

---

Product Manual

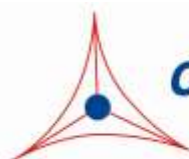
# OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)

## Catalog Number

STA-342	96 assays
STA-342-5	5 x 96 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.

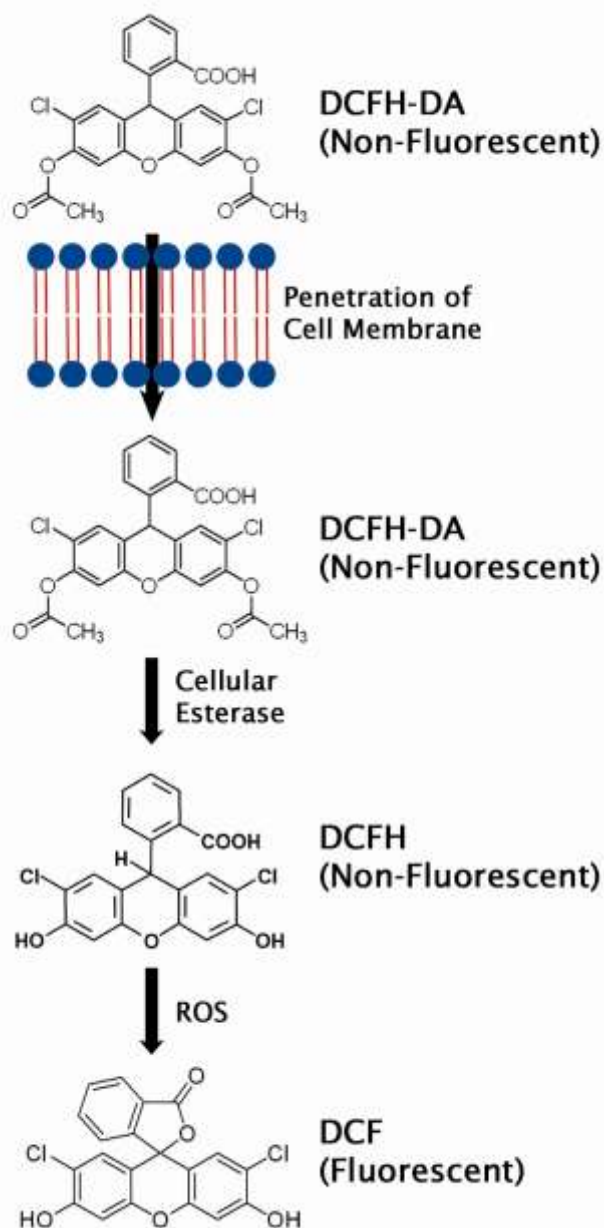
Cell Biolabs' OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence) is a cell-based assay for measuring hydroxyl, peroxy, or other reactive oxygen species activity within a cell. The assay employs the cell-permeable fluorogenic probe 2', 7'-Dichlorodihydrofluorescein diacetate (DCFH-DA). In brief, 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 kit provides sufficient reagents to perform up to 96 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.

## **Related Products**

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
3. STA-341: OxiSelect™ Catalase Activity Assay Kit
4. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit
5. STA-345: OxiSelect™ ORAC Activity Assay Kit
6. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
7. STA-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 assays
8. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
9. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit



**Figure 1.** Mechanism of DCF Assay

### **Kit Components**

1. 20X DCFH-DA (Part No. 234201): One 500  $\mu$ L amber tube of a 20 mM solution in methanol.
2. DCF Standard (Part No. 234202): One 100  $\mu$ L amber tube of a 1 mM solution in DMSO.
3. Hydrogen Peroxide (Part No. 234102): One 100  $\mu$ L amber tube of an 8.821 M solution.
4. 2X Cell Lysis Buffer (Part No. 234203): One 20 mL bottle.

## **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 µM) 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<sub>2</sub>O<sub>2</sub>): Prepare H<sub>2</sub>O<sub>2</sub> 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).

