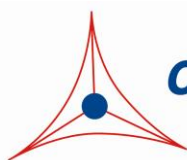

Product Manual

OxiSelect™ *In Vitro* ROS/RNS Assay Kit (Green Fluorescence)

Catalog Number

STA-347	96 assays
STA-347-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well-established molecules responsible for the deleterious effects of oxidative stress. Accumulation of free radicals 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, cancer, as well as ageing 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/RNS activity is crucial to suppressing or treating oxidative stress inducers.

The OxiSelect™ *In Vitro* ROS/RNS Assay Kit is an assay for measuring the total free radical presence of a sample. The assay employs a proprietary quenched fluorogenic probe, dichlorodihydrofluorescein DiOxyQ (DCFH-DiOxyQ), which is a specific ROS/RNS probe that is based on similar chemistry to the popular 2', 7'-dichlorodihydrofluorescein diacetates. The DCFH-DiOxyQ probe is first primed with a quench removal reagent, and subsequently stabilized in the highly reactive DCFH form. In this reactive state, ROS and RNS species can react with DCFH, which is rapidly oxidized to the highly fluorescent 2', 7'-dichlorodihydrofluorescein (DCF) (Figure 1). Fluorescence intensity is proportional to the total ROS/RNS levels within the sample. The DCFH-DiOxyQ probe can react with hydrogen peroxide (H₂O₂), peroxy radical (ROO·), nitric oxide (NO), and peroxynitrite anion (ONOO⁻). These free radical molecules are representative of both ROS and RNS, thus allowing for measurement of the total free radical population within a sample. OxiSelect™ *In Vitro* ROS/RNS Assay Kit can also be used to evaluate antioxidant's effect on free radicals. The kit has a detection sensitivity limit of 10 pM for DCF and 40 nM for H₂O₂ respectively. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

The OxiSelect™ *In Vitro* ROS/RNS Assay Kit is an *in vitro* assay for measuring total ROS/RNS free radical activity. Unknown ROS or RNS samples or standards are added to the wells with a catalyst that helps accelerate the oxidative reaction. After a brief incubation, the prepared DCFH probe is added to all wells and the oxidation reaction is allowed to proceed (Figure 1). Samples are measured fluorometrically against a hydrogen peroxide or DCF standard. The assay is performed in a 96-well fluorescence plate format that can be read on a standard fluorescence plate reader. The free radical content in unknown samples is determined by comparison with the predetermined DCF or hydrogen peroxide standard curve.

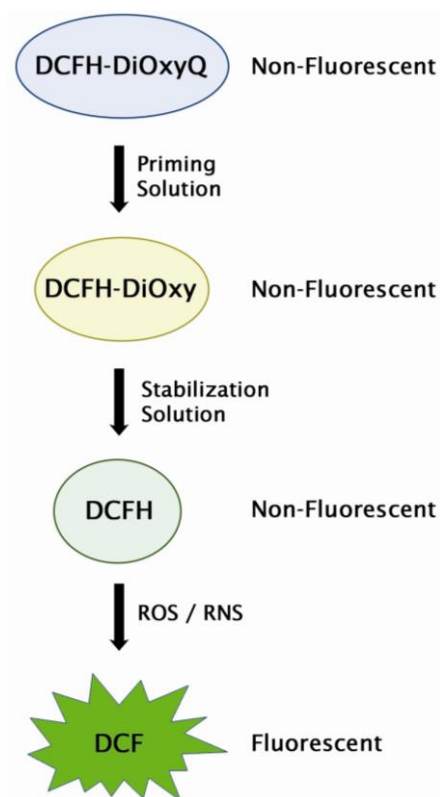


Figure 1. Mechanism of *In Vitro* ROS/RNS Assay.

Related Products

1. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
2. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
3. STA-341: OxiSelect™ Catalase Activity Assay Kit
4. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
5. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit

Kit Components

1. Priming Reagent (Part No. 234701): One 250 µL tube of solution.
2. Stabilization Solution (10X) (Part No. 234702): One 1.5 mL tube of solution.
3. Catalyst (250X) (Part No. 234703): One 20 µL tube of solution.
4. DCF-DiOxyQ (Part No. 234704): One 50 µL amber tube of solution in methanol.
5. DCF Standard (Part No. 234202): One 100 µL amber tube of a 1 mM solution in DMSO.
6. Hydrogen Peroxide (Part No. 234102): One 100 µL amber tube of an 8.821 M solution.

