
Product Manual

OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence), Trial Size

Catalog Number

STA-342-T

20 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Accumulation of reactive oxygen species (ROS) coupled with an increase in oxidative stress has been implicated in the pathogenesis of several disease states. The role of oxidative stress in vascular diseases, diabetes, renal ischemia, atherosclerosis, pulmonary pathological states, inflammatory diseases, and cancer has been well established. Free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to biomolecules, a process held in check by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. Measuring the effect of antioxidant therapies and ROS activity intracellularly is crucial to suppressing or treating oxidative stress inducers.

Cell Biolabs' OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence) is a cell-based assay for measuring hydroxyl, peroxy, or other reactive oxygen species activity within a cell. The assay employs the cell-permeable fluorogenic probe 2', 7'-Dichlorodihydrofluorescein diacetate (DCFH-DA). In brief, DCFH-DA is diffused into cells and is deacetylated by cellular esterases to non-fluorescent 2', 7'-Dichlorodihydrofluorescein (DCFH), which is rapidly oxidized to highly fluorescent 2', 7'-Dichlorodihydrofluorescein (DCF) by ROS (Figure 1). The fluorescence intensity is proportional to the ROS levels within the cell cytosol. The effect of antioxidant or free radical compounds on DCFH-DA can be measured against the fluorescence of the provided DCF standard. The kit has a DCF detection sensitivity limit of 10 pM. Each Trial Size Intracellular ROS Assay Kit provides sufficient reagents to perform up to 20 assays, including standard curve and unknown samples.

Assay Principle

The OxiSelect™ Intracellular ROS Assay Kit is a cell-based assay for measuring antioxidant or ROS activity. Cells are cultured in a 96-well cell culture plate and then pre-incubated with DCFH-DA, which is cell-permeable (Figure 1). The unknown antioxidant or ROS samples are then added to the cells. After a brief incubation, the cells can be read on a standard fluorescence plate reader. The ROS or antioxidant content in unknown samples is determined by comparison with the predetermined DCF standard curve.

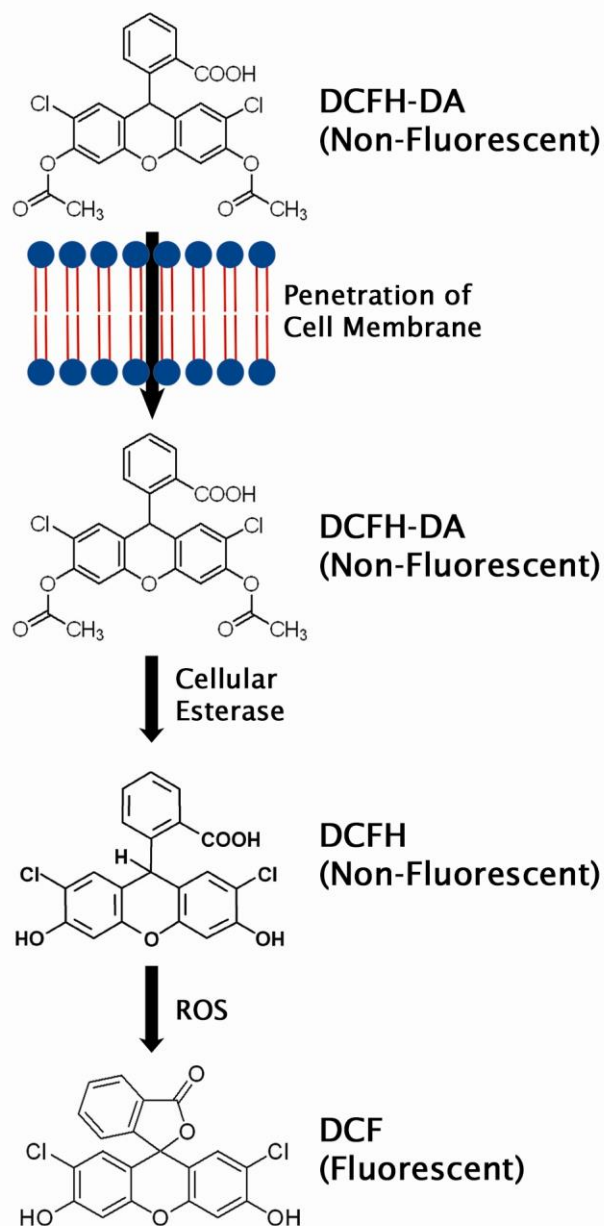


Figure 1. Mechanism of DCF Assay

Related Products

1. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit
2. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
3. STA-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 assays
4. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
5. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. 20X DCFH-DA (Part No. 234201-T): One 100 μ L amber tube of a 20 mM solution in methanol.
2. DCF Standard (Part No. 234202-T): One 20 μ L amber tube of a 1 mM solution in DMSO.
3. Hydrogen Peroxide (Part No. 234102-T): One 20 μ L amber tube of an 8.821 M solution.
4. 2X Cell Lysis Buffer (Part No. 234203-T): Two 2 mL tubes.

