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Product Manual

# OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit (Colorimetric)

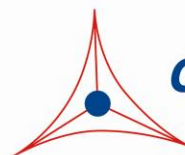
Catalog Number

STA-844

500 assays

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

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*Creating Solutions for Life Science Research*

## **Introduction**

Oxidative stress is a physiological condition where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. Research has shown that excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. Peroxides, such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), are some of the most well documented ROS produced under oxidative stress conditions. Hydrogen peroxide is an ROS that is a toxic product of normal aerobic metabolism and pathogenic ROS production involving oxidase and superoxide dismutase reactions. Hydrogen peroxide is poisonous to eukaryotic cells and in high doses can initiate oxidation of DNA, lipids, and proteins, which can lead to mutagenesis and cell death. The cellular damage caused by peroxides have been implicated in the development of many pathological conditions, such as ageing, asthma, arthritis, diabetes, cardiovascular disease, atherosclerosis, Down's Syndrome, and neurodegenerative diseases.

Cell Biolabs' OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit is a simple HTS-compatible assay for measuring hydrogen peroxide concentrations or peroxidase activities in biological samples without any need for pretreatment. The colorimetric probe reacts with  $\text{H}_2\text{O}_2$  and horseradish peroxidase enzyme (HRP) to produce a pink colored product. The probe has less background and greater stability than the commonly used Xylenol Orange (FOX) colorimetric assay for  $\text{H}_2\text{O}_2$ . The probe can be also used as an ultrasensitive assay for peroxidase activity when  $\text{H}_2\text{O}_2$  is in excess. The kit has a detection sensitivity limit of  $0.8 \mu\text{M}$  ( $\text{H}_2\text{O}_2$ ) or  $0.16 \text{ mU/mL}$  (peroxidase). Each kit provides sufficient reagents to perform up to 500 assays, including standard curve and unknown samples.

## **Assay Principle**

The OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit is a sensitive quantitative colorimetric assay for hydrogen peroxide or peroxidase. In the presence of peroxidase, the probe reacts with  $\text{H}_2\text{O}_2$  in a 1:1 stoichiometry to produce a bright pink colored product. This product can be easily read by a standard colorimetric microplate reader with a filter in the 540-570 nm range. Absorbance values are proportional to the  $\text{H}_2\text{O}_2$  or peroxidase levels within the samples, depending on the assay employed. The  $\text{H}_2\text{O}_2$  or peroxidase content in unknown samples is determined by comparison with its respective standard curve.

## **Related Products**

1. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
2. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
3. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
4. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
5. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

## **Kit Components**

1. Colorimetric Probe (100X) (Part No. 284401): One 250 µL amber tube of solution.
2. HRP (Part No. 234402): One 100 µL tube of a 100 U/mL solution in glycerol\*.
3. Hydrogen Peroxide (Part No. 234102): One 100 µL amber tube of an 8.8 M solution.
4. 10X Assay Buffer (Part No. 234403): One 25 mL bottle.

*\*Note: One unit is defined as the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 and 20°C.*

## **Materials Not Supplied**

1. Distilled or deionized water
2. 1X PBS for sample 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. Standard 96-well microtiter plate
6. Multichannel micropipette reservoir
7. Spectrophotometric microplate reader capable of reading in the 540-570 nm absorbance range.
8. Superoxide dismutase (optional)

## **Storage**

Upon receipt, aliquot and store the Colorimetric Probe and HRP at -20°C. Avoid multiple freeze/thaw cycles. Store the remaining kit components at 4°C. The Colorimetric Probe is light sensitive and must be stored accordingly.

## **Preparation of Reagents**

*Note: All reagents must be brought to room temperature prior to use.*

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Hydrogen Peroxide Working Solution (Hydrogen Peroxide Assay): If measuring hydrogen peroxide, prepare a working solution by diluting the Colorimetric Probe 1:100 and HRP to a final

concentration of 0.2 U/mL in 1X Assay Buffer (eg. Add 50  $\mu$ L Colorimetric Probe stock solution and 10  $\mu$ L HRP stock solution to 4.940 mL 1X Assay Buffer). This volume is enough for ~100 assays. The Hydrogen Peroxide Working Solution should be protected from light and used within 4 hours. Prepare only enough for immediate use.

