

DCFDA / H2DCFDA - Cellular ROS Assay Kit ab113851

★★★★★ [11 Abreviews](#) [605 References](#) [画像数 8](#)

製品の概要

製品名	DCFDA / H2DCFDA - Cellular ROS Assay Kit
検出方法	Fluorescent
サンプルの種類	Adherent cells, Suspension cells
アッセイタイプ	Cell-based (quantitative)
全工程の試験時間	0h 40m
製品の概要	DCFDA - Cellular ROS Assay Kit / Reactive Oxygen Species Assay Kit (ab113851) uses the cell permeant reagent 2',7' -dichlorofluorescein diacetate (DCFDA, also known as H2DCFDA, DCFH-DA, and DCFH) to quantitatively assess reactive oxygen species in live cell samples.

DCFDA / H2DCFDA / DCFH-DA / DCFH is a fluorogenic dye that measures hydroxyl, peroxy and other reactive oxygen species (ROS) activity within the cell. NB: DCFDA and DCFA are also available as free molecules as [ab145286](#) (Carboxy-DCFDA N-succinimidyl ester) and [ab145439](#) (5(6)-Carboxy-2',7'-dichlorofluorescein).

The DCFDA assay protocol is based on the diffusion of DCFDA / H2DCFDA / DCFH-DA / DCFH into the cell. It is then deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2', 7' -dichlorofluorescein (DCF). DCF is highly fluorescent and is detected by fluorescence spectroscopy with excitation / emission at 485 nm / 535 nm.

DCFDA assay protocol / ROS assay protocol summary (microplate):

- collect suspension cells in tube / seed and allow attachment of adherent cells in 96-well plate
- wash in buffer
- stain with DCFDA for 30 min (suspension) / 45 min (adherent), wash with buffer
- if suspension cells, transfer to microplate
- analyze with microplate reader

DCFDA assay protocol / ROS assay protocol summary (flow cytometry):

- collect cells in tubes
- stain with DCFDA for 30 min (without washing)
- analyze with flow cytometer

DCFDA assay protocol / ROS assay protocol summary (fluorescent microscopy):

- wash adherent cells with buffer

- stain with DCFDA for 45 min
- wash in buffer
- analyze with fluorescent microscope
- maintain low light conditions to reduce photo-bleaching

特記事項

Previously called DCFDA / H2DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit.

This kit contains sufficient materials for approximately 300 measurements in microplate format and 70 measurements (35 mL) by flow cytometry. The number of measurements in microscopy is dependent on experimental setup.

This kit is not compatible with fixed samples. Stained cells must be measured live.

Related products

Review the [oxidative stress marker and assay guide](#), or the full [metabolism assay guide](#) to learn about more assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also how to assay metabolic function in live cells using your plate reader.

For reactive oxygen species, the most popular live cell ROS assay is DCFDA Assay [ab113851](#) (green). Alternative ROS assays are available in orange ([ab186028](#)), red ([ab186027](#)), and deep red ([ab186029](#)). [ab238535](#) is used to measure ROS in biofluids, culture supernatants and cell lysates.

For assays designed to differentiate ROS, superoxides, and reactive nitrogen species: to assay ROS and superoxides use [ab139476](#); to assay ROS, superoxides, and reactive nitrogen species use [ab139473](#); to assay superoxides use [ab219943](#).

試験プラットフォーム

Microplate reader, Fluor. microscope, Flow cyt.

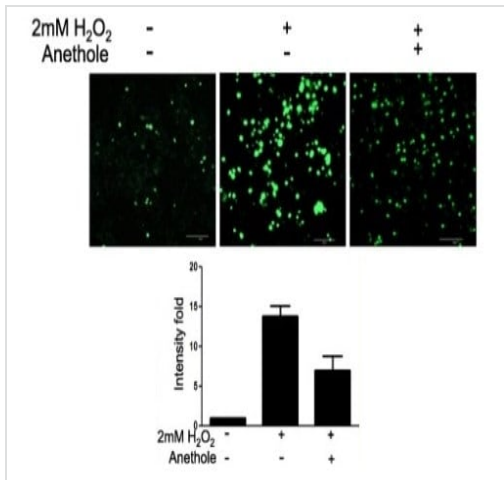
製品の特性

保存方法

Store at -20°C. Please refer to protocols.

内容	300 tests
10X Dilution Buffer (Sterile)	1 x 10ml
20 mM DCFDA (Label)	1 x 35µl
55mM TBHP	1 x 50µl

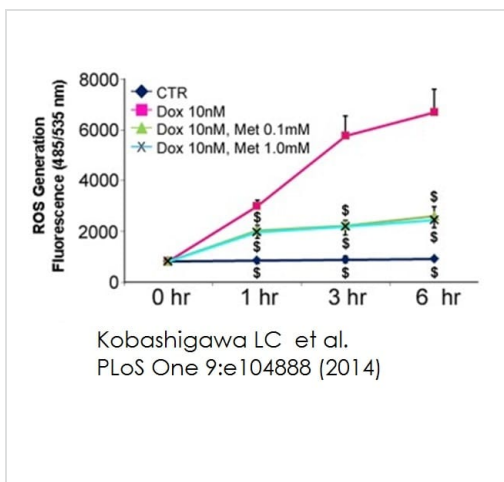
画像



Effect of anethole on excessive ROS generation in hMSCs.