Materials Not Supplied

1. Sterile DPBS for washes and buffer dilutions
2. Hank's Balanced Salt Solution (HBSS)
3. Cell culture medium (ie: DMEM +/-10% FBS)
4. 96-well black or fluorescence microtiter plate
5. Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

Storage

Upon receipt, store the DCFH-DA and DCF Standard at -20°C . Avoid multiple freeze/thaw cycles. Store the Cell Lysis Buffer and Hydrogen Peroxide at 4°C .

Preparation of Reagents

- 1X DCFH-DA: Dilute the 20X DCFH-DA stock solution to 1X in cell culture media, preferably without FBS. Stir or vortex to homogeneity. Prepare only enough for immediate applications.

Notes:

- *1X DCFH-DA/media solution contains 5% methanol. For cells that are sensitive to methanol, we recommend instead preparing a 0.1X (100 μ M) solution of DCFH-DA in cell culture media.*
- *Due to light-induced auto-oxidation, DCFH-DA solutions at any concentration must be protected from light.*
- Hydrogen Peroxide (H_2O_2): Prepare H_2O_2 dilutions in DMEM or DPBS as necessary. Do not store diluted solutions. Hydrogen Peroxide may be used as a positive control in the assay, or as a cell treatment.

Preparation of Standard Curve

1. Prepare a 1:10 dilution series of DCF standards in the concentration range of 0 μM – 10 μM by diluting the 1 mM DCF stock in cell culture media (see Table 1).

Standard Tubes	DCF Standard (μL)	Culture Medium (μL)	DCF (nM)
1	10	990	10,000
2	100 of Tube #1	900	1000
3	100 of Tube #2	900	100
4	100 of Tube #3	900	10
5	100 of Tube #4	900	1
6	100 of Tube #5	900	0.1
7	100 of Tube #6	900	0.01
8	0	1000	0

Table 1. Preparation of DCF Standards

2. Transfer 75 μL of each DCF standard to a 96-well plate suitable for fluorescence measurement. Add 75 μL of the 2X Cell Lysis Buffer.
3. Read the fluorescence with a fluorescence plate reader at 480 nm excitation /530 nm emission.

Assay Protocol

I. DCF Dye Loading

1. Prepare and mix all reagents thoroughly before use. Each unknown sample should be assayed in duplicate or triplicate.
2. Culture cells in either a clear or black 96-well cell culture plate.
Note: If using a black plate, choose an appropriate plate based on your fluorometer's reader (i.e. choose a clear bottom black plate for bottom readers).
3. Remove media from all wells and discard. Wash cells gently with DPBS or HBSS 2-3 times. Remove the last wash and discard.
4. Add 100 μL of 1X DCFH-DA/media solution to cells. Incubate at 37°C for 30-60 minutes.
5. Remove solution. Repeat step three using multiple washes with DPBS or HBSS. Remove the last wash and discard.
6. Treat DCFH-DA loaded cells with desired oxidant or antioxidant in 100 μL medium.

II. Quantitation of Fluorescence

- Fluorescence microscopy or Flow cytometry: Fluorescence can be analyzed on an inverted fluorescence microscope or by flow cytometry using excitation and emission wavelengths of 480 nm and 530 nm, respectively.
- Fluorescence Plate Reader:
 - Assays performed in black cell culture fluorometric plates: Plate may be read immediately for kinetic analysis or after 1 hour for static analysis. Plates read for

kinetic analysis may be read in increments of 1 and 5 minutes up to 1 hour or more as necessary. Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm.

- Assays performed in clear cell culture plates: After treatment with desired oxidant or antioxidant, carefully remove treatment media from all wells and discard. Wash cells gently with DPBS or HBSS 2-3 times. Remove the last wash and discard. Add 100 μ L of medium to each well. Add 100 μ L of the 2X Cell Lysis Buffer, mix thoroughly and incubate 5 minutes. Transfer 150 μ L of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm.