<b>Standard Tubes</b>	<b>DCF Standard (µL)</b>	<b>Culture Medium (µL)</b>	<b>DCF (nM)</b>
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  $\mu\text{L}$  of each DCF standard to a 96-well plate suitable for fluorescence measurement. Add 75  $\mu\text{L}$  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**

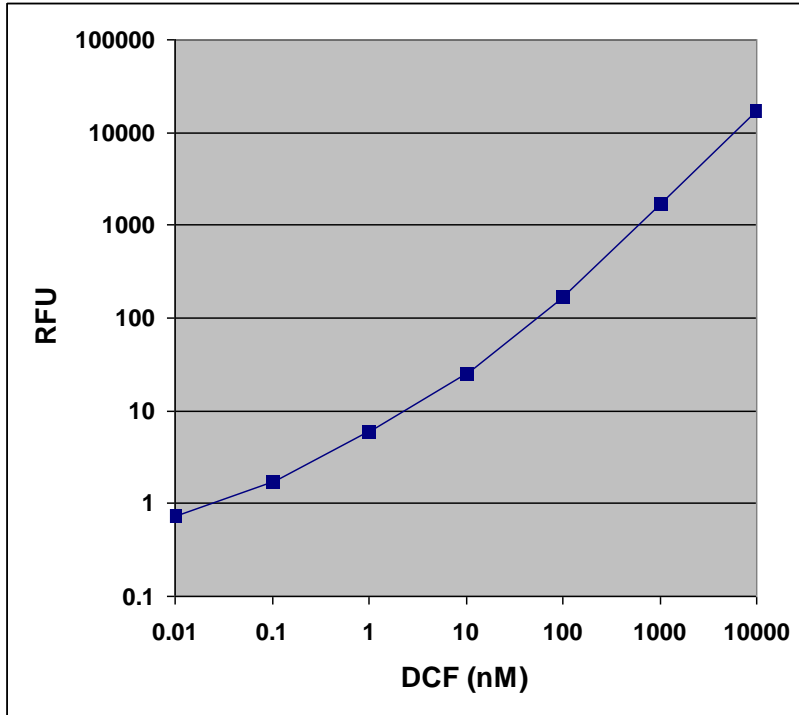
1. Prepare and mix all reagents thoroughly before use. Each unknown sample should be assayed in duplicate or triplicate.
2. Culture cells in either a clear or black 96-well cell culture plate.  
*Note: If using a black plate, choose an appropriate plate based on your fluorometer's reader (i.e. choose 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  $\mu\text{L}$  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  $\mu\text{L}$  medium.

### **II. Quantitation of Fluorescence**

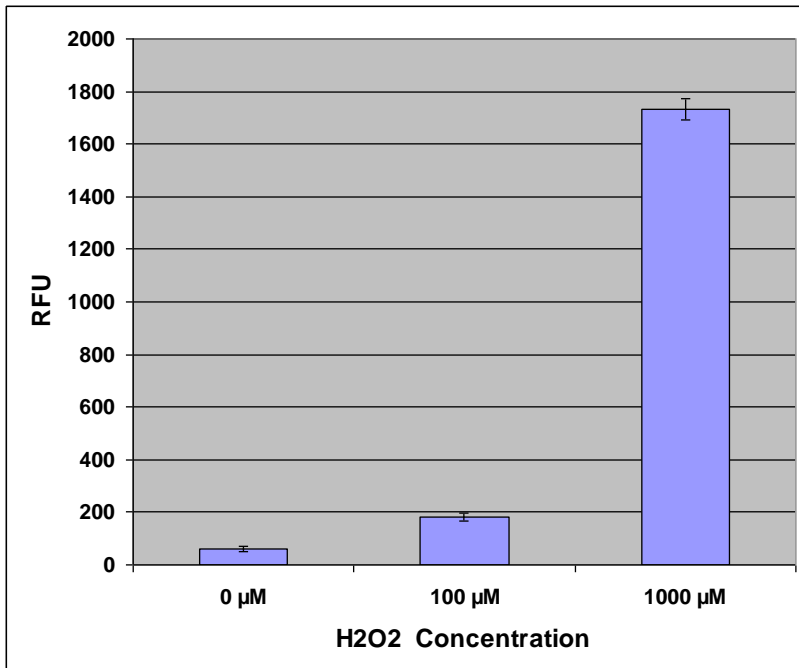
- Fluorescence microscopy or Flow cytometry: Fluorescence can be analyzed on an inverted fluorescence microscope or by flow cytometry using excitation and emission wavelengths of 480 nm and 530 nm, respectively.
- Fluorescence Plate Reader:
  - Assays performed in black cell culture fluorometric plates: 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  $\mu\text{L}$  of medium to each well. Add 100  $\mu\text{L}$  of the 2X Cell Lysis Buffer, mix thoroughly and incubate 5 minutes. Transfer 150  $\mu\text{L}$  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**