- Peroxidase Working Solution (Peroxidase Assay): If measuring peroxidases, prepare a working solution by diluting the Colorimetric Probe 1:100 and H<sub>2</sub>O<sub>2</sub> to a final concentration of 2 mM in 1X Assay Buffer. First perform a 1:1000 dilution of the stock H<sub>2</sub>O<sub>2</sub> in 1X Assay Buffer. Use only enough for immediate applications (eg. Add 5  $\mu$ L of H<sub>2</sub>O<sub>2</sub> to 4.995 mL 1X Assay Buffer). This solution has a concentration of 8.8 mM. Use this 8.8 mM H<sub>2</sub>O<sub>2</sub> solution to prepare a 2 mM H<sub>2</sub>O<sub>2</sub> solution in Probe/1X Assay Buffer (eg. Add 50  $\mu$ L Colorimetric Probe stock solution and 1.14 mL of the prepared 8.8 mM H<sub>2</sub>O<sub>2</sub> solution to 3.81 mL 1X Assay Buffer). This volume is enough for ~100 assays. The Peroxidase Working Solution should be protected from light and used within 4 hours. Prepare only enough for immediate use.

### **Preparation of Samples**

- Cell Culture Supernatant: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the H<sub>2</sub>O<sub>2</sub> standard curve in the same non-conditioned media. Serum should be avoided, as it interferes with the assay. *Note: Maintain pH between 7 and 8 for optimal working conditions as the probe is unstable at high pH (>8.5).*
- Cell lysate: Resuspend cells at 1-2 x 10<sup>6</sup> cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or titrated as necessary.
- Plasma, Serum or Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Undiluted serum or plasma may interfere with the assay.

#### *Notes:*

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *A serial dilution will be necessary depending on the total H<sub>2</sub>O<sub>2</sub> or peroxidase present. Extremely high levels of H<sub>2</sub>O<sub>2</sub> ( $\geq 500 \mu$ M final concentration) or peroxidase ( $\geq 100$  mU/mL) can lower the absorbance because excess H<sub>2</sub>O<sub>2</sub> or peroxidase can further oxidize the reaction product.*
- *Samples with NADH concentrations above 10  $\mu$ M and glutathione concentrations above 50  $\mu$ M will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Tatyana et al, Ref. 2).*
- *Avoid samples containing DTT or  $\beta$ -mercaptoethanol since the probe is not stable in the presence of thiols (above 10  $\mu$ M).*

## Preparation of Standard Curves

- **H<sub>2</sub>O<sub>2</sub> Standard:** To prepare the H<sub>2</sub>O<sub>2</sub> standards, first perform a 1:1000 dilution of the stock H<sub>2</sub>O<sub>2</sub> in 1X Assay Buffer. Prepare only enough for immediate use (e.g., Add 5 µL of H<sub>2</sub>O<sub>2</sub> to 4.995 mL 1X Assay Buffer). This solution has a concentration of 8.8 mM. Use this 8.8 mM H<sub>2</sub>O<sub>2</sub> solution to prepare standards in the concentration range of 0 µM – 100 µM by further diluting in 1X Assay Buffer (e.g., Add 11.5 µL of H<sub>2</sub>O<sub>2</sub> to 988.5 µL 1X Assay Buffer - see Table 1 below). H<sub>2</sub>O<sub>2</sub> diluted solutions and standards should be prepared fresh.

