}H_{16}Cl_2O_9$	531.29	290/none	5600	DMSO, DMF basic H_2O
6-Carboxy-H_2DCFDA, AM 6-Carboxy-2',7'-dichlorodihydrofluorescein diacetate diacetoxymethyl ester FP-963055, 5 mg	$C_{31}H_{24}Cl_2O_{13}$	675.42	292/none	5850	DMSO, DMF, CH_3CN and $CHCl_3$

* Dihydrofluorescein diacetates are nonfluorescent until both of the acetate groups are hydrolyzed and the products are subsequently oxidized to fluorescein derivatives. The oxidation products are 2',7'-dichlorofluorescein derivatives with $\lambda_{exc}\backslash\lambda_{em.\ max}$(pH 4) = 495 / 529 and $\lambda_{exc}\backslash\lambda_{em.\ max}$(pH 8) = 504 / 529

Storage: –20°C >1 year. (M) Protect from light and moisture

Applications: ROS detection, viability and cytotoxicity assays, apoptosis.

Introduction

H_2DCFDA is a standard probe to detect reactive oxygen species (COO-, ONOO-) in cells (neutrophils, macrophages).
It is colorless and non fluorescent until cleavage of the diacetate group. It is non-polar compound which is hydrolyzed by intracellular esterases to become a non-fluorescent polar derivative (H2DCF), which is oxidized rapidly to give the highly green fluorescent 2',7'-dichlorofluorescein (FP-46629A) in the presence of intracellular ROS.

It can be used with Propidium iodide or Ethidium Homodimer III to follow oxidant production and nuclear injury.

H_2DCFDA-SE is an amine-reactive probe for preparation of oxidation-sensitive conjugates.

LIFE SCIENCES
interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B

FT-467312

Carboxy-H_2DCFDA is a fluorogenic probe for detection of reactive oxygen intermediates in neutrophils and macrophages. Cell-permeable H_2DCFDA may also be useful for assessing the overall oxidative stress in toxicological phenomena. The hydrolyzed dye fluoresces on interaction with reactive oxygen species, including hydrogen peroxide and hydroxyl radical but not superoxide anion. Its additional negative charges improve its retention compared to non-carboxylated forms.

Carboxy-H_2DCFDA, AM is for detection of cytosolic reactive oxygen molecules.

Directions for use

Handling and Storage

It can be dissolved in EtOH, DMSO and DMF at least 10 mg/ml. DMF is probably a better solvent for better stability. The stock solution is stable at least 6 months at –20°C. If H_2DCFDA is prepared in aqueous buffer, dissolve before in 0.1M Na_2CO_3 and then in PBS to the desired concentration or pH. Do not store more one day.

Protocol 1 (r) - Guidelines for use – in Flow cytometer

1- Treat 2 × 10^6 cells (in PBS) were harvested with 1× trypsin-EDTA.

2- Cells were subsequently washed again with PBS and then re-suspended in 1 ml of PBS. Incubate with H_2DCFDA (0.5 to 1.0 µM) in the dark at 37°C for 30 min.

3- Cells were then washed twice with PBS and analyzed Flow Cytometer.

Protocol 2 - Guidelines for use - Determination of intracellular ROS levels **in spectrofluorometer.** (Pines, 2005 **r)**

1- Seed the cells at a density of 40 000 cells/cm^2.

2- Remove the medium with serum, wash the cells in the plates with PBS.

3- Incubate in 5% CO_2/95% air at 37°C and expose eventually to specific stimulus.

4- Add H_2DCFDA at 10 µM for 30 min before finishing cellular treatment. The cells were washed twice with PBS, harvested with trypsin and centrifuged at 800 *g* for 5 min and finally washed twice in PBS. Measure the fluorescence intensity with a spectrofluorometer with the excitation and emission wavelengths at 505 and 530 nm, respectively.

Protocol 3 (r) - Guidelines for use – on primary bovine aortic endothelial cells (BAEC) in confocal microscopy

1- Incubate Seed BAEC on fibronectin-coated, eight-chamber tissue culture glass slides at concentrations of 10 000-20 000 cells/well. Cells were pretreated with potent antioxidants for 1 h.