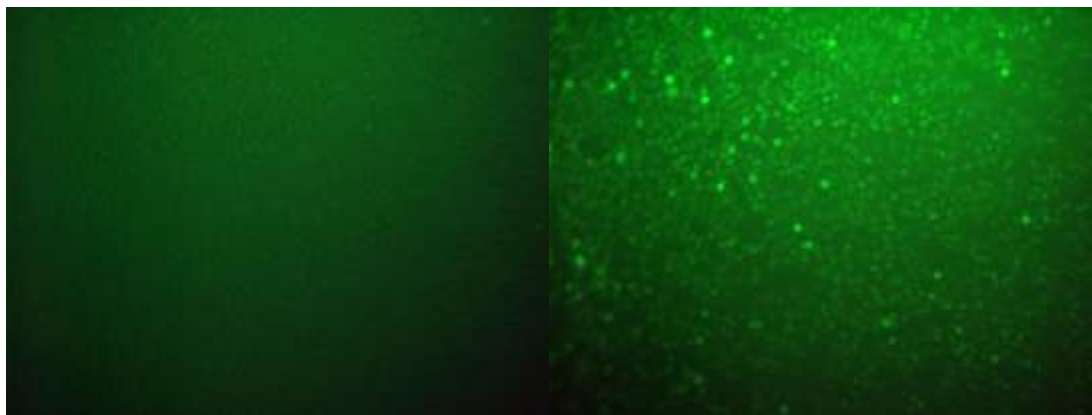
The following figures demonstrate typical ROS Assay results. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual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



**Figure 2. DCF Standard Curve.**



**Figure 3. ROS in HeLa cells treated with H<sub>2</sub>O<sub>2</sub>.** 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 H<sub>2</sub>O<sub>2</sub> for 20 minutes.



**Figure 4. DCF Fluorescence in H<sub>2</sub>O<sub>2</sub> 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. Tylek, K. et al. (2021). Time-Dependent Protective and Pro-Resolving Effects of FPR2 Agonists on Lipopolysaccharide-Exposed Microglia Cells Involve Inhibition of NF-κB and MAPKs Pathways. *Cells.* **10**(9):2373. doi: 10.3390/cells10092373.
2. 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.
3. Andrikopoulos, N. et al. (2021). Inhibition of Amyloid Aggregation and Toxicity with Janus Iron Oxide Nanoparticles. *Chem. Mater.* doi: 10.1021/acs.chemmater.1c01947.
4. 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.
5. 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.
6. 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.
7. Wu, X. et al. (2021). Carboxymethylated chitosan alleviated oxidative stress injury in retinal ganglion cells via lncRNA-THOR/IGF2BP1 axis. *Genes Genomics.* doi: 10.1007/s13258-021-01085-0.



