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Product Manual

# OxiSelect™ Cellular Antioxidant Activity Assay Kit (Green Fluorescence)

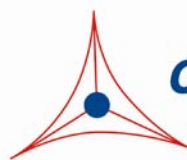
Catalog Number

STA- 349

192 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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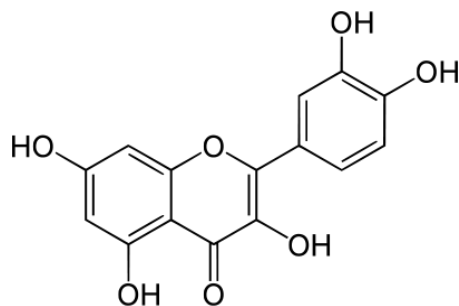
**CELL BIOLABS, INC.**

*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, phytochemical screening, and dietary monitoring of ROS activity within cells is crucial to suppressing or treating oxidative stress inducers.

Although many antioxidant activity assays exist, most do not measure *in vivo* activity accurately because they do not account for physiological conditions such as pH, temperature, or antioxidant bioavailability. Cell Biolabs' OxiSelect™ Cellular Antioxidant Assay Kit (Green Fluorescence) is a cell-based assay for measuring antioxidant activity within a cell in a standard cell culture environment. This environment accounts for the temperature, pH, uptake, metabolism and efficacy of antioxidants in a whole cell. The assay employs the cell-permeable fluorogenic probe dye 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 by free radicals to highly fluorescent 2', 7'-Dichlorodihydrofluorescein (DCF) (Figure 1). The fluorescence intensity is proportional to the ROS levels within the cell cytosol. The effect of antioxidant compounds on DCF formation can be measured against the fluorescence inhibition of the Quercetin standard. Quercetin is a plant flavonoid widely found in fruits and vegetables. The kit has a Quercetin detection sensitivity limit of approximately 10 µM. Each kit provides sufficient reagents to perform up to 192 assays, including standard curve and unknown samples.



Quercetin

## **Assay Principle**

The OxiSelect™ Cellular Antioxidant Assay Kit is a cell-based assay for measuring antioxidant activity within adherent cells. Cells are first cultured in a 96-well black fluorescence cell culture plate until confluent. Then the cells are pre-incubated with a cell-permeable DCFH-DA fluorescence probe dye and the bioflavonoid Quercetin, or the antioxidant sample being tested. After a brief incubation, the cells are washed, and the reaction started by adding the Free Radical Initiator. The Free Radical Initiator creates free radicals that convert the probe to highly fluorescent DCF. The Quercetin inhibits the formation of free radicals, and thus DCF formation, in a concentration dependent manner.

Fluorescence is measured over time in a standard microplate fluorometer. This fluorescence correlates to the Quercetin's ability to quench free radicals. Test antioxidant values can be compared to Quercetin to determine antioxidant activity within the cell.