2- Remove the medium and add PBS supplemented with H2DCFDA (5 µM) and TNF-α.

3- Follow H2DCFDA oxidation in the cultures with confocal microscopy. The system was equipped with an air-cooled argon laser. Fluorescent images were collected by using a 63 × 1.2 C-apochromate water-immersion lens (Zeiss), and oxidation level was standardized to fluorescence per cell number by using image-pro software.

Related products

- Propidium Iodide, 10mg/ml, FP-36774A
- Hoechst 33342, 10mg/ml, FP-59046A
- Ethidium Homodimer III, 1mM, FP-BP9341
- *tert*-Butyl hydroperoxide (TBHP), 07016E
- Dimethylsulfoxide (DMSO), FP-JW7390
- Carboxy-H_2DFFDA, FP-M1984A

References

H2DCFDA

- **Brubacher JL**, *et al.* « Chemically de-acetylated 2',7'-dichlorodihydrofluorescein diacetate as a probe of respiratory burst activity in mononuclear phagocytes. », *J Immunol Methods*, **251**(1-2),81 (2001) Abstract

LIFE SCIENCES interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B

FT-467312

- **Crow, J.P.**, « Dichlorodihydrofluorescein and dihydrorhodamine 123 are sensitive indicators of peroxynitrite in vitro: Implications for intracellular measurement of reactive nitrogen and oxygen species. », *Nitric Oxide: Biology and Chemistry* 1, 145 (1997).
- **Fribley A.**, *et al.*, « Proteasome Inhibitor PS-341 Induces Apoptosis through Induction of Endoplasmic Reticulum Stress-Reactive Oxygen Species in Head and Neck Squamous Cell Carcinoma Cells », *Mol Cell Biol*., **24**(22), 9695 (2004) Article
- **Honda S**, *et al.*,« The use of hyperoxia to induce chronic mild oxidative stress in RPE cells in vitro », *Molecular Vision*, **7**, 63 (2001) Article
- **Hamano T**., *et al.*, « The molecular mechanism in activation-induced cell death of an Ag-reactive B cell clone », *Clinical & Experimental Immunology*, **128**, 436 (2002) Article
- **Kooy N.W**., *et al.*, « Oxidation of 2',7'-dichlorofluorescin by peroxynitrite. », *Free Rad.Res.* **27**, 245 (1997).
- **Pines A.**, *et al*., « Activation of APE1/Ref-1 is dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP », *Nucleic Acids Res.*, 33, 4379 (2005) Article
- **Woo Ju-Hyung**, *et al.*, **«** Molecular mechanisms of curcumin-induced cytotoxicity: induction of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-X_L and IAP, the release of cytochrome *c* and inhibition of Akt », *Carcinogenesis*, **24**, 1199 (2003)Article

H2DCFDA-SE

- **Dong Xu**, et al., Mitochondrial aldehyde dehydrogenase attenuates hyperoxia-induced cell death through activation of ERK/MAPK and PI3K-Akt pathways in lung epithelial cells, Am J Physiol Lung Cell Mol Physiol; 291: L966 - L975 (2006) Article

carboxy-H_2DCFDA

- **Collesa S**. *et al*., Lysophosphatidylcholine-induced cellular injury in cultured fibroblasts involves oxidative events, *Journal of Lipid Research*, Vol. 41, 1188-1198 (2000)
- **Egan M**. *et al*., Generation of reactive oxygen species by fungal NADPH oxidases is required for rice blast disease, *PNAS* vol. 104, no. 28:11772-11777 (2007) Abstract
- **Lambert I**., Activation and inactivation of the volume-sensitive taurine leak pathway in NIH3T3 fibroblasts and Ehrlich Lettre ascites cells, *Am J Physiol Cell Physiol* 293: C390-C400 (2007) Abstract
- **Mattiazzi M**. *et al*., The mtDNA T8993G (NARP) mutation results in an impairment of oxidative phosphorylation that can be improved by antioxidants, *Human Molecular Genetics*, Vol. 13, No. 8 869-879 (2004)

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

Rev.F09VB

LIFE SCIENCES
interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B