Example of Results

The following figures demonstrate typical ROS Assay results. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

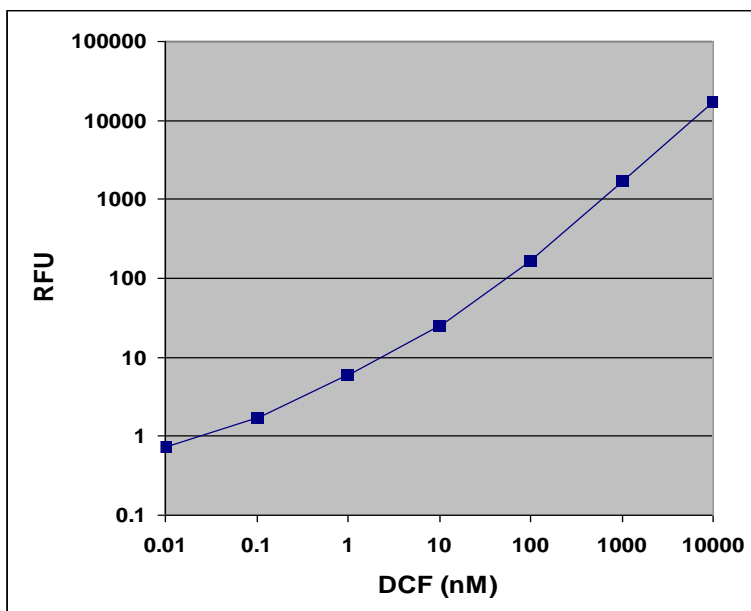


Figure 2. DCF Standard Curve.

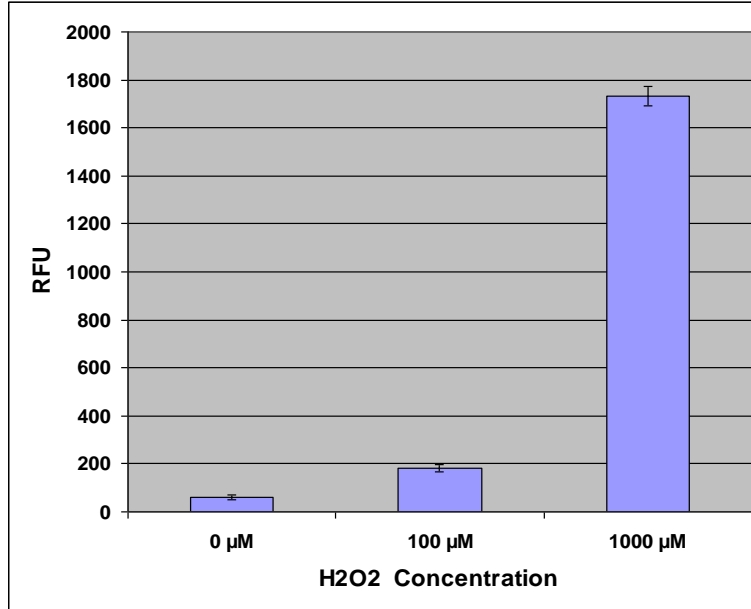


Figure 3. ROS in HeLa cells treated with H₂O₂. 50,000 HeLa cells in a 96-well plate were first pretreated with 1 mM DCFH-DA for 60 minutes at 37°C. Cells were then treated with various concentrations of H₂O₂ for 20 minutes.

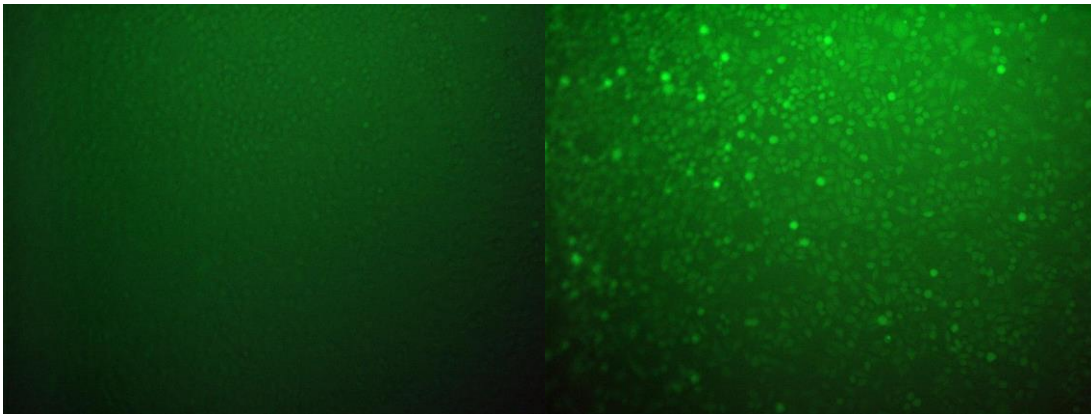


Figure 4. DCF Fluorescence in H₂O₂ treated HeLa cells after 1 hour. Left: 0 μM; Right: 1000 μM.

