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

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 (H_2O_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 H_2O_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 H_2O_2. The probe can be also used as an ultrasensitive assay for peroxidase activity when H_2O_2 is in excess. The kit has a detection sensitivity limit of 500 nM (H_2O_2) or 0.16 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 H_2O_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 H_2O_2 or peroxidase levels within the samples, depending on the assay employed. The H_2O_2 or peroxidase content in unknown samples is determined by comparison with its respective 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-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
5. STA-345: OxiSelect™ ORAC Activity Assay Kit
6. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
7. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
8. 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 20ºC.*

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 -20ºC. Avoid multiple freeze/thaw cycles. Store the remaining kit components at 4ºC. 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 µL Colorimetric Probe stock solution and 10 µL 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_2O_2 to a final concentration of 2 mM in 1X Assay Buffer. First perform a 1:1000 dilution of the stock H_2O_2 in 1X Assay Buffer. Use only enough for immediate applications (eg. Add 5 µL of H_2O_2 to 4.995 mL 1X Assay Buffer). This solution has a concentration of 8.8 mM. Use this 8.8 mM H_2O_2 solution to prepare a 2 mM H_2O_2 solution in Probe/1X Assay Buffer (eg. Add 50 µL Colorimetric Probe stock solution and 1.14 mL of the prepared 8.8 mM H_2O_2 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_2O_2 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 10^6 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 or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary.

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_2O_2 or peroxidase present. Extremely high levels of H_2O_2 (≥ 500 µM final concentration) or peroxidase (≥ 100 mU/mL) can lower the absorbance because excess H_2O_2 or peroxidase can further oxidize the reaction product.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM 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 β-mercaptoethanol since the probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curves

- **H_2O_2 Standard:** To prepare the H_2O_2 standards, first perform a 1:1000 dilution of the stock H_2O_2 in 1X Assay Buffer. Prepare only enough for immediate use (e.g. Add 5 µL of H_2O_2 to 4.995 mL 1X Assay Buffer). This solution has a concentration of 8.8 mM. Use this 8.8 mM H_2O_2 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_2O_2 to 988.5 µL 1X Assay Buffer - see Table 1 below). H_2O_2 diluted solutions and standards should be prepared fresh.

Standard Tubes	8.8 mM H_2O_2 Standard (µL)	1X Assay Buffer (µL)	H_2O_2 (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_2O_2 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 µL of each sample (H_2O_2 standard, control or unknown) into an individual microtiter plate well.
3. Add 50 µL 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 H_2O_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 µL of each sample (HRP standard, control or unknown) into an individual microtiter plate well.
3. Add 50 µL 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.