Image from Rhee YH et al., BMC Cell Biol, 2018, 19: 12, Fig. 3B.; doi: 10.1186/s12860-018-0163-2 Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Effect of anethole on excessive ROS generation in hMSCs. hMSCs were exposed at 2 mM H₂O₂ for 30 min and incubated for 2 days in presence or absence of 50 µM anethole. ROS was measured by staining the cells with DCFDA cellular ROS detection assay kit according to the manufacturer's instructions. ROS generation was observed under a fluorescence microscope at 200× magnification.



Kobashigawa LC et al. PLoS One 9:e104888 (2014)

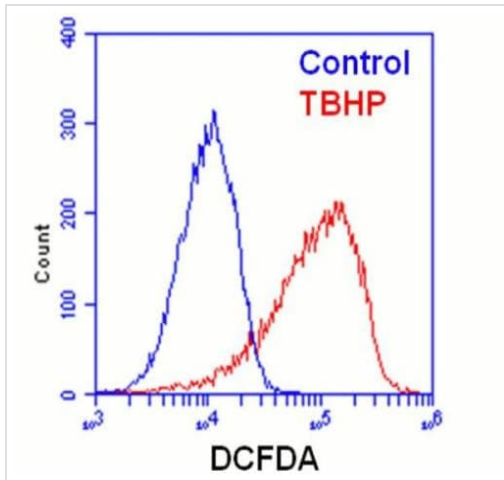
Kobashigawa et al. (Pubmed 25127116) used the DCFDA ROS assay ab113851 to investigate the causes of the protective effects of metformin (Met) treatment in Doxorubicin (Dox) induced cardiotoxicity.

They identified that in metformin treated H9c2 rat immortalized cardiomyoblasts, Met treatment reduced ROS levels induced by Dox (A). Values represent mean ± S.D. (n=4).

In combination with other assays, they developed the hypothesis that Dox induces increased ROS expression, leading to increased calcium levels and cell death, and that Met reduces this effect by increasing AMPK expression.

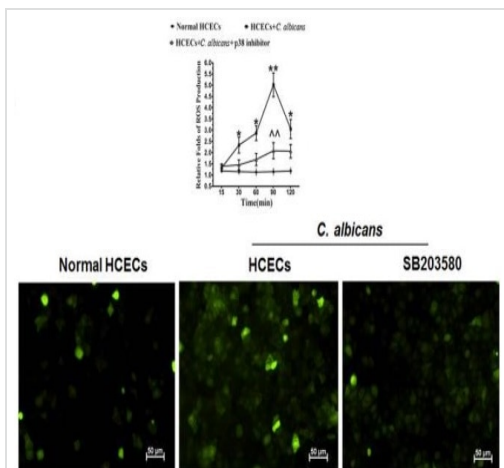
DCFDA ROS assay used to study Doxorubicin cardiotoxicity

Kobashigawa et al. (Pubmed 28056084)



Flow Cytometry - DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit (ab113851)

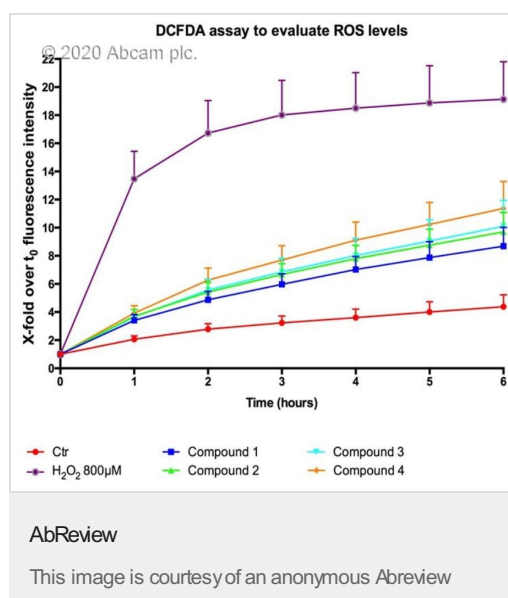
ab113851 (DCFDA) labeled and unlabeled Jurkat cells were treated with 50 μ M tert-butyl Hydrogen Peroxide (tbHP), then analyzed by flow cytometry.



p38 MAPK pathway involved in oxidative injury to HCECs challenged with *C. albicans*. Increased ROS generation in HCECs challenged with *C. albicans* and inhibition by the p38 activation inhibitor SB203580.

p38 MAPK pathway involved in oxidative injury to HCECs challenged with *C. albicans*.