Standard Tubes	8.8 mM H <sub>2</sub> O <sub>2</sub> Standard (µL)	1X Assay Buffer (µL)	H <sub>2</sub> O <sub>2</sub> (µM)
1	11.5	988.5	100
2	500 of Tube #1	500	50
3	500 of Tube #2	500	25
4	500 of Tube #3	500	12.5
5	500 of Tube #4	500	6.25
6	500 of Tube #5	500	3.125
7	500 of Tube #6	500	1.56
8	500 of Tube #7	500	0.78
9	0	500	0

**Table 1. Preparation of H<sub>2</sub>O<sub>2</sub> Standards**

- **Peroxidase Standard:** To prepare the peroxidase standards, first perform a 1:1000 dilution of the stock HRP in 1X Assay Buffer (e.g., Add 5 µL of HRP stock to 4.995 mL 1X Assay Buffer). Prepare only enough for immediate use. This solution has a concentration of 100 mU/mL. Use this 100 mU/mL solution to prepare standards in the concentration range of 0 mU/mL – 10 mU/mL by further diluting in 1X Assay Buffer (see Table 2 below). HRP diluted solutions and standards should be prepared fresh.

Standard Tubes	100 mU/mL HRP Standard (µL)	1X Assay Buffer (µL)	HRP (mU/mL)
1	100	900	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.63
6	500 of Tube #5	500	0.32
7	500 of Tube #6	500	0.16
8	0	500	0

**Table 2. Preparation of HRP Standards**

## **Assay Protocol**

### **I. Hydrogen Peroxide**

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50  $\mu\text{L}$  of each sample ( $\text{H}_2\text{O}_2$  standard, control or unknown) into an individual microtiter plate well.
3. Add 50  $\mu\text{L}$  of Hydrogen Peroxide Working Solution to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature protected from light.

*Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the kinetics of the reactions.*

4. Read the plate absorbance with a microplate reader in the 540-570 nm range.
5. Calculate the concentration of peroxide within samples by comparing the sample absorbance to the standard curve. Subtract the value from the zero  $\text{H}_2\text{O}_2$  control.

### **II. Peroxidase**

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50  $\mu\text{L}$  of each sample (HRP standard, control or unknown) into an individual microtiter plate well.
3. Add 50  $\mu\text{L}$  of Peroxidase Working Solution to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature protected from light.

*Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the kinetics of the reactions.*

4. Read the plate absorbance with a microplate reader in the 540-570 nm range.
5. Calculate the concentration of peroxidase within samples by comparing the sample absorbance to the standard curve. Subtract the value from the zero HRP control.

## **Example of Results**

The following figures demonstrate typical Hydrogen Peroxide/Peroxidase Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