References

1. Bass DA, Parce JW, Dechatelet LR, Szejda P, Seeds MC, Thomas M. Flow cytometric studies of oxidative product formation by neutrophils: A graded response to membrane stimulation. *J Immunol.* 1983; **130**:1910-1917.
2. Brandt R, Keston AS. Synthesis of diacetyldichlorofluorescein: A stable reagent for fluorometric analysis. *Anal Biochem.* 1965; **11**:6-9.
3. Keston AS, Brandt R. The fluorometric analysis of ultramicro quantities of hydrogen peroxide. *Anal Biochem.* 1965; **11**:1-5.

Recent Product Citations

1. Abd-Elraoof, W.A. et al. (2023). Characterization and antimicrobial activity of a chitosan-selenium nanocomposite biosynthesized using *Posidonia oceanica*. *RSC Adv.* **13**(37):26001-26014. doi: 10.1039/d3ra04288j.
2. Gallo, G. et al. (2023). Polymorphic variants at NDUFC2, encoding a mitochondrial complex I subunit, associate with cardiac hypertrophy in human hypertension. *Mol Med.* **29**(1):107. doi: 10.1186/s10020-023-00701-x.
3. Abu Bakar, S.A. et al. (2023). Goniotalamin Inhibits Cell Growth, Perturbs Cell Cycle and Induces Apoptosis in Human Osteosarcoma SAOS-2 Cells. *JUMMEC*. doi: 10.22452/jummec.sp2023no1.9.
4. Yang, H. et al. (2023). MEK1 drives oncogenic signaling and interacts with PARP1 for genomic and metabolic homeostasis in malignant pleural mesothelioma. *Cell Death Discov.* **9**(1):55. doi: 10.1038/s41420-023-01307-2.
5. Zanotti, F. et al. (2023). Playing with Biophysics: How a Symphony of Different Electromagnetic Fields Acts to Reduce the Inflammation in Diabetic Derived Cells. *Int J Mol Sci.* **24**(2):1754. doi: 10.3390/ijms24021754.
6. Kang, J.R. et al. (2022). Anti-inflammatory activities of polysaccharides isolated from *Aloe saponaria* Haw grown in Namhae. *Korean J Food Preserv.* **29**(7):1189-1200. doi: 10.11002/kjfp.2022.29.7.1189.
7. Kim, Y.H. et al. (2022). The Antioxidant Auraptene Improves Aged Oocyte Quality and Embryo Development in Mice. *Antioxidants (Basel)*. **12**(1):87. doi: 10.3390/antiox12010087.
8. Shimizu, H. et al. (2022). Dimethyl Fumarate Protects Retinal Pigment Epithelium from Blue Light-Induced Oxidative Damage via the Nrf2 Pathway. *Antioxidants (Basel)*. **12**(1):45. doi: 10.3390/antiox12010045.
9. Michon, M. et al. (2022). In Vitro Biological Effects of E-Cigarette on the Cardiovascular System-Pro-Inflammatory Response Enhanced by the Presence of the Cinnamon Flavor. *Toxics*. **10**(12):784. doi: 10.3390/toxics10120784.
10. Kumawat, M. et al. (2022). Double functionalized haemocompatible silver nanoparticles control cell inflammatory homeostasis. *PLoS One*. **17**(10):e0276296. doi: 10.1371/journal.pone.0276296.
11. Campo-Sabariz, J. et al. (2022). Hydroxy-Selenomethionine, an Organic Selenium Source, Increases Selenoprotein Expression and Positively Modulates the Inflammatory Response of LPS-Stimulated Macrophages. *Antioxidants*. **11**(10):1876. doi: 10.3390/antiox11101876.
12. Park, S. et al. (2022). β -Escin overcomes trastuzumab resistance in HER2-positive breast cancer by targeting cancer stem-like features. *Cancer Cell Int.* **22**(1):289. doi: 10.1186/s12935-022-02713-9.
13. Walker, C.C.F. et al. (2022). Anandamide Alters Barrier Integrity of Bovine Vascular Endothelial Cells during Endotoxin Challenge. *Antioxidants (Basel)*. **11**(8):1461. doi: 10.3390/antiox11081461.
14. Zwolak, I. & Wnuk, E. (2022). Effects of Sodium Pyruvate on Vanadyl Sulphate-Induced Reactive Species Generation and Mitochondrial Destabilisation in CHO-K1 Cells. *Antioxidants*. **11**(5):909. doi: 10.3390/antiox11050909.
15. Whitmore, C.A. et al. (2022). Longitudinal Consumption of Ergothioneine Reduces Oxidative Stress and Amyloid Plaques and Restores Glucose Metabolism in the 5XFAD Mouse Model of Alzheimer's Disease. *Pharmaceuticals (Basel)*. **15**(6):742. doi: 10.3390/ph15060742.
16. Acharya, A. et al. (2022). Microgravity-induced stress mechanisms in human stem cell-derived cardiomyocytes. *iScience*. doi: 10.1016/j.isci.2022.104577.
17. Ahmed, S. et al. (2022). HMGB1-Like Dorsal Switch Protein 1 Triggers a Damage Signal in Mosquito Gut to Activate Dual Oxidase via Eicosanoids. *J Innate Immun.* doi: 10.1159/000524561.