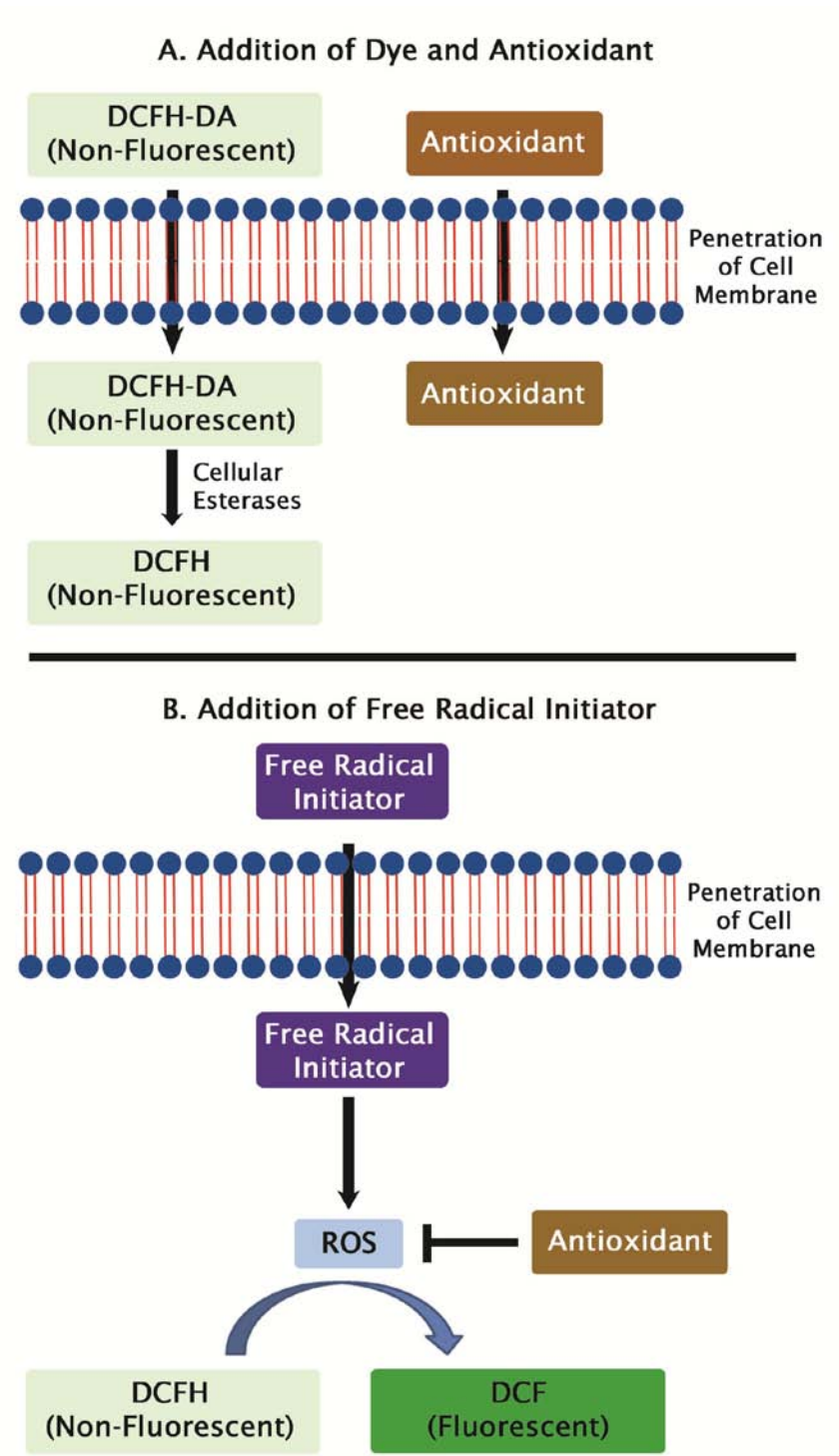


Figure 1. Mechanism of Cellular Antioxidant Assay

## **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-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
5. STA-343: OxiSelect™ Hydrogen Peroxide Assay Kit
6. STA-345: OxiSelect™ ORAC Activity Assay Kit
7. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
8. STA-360: OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit
9. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
10. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

## **Kit Components**

1. 96-well Cell Culture Microtiter Plate (Part No. 234904): Two 96-well tissue culture treated clear bottom black plates.
2. DCFH-DA Probe (1000X) (Part No. 234901): One 100 µL amber tube of a 1000X solution in methanol.
3. Free Radical Initiator (Part No. 234902): One 200 mg tube of powder.
4. Quercetin (Part No. 234903): One 250 µL tube of a 50 mM solution in DMSO.

## **Materials Not Supplied**

1. Adherent cell lines and test samples
2. Sterile DPBS or HBSS for washes and component dilutions
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Cell culture incubator (37°C, 5% CO<sub>2</sub> atmosphere)
7. Cell culture medium (ie: DMEM +/-10% FBS)
8. Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

## **Storage**

Upon receipt, store the DCFH-DA Probe (1000X) and Quercetin at -20°C. Avoid multiple freeze/thaw cycles. Store the remaining components at 4°C.

## **Preparation of Reagents**

- 2X DCFH-DA Probe: Dilute the DCFH-DA Probe (1000X) stock solution to 2X in cell culture media, preferably without FBS. Stir or vortex to homogeneity. Label this as 2X DCFH-DA Probe/Media Solution. Prepare only enough for immediate applications.

*Note: Due to light-induced auto-oxidation, DCFH-DA fluorescence probe solutions at any concentration must be protected from light.*

- Free Radical Initiator: Freshly weigh out and prepare a 2.8 % solution of the Free Radical Initiator in 1X sterile DPBS. (eg. Reconstitute 28 mg of powder in 1000  $\mu$ L DPBS). This is a 100X solution. Prior to use, dilute the Free Radical Initiator 1:100 in DPBS. Vortex thoroughly. The 100X Free Radical Initiator solution is stable for 1 week when stored at  $-20^{\circ}\text{C}$ .

## **Preparation of Samples**

*Note: Samples should be stored at  $-70^{\circ}\text{C}$  prior to performing the assay. Samples should be prepared at the discretion of the user. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.*

Prepare all samples by diluting and titrating in cell culture media. Samples will be diluted 1:2 final within the assay.