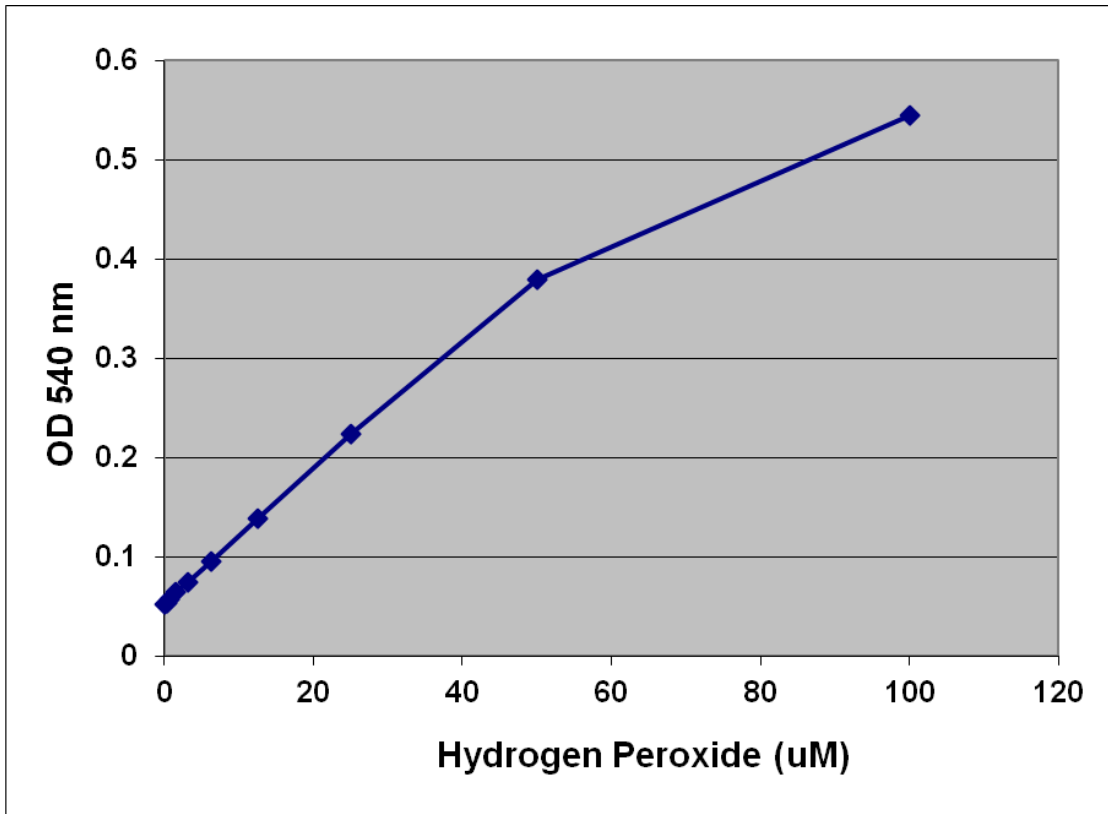


Figure 1. H<sub>2</sub>O<sub>2</sub> Standard Curve.

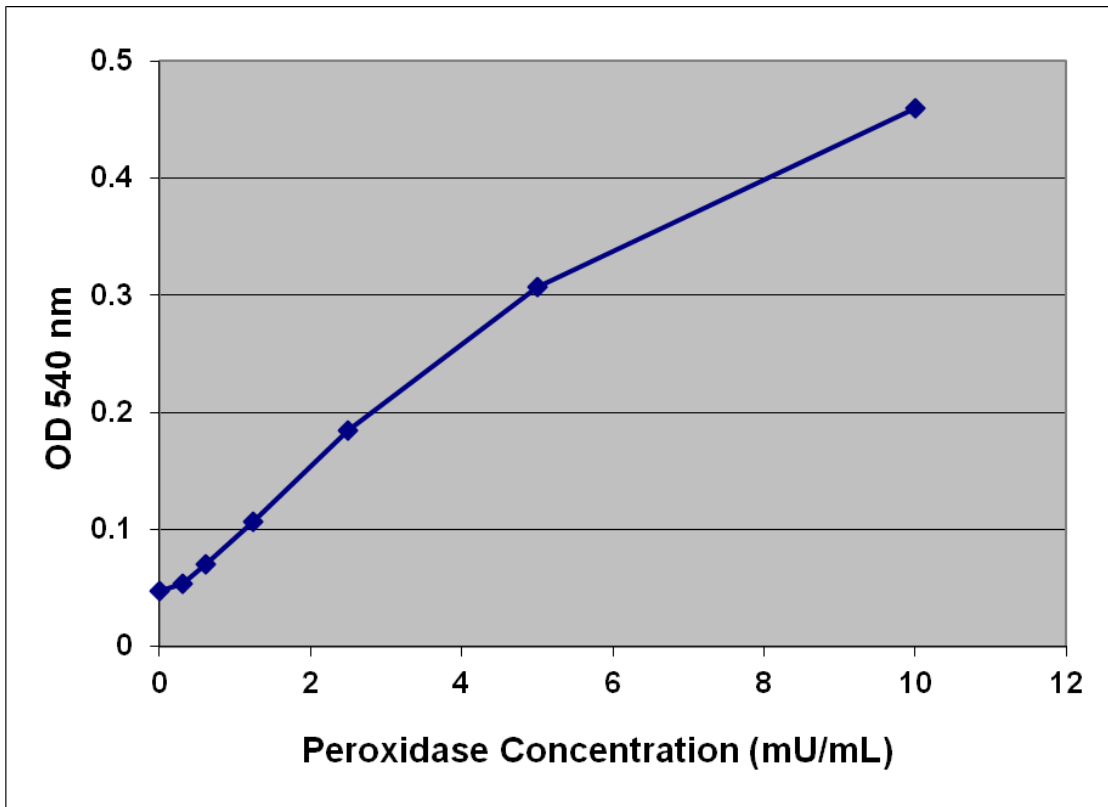


Figure 2. HRP Standard Curve.

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