Materials Not Supplied

1. Phosphate Buffered Saline for sample preparations and dilutions
2. 96-well black or fluorescence microtiter plate

Storage

Upon receipt, store the DCF-DiOxyQ and DCF Standard at -20°C. Avoid multiple freeze/thaw cycles. Store all other components at 4°C.

Preparation of Reagents

- 1X Stabilization Solution: Dilute the 10X Stabilization Solution 1:10 by adding 1.5 mL of solution to 13.5 mL of deionized water. Stir or vortex to homogeneity. Store the solution at 4°C.
- 1X Catalyst: Prior to use, dilute the 250X Catalyst 1:250 in PBS. Vortex thoroughly. Prepare only enough for immediate applications (e.g., add 10 µL of Catalyst to 2.49 mL PBS for 50 wells).
- DCFH Solution: Prepare only enough DCFH Solution for immediate applications in an amber tube or aluminum foil covered tube. Prepare DCFH Solution by diluting the stock solution of DCF-DiOxyQ 1:5 with Priming Reagent (e.g., for 50 assays, add 25 µL DCF-DiOxyQ to 100 µL Priming Reagent). Vortex to homogeneity. Incubate the solution for 30 minutes at room temperature. Next, dilute the reaction 1:40 with 1X Stabilization Solution (e.g., for 50 assays, add 125 µL DCF-DiOxyQ/ Priming Reagent reaction to 4.875 mL of Stabilization Solution). Vortex to homogeneity. Protect the solution from light. This solution is now stable in the DCFH form and ready to use. The solution may be stored at -20°C for up to one week when protected from light.

Note: Due to light-induced auto-oxidation, the stock DCF-DiOxyQ solution and all subsequent DCF-DiOxy and DCFH solutions must be protected from light.

Preparation of Samples

All samples should be assayed immediately or stored at -80°C for up to 1-2 months. The assay may be used on cell or tissue lysates, cell culture supernatants, serum, plasma, urine, and other biological fluids. Always run a standard curve with samples. Use PBS for dilution and preparation of samples.

Some common detergents and denaturants have been tested for compatibility in the assay (below table). Dilution of samples, and interfering substances, may be necessary for assay compatibility.

Substance	Compatible Concentration
Triton X-100	≤1%
NP-40	≤1%
SDS	≤0.1%
Deoxycholate	≤1%
Tween-20	≤0.1%
EDTA	≤10 mM
EGTA	≤10 mM
Glycerol	≤10%

Table 1. Substance Compatibility Table

- Cells or Tissues: Resuspend cells at 1-2 x 10⁷ cells/mL or tissues at 10-50 mg/mL in PBS. Homogenize or sonicate on ice. To remove insoluble particles, spin at 10,000 g for 5 min. The homogenate can be assayed directly or stored at -80°C as necessary.
- Serum, Plasma, Urine or Cell Culture Supernatants: To remove insoluble particles, spin at 10,000 g for 5 min. The supernatant can be assayed directly or stored at -80°C as necessary.

Preparation of the DCF Standard Curve

1. Prepare a 1:10 dilution series of DCF standards in the concentration range of 0 μM – 10 μM by diluting the 1mM DCF stock in 1X PBS (see Table 2).

Standard Tubes	DCF Standard (μL)	PBS (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	0	1000	0

Table 2. Preparation of DCF Standards

2. Transfer 200 μL of each DCF standard to a 96-well plate suitable for fluorescence measurement.
3. Read the relative fluorescence with a fluorescence plate reader at 480 nm excitation / 530 nm emission.

Preparation of the H₂O₂ Standard Curve

1. To prepare the Hydrogen Peroxide standards, first perform a 1:4400 dilution of the stock Hydrogen Peroxide in deionized water. Use only enough for immediate applications (e.g., Add 5 μL of Hydrogen Peroxide to 22 mL deionized water). This solution has a concentration of 2 mM.
2. Use the 2 mM H₂O₂ solution to prepare standards in the concentration range of 0 μM – 20 μM by further diluting in PBS (see Table 3). H₂O₂ diluted solutions and standards should be prepared fresh. Use the table below as a reference guide only. The volumes and concentrations of the standard may be adjusted by the user.