- Nutrition Extracts: Results may vary depending on sample source and purification. Dilution and preparation of these samples is at the discretion of the user, but use the following guidelines:

Weigh solid sample and then blend with a Waring blender for 5 min. in chilled 80% acetone (1:2 w/v, eg. 1 g sample in 2 mL of 80% acetone). Next, homogenize the samples for 3 min. Filter through Whatman paper and evaporate filtrate under vacuum. Reconstitute samples in 70% methanol and store at  $-80^{\circ}\text{C}$ . Before use, evaporate methanol, preferably under nitrogen, and reconstitute extracts with deionized water or DPBS. Samples may also be diluted in cell culture medium. Final treatment solutions should contain 2% or less of solvent to prevent cytotoxicity.

## **Preparation of Standard Curve**

Immediately before use, prepare a 1:25 dilution of the stock Quercetin in cell culture media. Use this Quercetin solution to prepare a series of Quercetin standards in the concentration range of 0  $\mu\text{M}$  – 2000  $\mu\text{M}$  in cell culture media according to the table below (see Table 1).

Standard Tubes	Quercetin Standard (μL)	Cell Culture Media (μL)	Quercetin (μM)
1	40	960	2000
2	500 of Tube #1	500	1000
3	500 of Tube #2	500	500
4	500 of Tube #3	500	250
5	500 of Tube #4	500	125
6	500 of Tube #5	500	62.5
7	500 of Tube #6	500	31.3
8	0	1000	0