18. Taguchi, N. et al. (2022). Protective effect of hydroxygenkwanin against hair graying induced by X-ray irradiation and repetitive plucking. *JID Innov.* doi: 10.1016/j.xjidi.2022.100121.
19. Tatullo, M. et al. (2022). Potential impact of functional biomolecules-enriched foods on human health: A randomized controlled clinical trial. *Int J Med Sci.* **19**(3):563-571. doi: 10.7150/ijms.70435.
20. Lu, G.L. et al. (2022). The Surface Amine Group of Ultrasmall Magnetic Iron Oxide Nanoparticles Produce Analgesia in the Spinal Cord and Decrease Long-Term Potentiation. *Pharmaceutics.* **14**(2):366. doi: 10.3390/pharmaceutics14020366.
21. Fares, M. et al. (2022). COL-3-Induced Molecular and Ultrastructural Alterations in K562 Cells. *J Pers Med.* **12**(1):42. doi: 10.3390/jpm12010042.
22. Tang, H. et al. (2021). Graphene Quantum Dots Obstruct the Membrane Axis of Alzheimer's Amyloid Beta. *Phys. Chem. Chem. Phys.* doi: 10.1039/D1CP04246G.
23. Truman, J.P. et al. (2021). 1-deoxysphinganine initiates adaptive responses to serine and glycine starvation in cancer cells via proteolysis of sphingosine kinase. *J Lipid Res.* doi: 10.1016/j.jlr.2021.100154.
24. Raghubir, M. et al. (2021). Riluzole-induced apoptosis in osteosarcoma is mediated through Yes-associated protein upon phosphorylation by c-Abl Kinase. *Sci Rep.* **11**(1):20974. doi: 10.1038/s41598-021-00439-8.
25. Tylek, K. et al. (2021). Time-Dependent Protective and Pro-Resolving Effects of FPR2 Agonists on Lipopolysaccharide-Exposed Microglia Cells Involve Inhibition of NF- κ B and MAPKs Pathways. *Cells.* **10**(9):2373. doi: 10.3390/cells10092373.
26. Nelson, M.T et al. (2021). Examining cellular responses to reconstituted antibody protein liquids. *Sci Rep.* **11**(1):17066. doi: 10.1038/s41598-021-96375-8.
27. Andrikopoulos, N. et al. (2021). Inhibition of Amyloid Aggregation and Toxicity with Janus Iron Oxide Nanoparticles. *Chem. Mater.* doi: 10.1021/acs.chemmater.1c01947.
28. Tsutsumi-Arai, C. et al. (2021). Microbicidal effect of 405-nm blue LED light on *Candida albicans* and *Streptococcus mutans* dual-species biofilms on denture base resin. *Lasers Med Sci.* doi: 10.1007/s10103-021-03323-z.
29. Ng, C.H. et al. (2021). Synthesis, characterization and multiple targeting with selectivity: Anticancer property of ternary metal phenanthroline-maltol complexes. *J Inorg Biochem.* **220**:111453. doi: 10.1016/j.jinorgbio.2021.111453.
30. Lakshmi, B.A. et al. (2021). Ruthenium(II)-Curcumin Liposome Nanoparticles: synthesis, characterization, and effects against cervical cancer. *Colloids Surf B Biointerfaces.* doi: 10.1016/j.colsurfb.2021.111773.

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