8. Nurrahmah, Q.I. et al. (2021). Retinoic acid abrogates LPS-induced inflammatory response via negative regulation of NF-kappa B/miR-21 signaling. *Immunopharmacol Immunotoxicol*. doi: 10.1080/08923973.2021.1902348.
9. Dong, H. et al. (2021). Paeoniflorin and Plicyrrhetic Acid Synergistically Alleviate MPP+/MPTP-Induced Oxidative Stress through Nrf2-Dependent Glutathione Biosynthesis Mechanisms. *ACS Chem Neurosci*. doi: 10.1021/acschemneuro.0c00544.
10. Ashibe, S. et al. (2021). Mechanism of the adverse effect of hyaluronidase used for oocyte denudation on early development of bovine embryos. *Zygote*. doi: 10.1017/S0967199421000010.
11. Codenotti, S. et al. (2021). Caveolin-1 promotes radioresistance in rhabdomyosarcoma through increased oxidative stress protection and DNA repair. *Cancer Lett*. doi: 10.1016/j.canlet.2021.02.005.
12. Lazarova, D. et al. (2021). Quantum Sensors To Track Total Redox-Status and Oxidative Stress in Cells and Tissues Using Electron-Paramagnetic Resonance, Magnetic Resonance Imaging, and Optical Imaging. *Anal Chem*. doi: 10.1021/acs.analchem.0c04116.
13. Truman, J.P. et al. (2021). Sphingosine kinase 1 downregulation is required for adaptation to serine deprivation. *FASEB J*. **35**(2):e21284. doi: 10.1096/fj.202001814RR.
14. Gupta, S. et al. (2021). Glutathione is a potential therapeutic target for acrolein toxicity in the cornea. *Toxicol Lett*. doi: 10.1016/j.toxlet.2021.01.005.
15. Hoque, S.A.M. et al. (2020). Adverse effect of superoxide-induced mitochondrial damage in granulosa cells on follicular development in mouse ovaries. *Free Radic Biol Med*. doi: 10.1016/j.freeradbiomed.2020.12.434.
16. Pan, W. et al. (2021). Deubiquitinating enzyme USP30 negatively regulates mitophagy and accelerates myocardial cell senescence through antagonism of Parkin. *Cell Death Discov*. **7**(1):187. doi: 10.1038/s41420-021-00546-5.
17. Kim, E.Y. & Dryer, S.E. (2021). RAGE and  $\alpha$ V $\beta$ 3-integrin are essential for suPAR signaling in podocytes. *Biochim Biophys Acta Mol Basis Dis*. **1867**(10):166186. doi: 10.1016/j.bbadis.2021.166186.
18. Blagojevic, M. et al. (2021). Candidalysin triggers epithelial cellular stresses that induce necrotic death. *Cell Microbiol*. doi: 10.1111/cmi.13371.
19. Chang, M.S. et al. (2021). Study of Ojayeonjonghwan on hydrogen peroxide-induced oxidative stress in male reproductive GC-1 germ cell lines. *Herb. Formula Sc*. **29**(1):1-8. doi: 10.14374/HFS.2021.29.1.1.
20. Yen, I.C. et al. (2021). 4-Acetylanthroquinonol B ameliorates nonalcoholic steatohepatitis by suppression of ER stress and NLRP3 inflammasome activation. *Biomed Pharmacother*. **138**:111504. doi: 10.1016/j.biopha.2021.111504.
21. Kuhn, M.J. et al. (2021). Vitamin E analogs limit in vitro oxidant damage to bovine mammary endothelial cells. *J Dairy Sci*. doi: 10.3168/jds.2020-19675.
22. Nukala, S.B. et al. (2021). Protein network analyses of pulmonary endothelial cells in chronic thromboembolic pulmonary hypertension. *Sci Rep*. **11**(1):5583. doi: 10.1038/s41598-021-85004-z.
23. Sangweni, N.F. et al. (2021). The triterpene, methyl-3 $\beta$ -hydroxylanosta-9,24-dien-21-oate (RA3), attenuates high glucose-induced oxidative damage and apoptosis by improving energy metabolism. *Phytomedicine*. doi: 10.1016/j.phymed.2021.153546.
24. Panchariya, V. et al. (2021). Chromatic intervention and biocompatibility assay for biosurfactant derived from *Balanites aegyptiaca* (L.) Del. *Sci Rep*. **11**(1):4186. doi: 10.1038/s41598-021-83573-7.



25. Zhang, L. et al. (2021). microRNA-204 Silencing Reduces Mitochondrial Autophagy and Reactive Oxygen Species Production in a murine Alzheimer's Disease model via the TRPML1-Activated STAT3 Pathway. *Mol Ther Nucleic Acids*. doi: 10.1016/j.omtn.2021.02.010.
26. Kinoshita, C. et al. (2021). Inhibition of miR-96-5p in the mouse brain increases glutathione levels by altering NOVA1 expression. *Commun Biol*. **4**(1):182. doi: 10.1038/s42003-021-01706-0.
27. Buskaran, K. et al. (2021). Anticancer Molecular Mechanism of Protocatechuic Acid Loaded on Folate Coated Functionalized Graphene Oxide Nanocomposite Delivery System in Human Hepatocellular Carcinoma. *Materials (Basel)*. **14**(4):817. doi: 10.3390/ma14040817.
28. Moon, I.J. et al. (2021). Ursodeoxycholic Acid May Inhibit Environmental Aging-Associated Hyperpigmentation. *Antioxidants (Basel)*. **10**(2):267. doi: 10.3390/antiox10020267.
29. Dong, S. et al. (2021). Leukemia inhibitory factor protects photoreceptor cone cells against oxidative damage through activating JAK/STAT3 signaling. *Ann Transl Med*. **9**(2):152. doi: 10.21037/atm-20-8040.
30. Gauthier, A.G. et al. (2021). The Positive Allosteric Modulation of alpha7-Nicotinic Cholinergic Receptors by GAT107 Increases Bacterial Lung Clearance in Hyperoxic Mice by Decreasing Oxidative Stress in Macrophages. *Antioxidants (Basel)*. **10**(1):135. doi: 10.3390/antiox10010135.

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

## **Contact Information**

Cell Biolabs, Inc.  
7758 Arjons Drive  
San Diego, CA 92126  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2009-2021: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.