**Table 1. Preparation of Quercetin Standards**

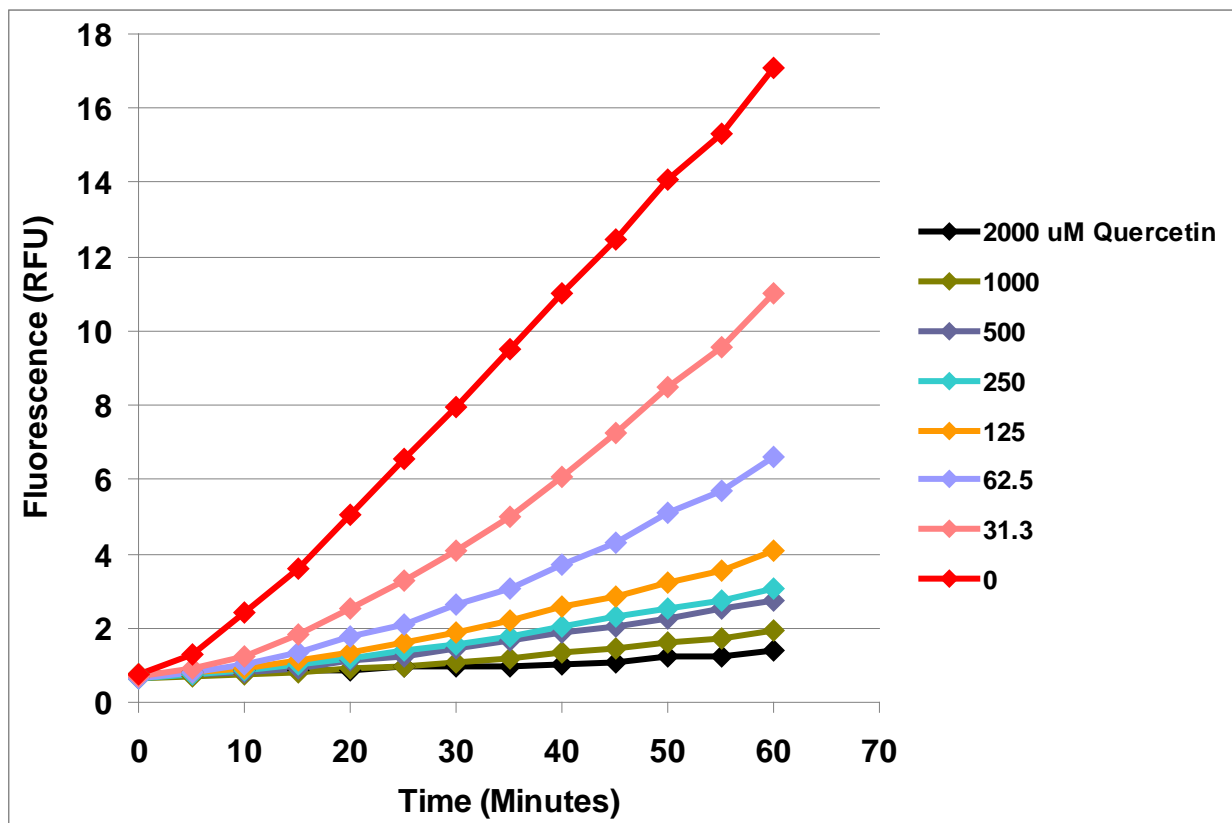
### **Assay Protocol**

*Note: Each Antioxidant Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.*

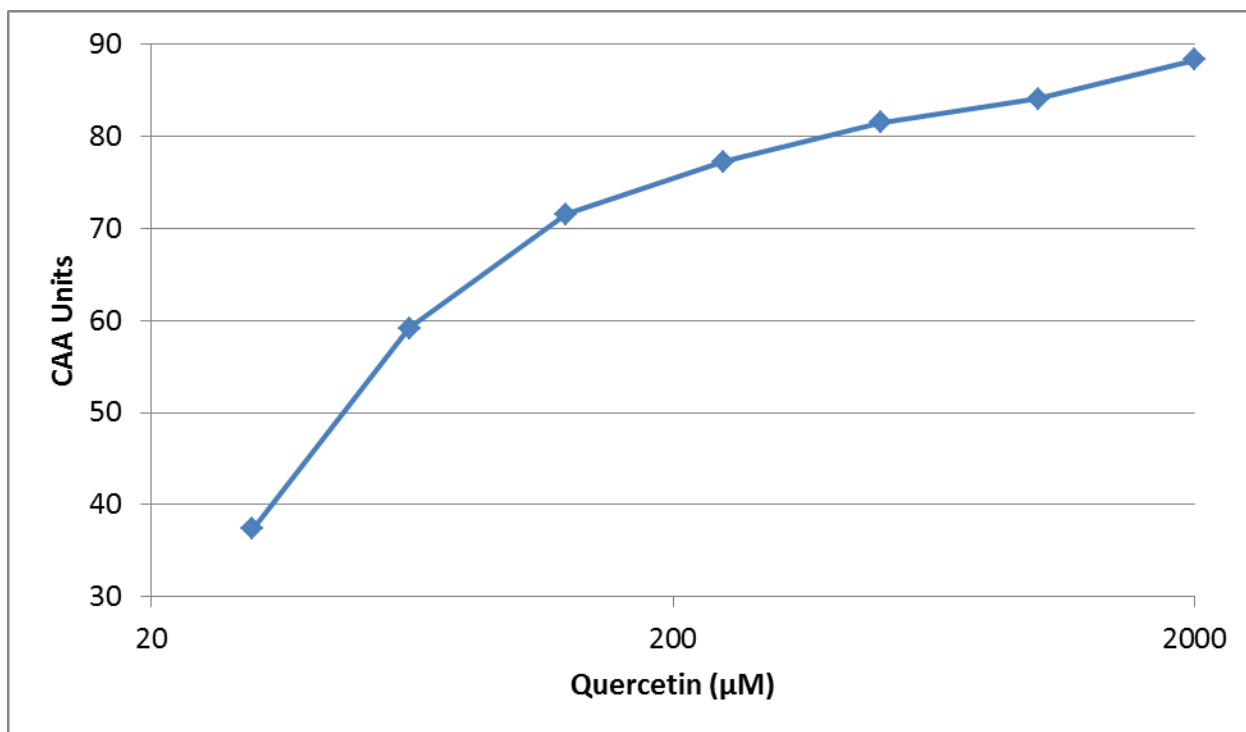
1. Culture cells such as HepG2 or HeLa in the 96-well Cell Culture Microtiter Plate until cells are 90% to 100% confluent.
2. Carefully remove media from all wells and discard. Wash cells gently 3 times with DPBS or HBSS. Remove the last wash and discard.
3. Add 50 μL of DCFH-DA Probe solutions to all wells with confluent cells to be tested.
4. Add 50 μL of Quercetin Standard or prepared sample to each well with confluent cells to be tested. Incubate at 37°C for 60 minutes.
5. Carefully remove the solution. Wash 3 times with DPBS or HBSS. Remove the last wash and discard.
6. Add 100 μL of the Free Radical Initiator solution to all wells. Immediately begin reading wells with a fluorescent microplate reader at 37°C with an excitation wavelength of 480nm and an emission wavelength of 530nm. Read the wells in increments between 1 and 5 minutes for a total of 60 minutes. Save values for Calculation of Results.

## Example of Results

The following figures demonstrate typical Intracellular Antioxidant 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. Cellular Antioxidant Activity of Quercetin in HeLa Cells.** 60,000 HeLa cells were seeded and cultured in a 96-well plate until confluent. Cells were then pretreated with DCFH-DA and Quercetin for 60 minutes at 37°C. Free Radical Initiator was then added to the cells to begin the assay, which was read every five minutes for 1 hour at 37°C.