Standard Tubes	2 mM H₂O₂ Standard (μL)	PBS (μL)	H₂O₂ (μM)
1	10	990	20
2	500 of Tube #1	500	10
3	500 of Tube #2	500	5
4	500 of Tube #3	500	2.5
5	500 of Tube #4	500	1.25
6	500 of Tube #5	500	0.625
7	500 of Tube #6	500	0.313
8	500 of Tube #7	500	0.156
9	500 of Tube #8	500	0.078
10	500 of Tube #9	500	0.039
11	0	1000	0

Table 3. Preparation of H₂O₂ Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknown(s) and standard(s), should be assayed in duplicate or triplicate.
2. Add 50 μL of unknown sample or hydrogen peroxide standard to wells of a 96-well plate suitable for fluorescence measurement.

3. Add 50 μL of Catalyst to each well. Mix well and incubate 5 minutes at room temperature.
4. Add 100 μL of DCFH solution to each well. Cover the plate reaction wells to protect them from light and incubate at room temperature for 15-45 minutes.
5. Read the fluorescence with a fluorescence plate reader at 480 nm excitation / 530 nm emission.

Example of Results

The following figures demonstrate typical Free Radical ROS/RNS 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.

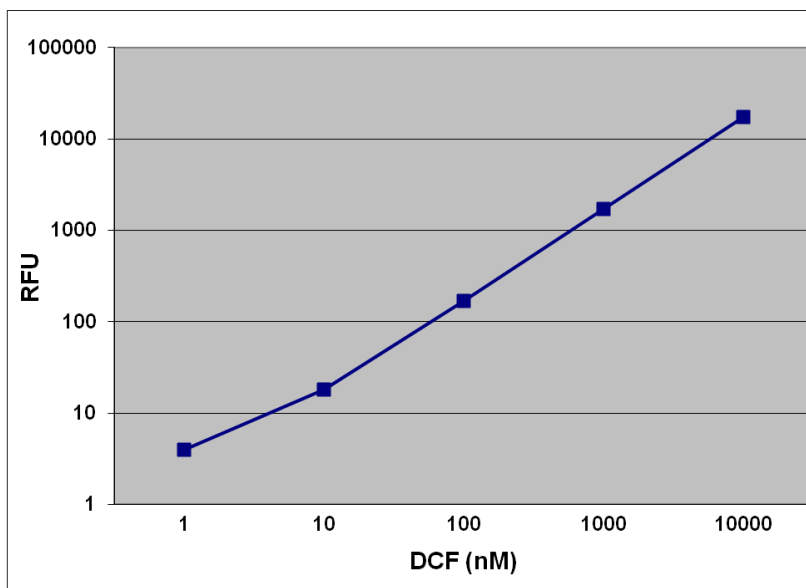


Figure 2. DCF Standard Curve.

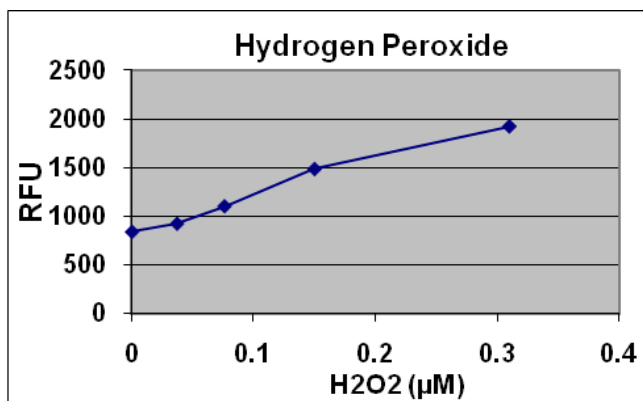
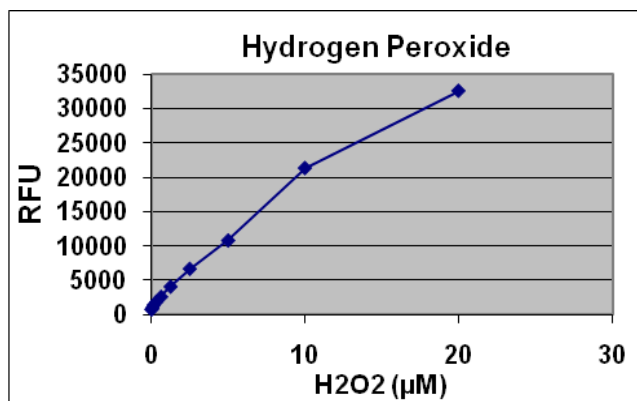


Figure 3. Hydrogen Peroxide Standard Curve.