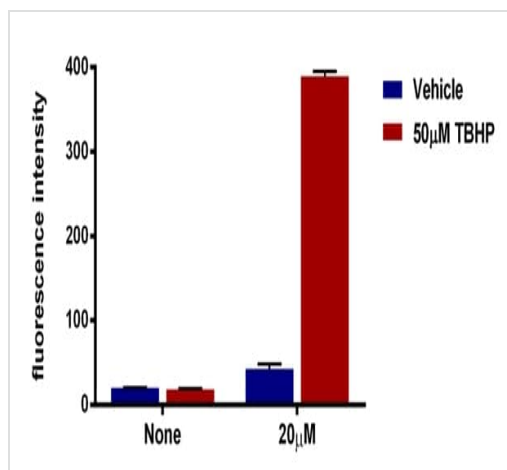
Image from Hua X et al., Sci Rep, 2017, 7: 10421, Fig. 4A; doi: 10.1038/s41598-017-09636-w Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>



ROS levels analysis in human immortalized cardiomyocytes cells with ab113851 under different treatment conditions.

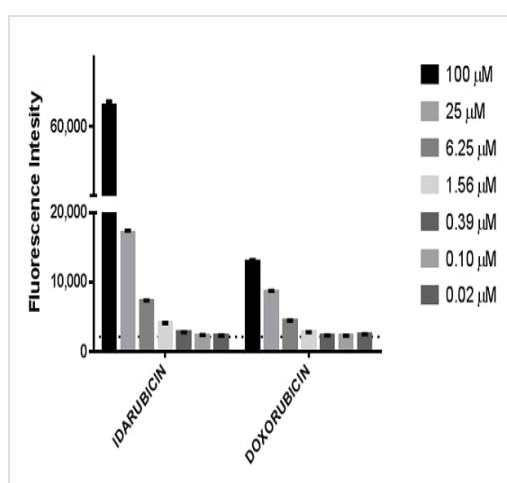
Data are mean \pm SEM of three different experiments. H₂O₂ 800µM was used as Positive Control. The values of fluorescence intensity at each time point are indicated as the ratio of the value at specific t-time point on the value at time point zero (first measurement) (t-time point/t₀). Briefly, cells were plated (seeding density 2.5 x 10⁴ cells/cm²) in FBS-supplemented medium w/o phenol red onto a 96 black well plate. After 24 hours the cells were washed one time with 1X buffer (provided in the kit), then the cells were incubated with DCFDA 10 µM for 30 min at 37° C protected from light. Following incubation the wells were washed with PBS and the cells were exposed to treatments of interest in FBS-supplemented medium w/o phenol red. ROS production was determined immediately by measuring the formation of fluorescent dichloro fluorescein (DCF), using a PerkinElmer VICTOR3, at an Ex-485 and Em-535 nm. Measurements were done every 30 min for six hours. The value of fluorescence intensity at each time point is reported. The value reported was obtained by the ratio of fluorescence at specific time point on fluorescence at time 0, which was measured immediately after DCFDA incubation.

See Abreview



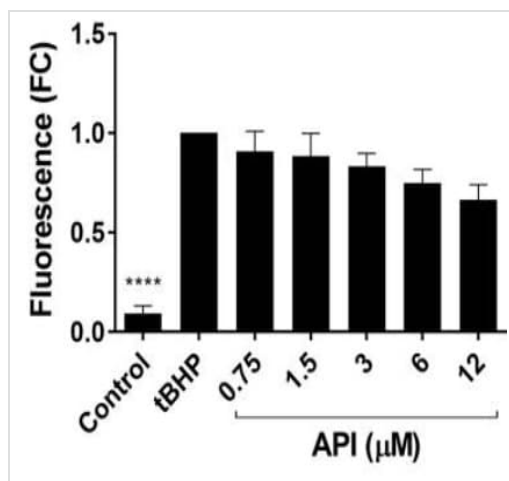
Functional Studies - DCFDA - Cellular Reactive
Oxygen Species Detection Assay Kit (ab113851)

Jurkat cells were labeled with DCFDA (20 μM) or unlabeled (none) and then cultured an additional 3 hours with or without 50 μM tert-butyl hydrogen peroxide (TBHP) according to the protocol. Cells were then analyzed on a fluorescent plate reader. Mean \pm standard deviation is plotted for 4 replicates from each condition. TBHP mimics ROS activity to oxidize DCFDA to fluorescent DCF.



Acute Effect of anthracyclines on ROS production in
HL60 cells.

Labeled HL60 cells were treated with idarubicin or doxorubicin for 4 hours at multiple doses according to the protocol. At the end of the treatment cells were read end point in a fluorescent plate reader (Perking Elmer-Wallac 1420 Victor 2 Multilabel plate reader). Mean \pm standard deviation is plotted for 3 replicates from each condition. The dotted line represents the mean of 24 replicates of HL60 cells treated with 0.5% DMSO.



Reactive oxygen species (ROS) measured using the DCFDA assay in human primary articular chondrocytes. Cells were treated with 100 μM tert-butyl-hydroperoxide (tBHP) alone (4 h) \pm pre-treatment with apigenin.

Reactive oxygen species (ROS) measured using the DCFDA assay

Image from Davidson et al., Sci Rep, 2018. Fig. 2B.; doi: 10.1038/s41598-018-35455-8 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

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