**Figure 3. Dose-Response Curve of Quercetin Standard.**

### Calculation of Results

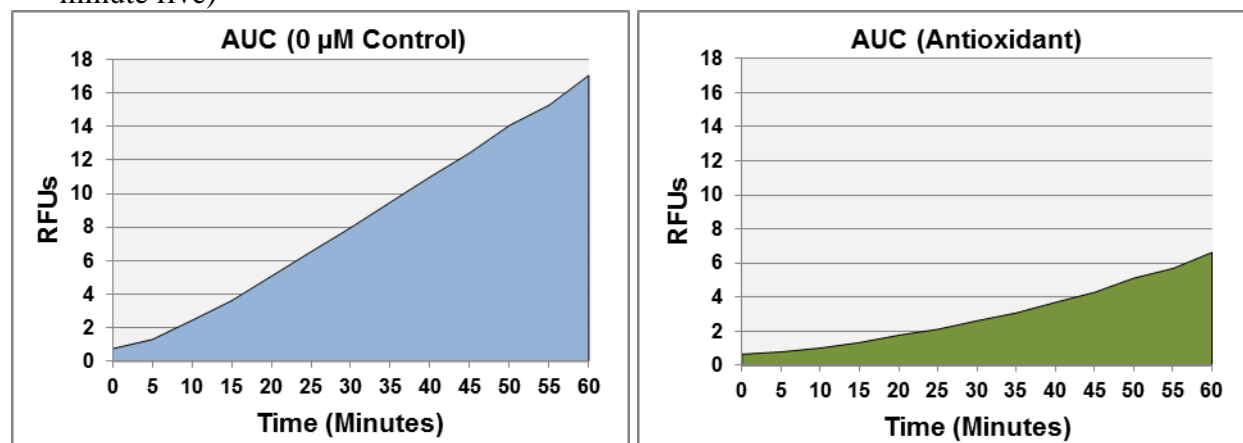
*Note: A spreadsheet application or plate reader software can be used to perform the calculations.*

1. Calculate the integrated area under the curve (AUC) for each sample and standard using the final assay values and the linear regression formula below:

$$AUC = 1 + RFU_1/RFU_0 + RFU_2/RFU_0 + RFU_3/RFU_0 + \dots + RFU_{59}/RFU_0 + RFU_{60}/RFU_0$$

**RFU<sub>0</sub>** = relative fluorescence value of time point zero.

**RFU<sub>x</sub>** = relative fluorescence value of time points (e.g. RFU<sub>5</sub> is relative fluorescence value at minute five)



**Figure 4. Example Data for Calculation of AUC for Antioxidant Sample and Negative Control.**



2. Use the AUC values to determine the Cellular Antioxidant Activity values according to the formula:

$$\text{CAA Units} = 100 - (\text{AUC}_{\text{Antioxidant}} / \text{AUC}_{\text{Control}}) \times 100$$

3. Plot a dose-response curve by graphing CAA units versus Quercetin concentration (see Figure 3). Based on the Quercetin antioxidant standard curve, determine the equivalent Quercetin Equivalents (QE) value of unknown samples.

## **References**

1. Martins, E.A., et al. (1991) *Mutat. Res.* **250**: 95-101.
2. Rice-Evans, C.A., et al. (1995) *Free Radic Biol. Med.* **22**: 375-383.
3. Sun, J., et al. (2002) *J. Agric. Food Chem.* **50**: 7449-7454.
4. Wang, H., et al. (1996) *J. Agric. Food Chem.* **44**: 701-705.

## **Recent product citations**

1. Pagano, I. et al. (2016). Chemical profile and cellular antioxidant activity of artichoke by-products. *Food Funct.* doi:10.1039/C6FO01443G.
2. Chiang, M. et al. (2016). Chemical-and cell-based antioxidant capacity of methanolic extracts of three commonly edible plants from Zingiberaceae family. *Free Radical Antioxid.* **7**:57-62.
3. Verbanac, D. et al. (2016). Synthesis and evaluation of antibacterial and antioxidant activity of novel 2-phenyl-quinoline analogs derivatized at position 4 with aromatically substituted 4 H-1, 2, 4-triazoles. *J Enzyme Inhib Med Chem.* doi:10.1080/14756366.2016.1190714.
4. Yamani, H. & Abbenante, G. (2015). The OxiSelect Cellular Antioxidant Assay (CAA) on the FLUOstar Omega. BMG Labtech Application Notes.

## **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.

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