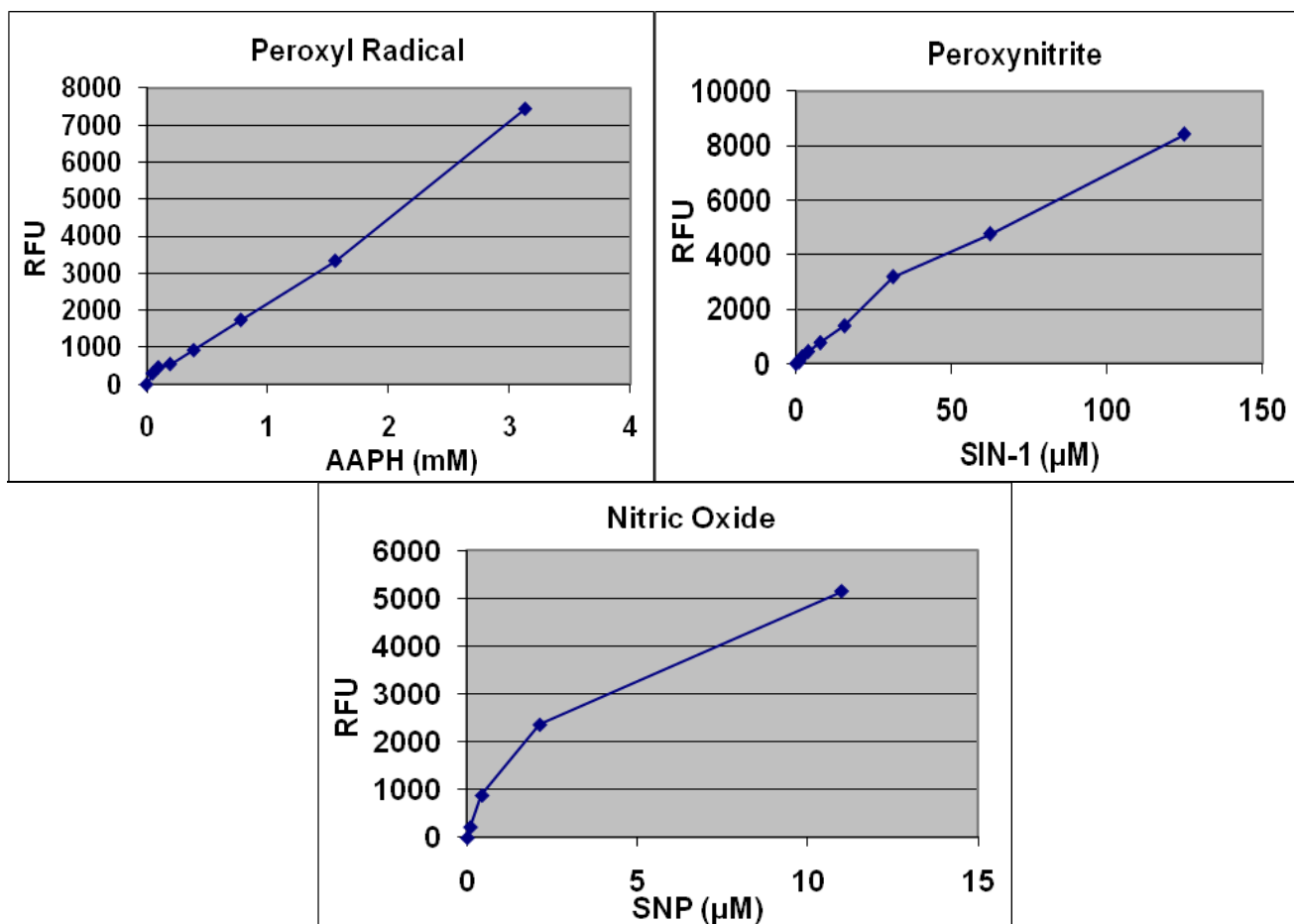


Figure 4. Detection of various free radical species using OxiSelect™ In Vitro ROS/RNS Assay Kit. DCF Fluorescence curves for AAPH (peroxyl radical generator, top), SIN-1 (peroxynitrite generator, center), and SNP (nitric oxide generator, bottom).