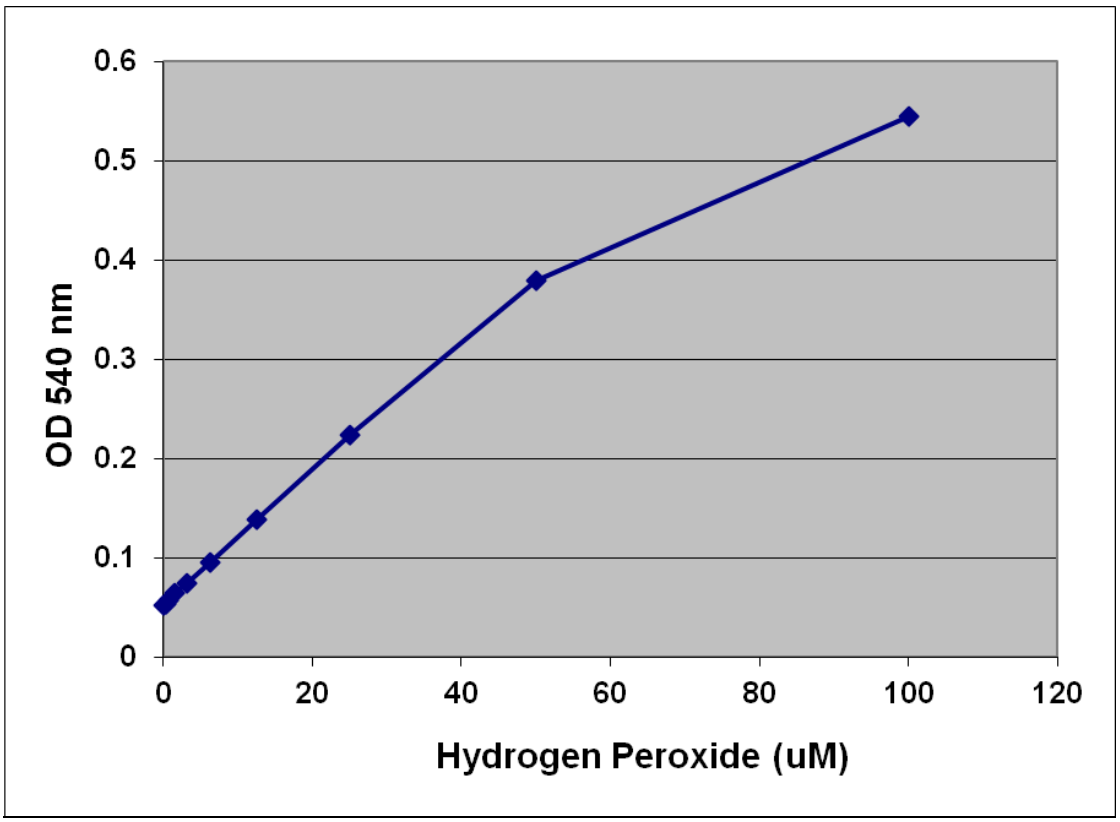

Figure 1. H_2O_2 Standard Curve.

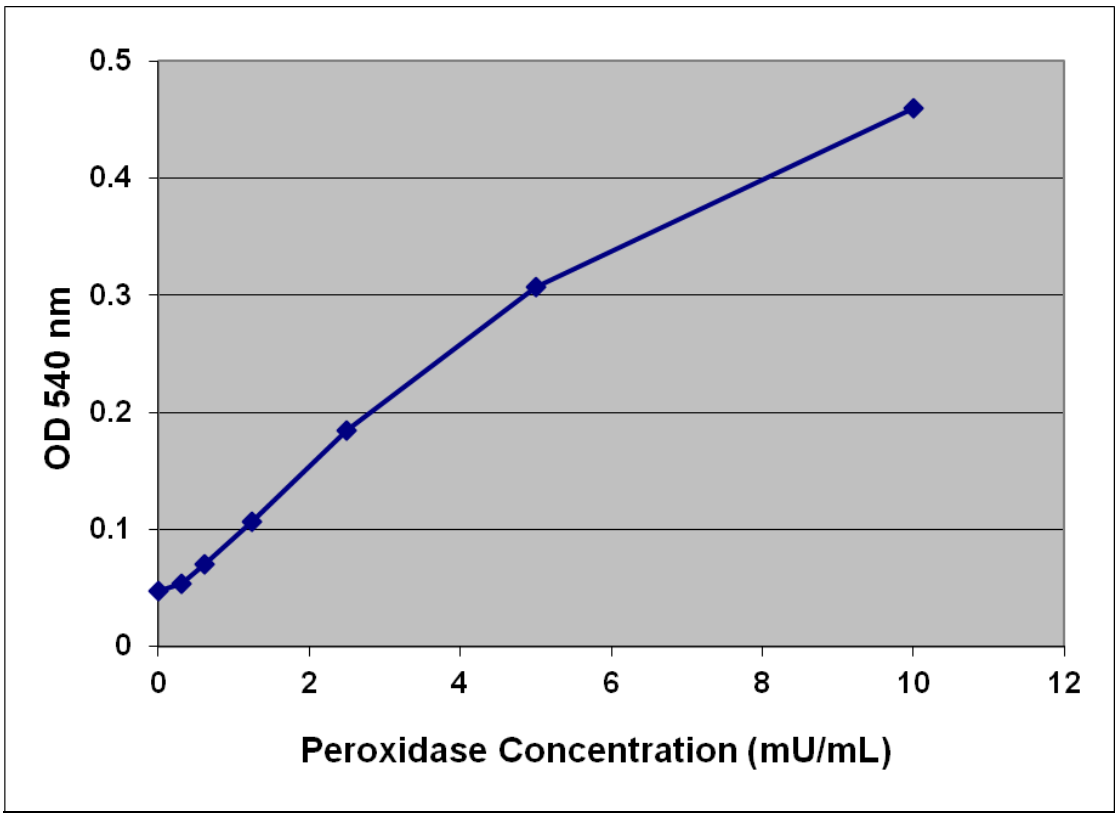

Figure 2. HRP Standard Curve.

References

1. Mohanty, J.G., et al. (1997) *J. Immunol. Methods* **202**: 133.
2. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.
3. Votyakova TV, and Reynolds IJ (2004) *Archives of Biochemistry and Biophysics*. **431**: 138-144.

Recent Product Citations

1. Otreba, M. et al (2017). Prochlorperazine interaction with melanin and melanocytes. *Die Pharmazie - An International Journal of Pharmaceutical Sciences*. **72** (3): 171-176(6).
2. Lee, S.H. et al. (2016). Proteomic analysis indicates activation of reactive oxygen species signaling during seed germination and seedlings growth in *Hordeum vulgare* (barley). *J. Prot. Proteom.* **7**:269-277.

Warranty

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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