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. Wang, H. et al. (2022). Toxicity Assessment of 91-Day Repeated Inhalation Exposure to an Indoor School Air Mixture of PCBs. *Environ Sci Technol.* doi: 10.1021/acs.est.1c05084.
2. Walayat, A. et al. (2021). Fetal e-cigarette exposure programs a neonatal brain hypoxic-ischemic sensitive phenotype via altering DNA methylation patterns and autophagy signaling pathway. *Am J Physiol Regul Integr Comp Physiol.* doi: 10.1152/ajpregu.00207.2021.

3. Chen, P. et al. (2023). Dietary dehulled adlay ameliorated alcoholic liver disease progression by modulating oxidative stress, inflammation, and gut-liver axis disruption in rats. *J Funct Foods*. doi: 10.1016/j.jff.2023.105759.
4. Han, K. et al. (2023). Boosting NAD preferentially blunts Th17 inflammation via arginine biosynthesis and redox control in healthy and psoriasis subjects. *Cell Rep Med*. doi: 10.1016/j.xcrm.2023.101157.
5. Aslan, M. et al. (2023). Effects of aurantiamide on a rat model of renovascular arterial hypertension. *Pflugers Arch*. **475**(10):1177-1192. doi: 10.1007/s00424-023-02850-8.
6. Swartz, T.H. et al. (2023). Effects of dietary rumen-protected choline supplementation to periparturient dairy cattle on inflammation, metabolism, and performance during an intramammary lipopolysaccharide challenge. *J Dairy Sci*. doi: 10.3168/jds.2023-23259.
7. Santacruz-Márquez, R. et al. (2023). Exposure to Zinc Oxide Nanoparticles Increases Estradiol Levels and Induces an Antioxidant Response in Antral Ovarian Follicles In Vitro. *Toxics*. **11**(7):602. doi: 10.3390/toxics11070602.
8. Barreby, E. et al. (2023). Human resident liver myeloid cells protect against metabolic stress in obesity. *Nat Metab*. doi: 10.1038/s42255-023-00834-7.
9. Zhang, K. et al. (2023). Cloak Scavenges the Reactive Oxygen Species around the Larvae of *Drino inconspicuosoides* (Diptera: Tachinidae). *Insects*. **14**(7):602. doi: 10.3390/insects14070602.
10. Gu, H. et al. (2023). Hepatic Anti-Oxidative Genes CAT and GPX4 Are Epigenetically Modulated by ROR γ /NRF2 in Alphacoronavirus-Exposed Piglets. *Antioxidants*. **12**(6):1305. doi: 10.3390/antiox12061305.
11. Won, S.Y. et al. (2023). Effect of individual or combination of dietary betaine and glycine on productive performance, stress response, liver health, and intestinal barrier function in broiler chickens raised under heat stress conditions. *Poult Sci*. **102**(7):102771. doi: 10.1016/j.psj.2023.102771.
12. Zhang, J. et al. (2023). Therapeutic Effect of Prolyl Endopeptidase Inhibitor in High-fat Diet-induced Metabolic Dysfunction-associated Fatty Liver Disease. *J Clin Transl Hepatol*. doi: 10.14218/jcth.2022.00110.
13. Azarova, I. et al. (2023). Single Nucleotide Polymorphisms of the RAC1 Gene as Novel Susceptibility Markers for Neuropathy and Microvascular Complications in Type 2 Diabetes. *Biomedicines*. **11**(3):981. doi: 10.3390/biomedicines11030981.
14. Kim, C.U. et al. (2023). Influenza viral matrix 1 protein aggravates viral pathogenicity by inducing TLR4-mediated reactive oxygen species production and apoptotic cell death. *Cell Death Dis*. **14**(3):228. doi: 10.1038/s41419-023-05749-5.
15. Casado-Barragán, F. et al. (2023). Increased Renal Medullary NOX-4 in Female but Not Male Mice during the Early Phase of Type 1 Diabetes: Potential Role of ROS in Upregulation of TGF- β 1 and Fibronectin in Collecting Duct Cells. *Antioxidants (Basel)*. **12**(3):729. doi: 10.3390/antiox12030729.
16. Mann, S. et al. (2023). The effect of heat treatment on colostral and newborn calf redox status and oxylipid biomarkers. *J Dairy Sci*. **106**(5):3537-3547. doi: 10.3168/jds.2022-22679.
17. Kang, J.S. et al. (2023). Long-term exposure changes the environmentally relevant bis(2-ethylhexyl) phthalate to be a neuro-hazardous substance disrupting neural homeostasis in emotional and cognitive functions. *Environ Pollut*. doi: 10.1016/j.envpol.2023.121387.
18. Martín, C. et al. (2023). Transcriptomic and physiological effects of polyethylene microplastics on *Zea mays* seedlings and their role as a vector for organic pollutants. *Chemosphere*. **322**:138167. doi: 10.1016/j.chemosphere.2023.138167.
19. Zhou, S. et al. (2023). The Anti-Aging Hormone Klotho Promotes Retinal Pigment Epithelium Cell Viability and Metabolism by Activating the AMPK/PGC-1 α Pathway. *Antioxidants (Basel)*. **12**(2):385. doi: 10.3390/antiox12020385.
20. Wang, J.C. et al. (2023). Hyperuricemia exacerbates abdominal aortic aneurysm formation through the URAT1/ERK/MMP-9 signaling pathway. *BMC Cardiovasc Disord*. **23**(1):55. doi: 10.1186/s12872-022-03012-x.

21. Zhivagui, M. et al. (2023). DNA damage and somatic mutations in mammalian cells after irradiation with a nail polish dryer. *Nat Commun.* **14**(1):276. doi: 10.1038/s41467-023-35876-8.
22. Kim, K.H. et al. (2023). Effect of N-acetyl-L-cysteine on Testicular Tissue in Busulfan-Induced Dysfunction in the Male Reproductive System. *World J Mens Health.* doi: 10.5534/wjmh.220100.
23. Radajewska, A. et al. (2023). Punica granatum L. Polyphenolic Extract as an Antioxidant to Prevent Kidney Injury in Metabolic Syndrome Rats. *Oxid Med Cell Longev.* doi: 10.1155/2023/6144967.
24. Jeon, H.J. et al. (2023). Developmental toxicity of chlorpyrifos-methyl and its primary metabolite, 3,5,6-trichloro-2-pyridinol to early life stages of zebrafish (Danio rerio). *Ecotoxicol Environ Saf.* doi: 10.1016/j.ecoenv.2022.114352.
25. Chen, Y. et al. (2023). Antioxidative behavior of α 2-macroglobulin in intervertebral disc degeneration. *J Med Biochem.* **42**:1-8. doi: 10.5937/jomb0-39557.
26. Liu, J. et al. (2022). Circulating hemopexin modulates anthracycline cardiac toxicity in patients and in mice. *Sci Adv.* **8**(51): eadc9245. doi: 10.1126/sciadv.adc9245.
27. Klysova, E. et al. (2022). A Polymorphism in the Gene Encoding Heat Shock Factor 1 (HSF1) Increases the Risk of Type 2 Diabetes: A Pilot Study Supports a Role for Impaired Protein Folding in Disease Pathogenesis. *Life (Basel).* **12**(11):1936. doi: 10.3390/life12111936.
28. Xu, H. et al. (2022). Molecular Mechanism of Epimedium Extract against Ischemic Stroke Based on Network Pharmacology and Experimental Validation. *Oxid Med Cell Longev.* doi: 10.1155/2022/3858314.
29. Härtel, J.A. et al. (2022). Influence of 24 h Simulated Altitude on Red Blood Cell Deformability and Hematological Parameters in Patients with Fontan Circulation. *Metabolites.* **12**(11):1025. doi: 10.3390/metabo12111025.
30. Hiramoto, K. et al. (2022). Skin, Liver, and Kidney Interactions Contribute to Skin Dryness in Aging KK-Ay/Tajcl Mice. *Biomedicines.* **10**(10):2648. doi: 10.3390/biomedicines10102648.

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
www.cellbiolabs.com

©2010-2023: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.