StressMarq Biosciences INC.

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StressXpress®

Hydrogen Peroxide Detection Kit

Catalog# SKT-216 (2 Plate kit)

Quantitative colorimetric measurement of H_2O_2

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-216A	Clear 96 well Half Area Plates	2 Plates	Corning Costar Plate 3695.
SKC-216B	Hydrogen Peroxide Standard	220 µL	Hydrogen Peroxide at 1,000 µM in a special stabilizing solution.
SKC-216C	Assay Buffer Concentrate	25 mL	A 5X buffer concentrate containing detergents and stabilizers.
SKC-216D	Colorimetric Substrate	5 mL	A solution of the substrate in a special stabilizing buffer.
SKC-216E	Horseradish Peroxidase Concentrate	120 µL	A 50X concentrated solution of HRP in a special stabilizing solution.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

! **WARNING: Not for human or animal disease diagnosis or therapeutic drug use.**

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product. The supplied hydrogen peroxide standard contains very dilute H_2O_2.

Storage

All components of this kit should be stored at 4°C until the expiration date of the kit.

Materials Needed But Not Supplied

- Repeater pipet with disposable tips capable of dispensing 25 µL.
- 96 well plate reader capable of reading at 560 nm (Acceptable Range 540-580 nm.). Set plate parameters for a 96-well Corning Costar 3695 plate. Plate Dimensions (in mm): Well Depth: 10.54; Plate Length: 127.8; Plate Width: 85.5; A1 Row Offset: 11.2; A1 Column Offset: 14.3.
- Software for converting colorimetric intensity readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Please read this booklet completely prior to using the product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Hydrogen peroxide was first described in 1818 by Louis Jacques Thénard. Today, industrially, hydrogen peroxide is manufactured almost exclusively by the autoxidation of a 2-alkyl-9,10-dihydroxyanthracene to the corresponding 2-alkyl anthraquinone in the Riedl-Pfleiderer or anthraquinone process.

In biological systems incomplete reduction of O_2 during respiration produces superoxide anion (O_2-·), which is spontaneously or enzymatically dismutated by superoxide dismutase to H_2O_2. Many cells produce low levels of O_2-· and H_2O_2 in response to a variety of extracellular stimuli, such as cytokines (TGF-ß1, TNF-a, and various interleukins), peptide growth factors (PDGF; EGF, VEGF, bFGF, and insulin), the agonists of heterotrimeric G protein–coupled receptors (GPCR) such as angiotensin II, thrombin, lysophosphatidic acid, sphingosine 1-phosphate, histamine, and bradykinin, and by shear stress (1). The addition of exogenous H_2O_2 or the intracellular production in response to receptor stimulation affects the function of various proteins, including protein kinases, protein phosphatases, transcription factors, phospholipases, ion channels, and G proteins. In 1894, Fenton (2) described the oxidation of tartaric acid by Fe2+ and H_2O_2. H_2O_2 and O_2 may participate in the production of singlet oxygen and peroxynitrite and the generation of these species may be concurrent with reactions involving iron, and under some circumstances they might be important contributors to H_2O_2 toxicity (3,4).

A substantial portion of H_2O_2 lethality involves DNA damage by oxidants generated from iron-mediated Fenton reactions(5,6). Damage by Fenton oxidants occurs at the DNA bases or at the sugar residues. Sugar damage is initiated by hydrogen abstraction from one of the deoxyribose carbons, and the predominant consequence is eventual strand breakage and base release (7,8).

Assay Overview

The Hydrogen Peroxide *StressXpress*® Colorimetric Detection Kit is designed to quantitatively measure H_2O_2 in a variety of samples. Please read the complete kit insert before performing this assay. A hydrogen peroxide standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are mixed with the Colorimetric Substrate and the reaction initiated by addition of horseradish peroxidase. The reaction is incubated at room temperature for 15 minutes. The HRP reacts with the substrate in the presence of hydrogen peroxide to convert the colorless substrate into a colored product. The pink product is read at 560 nm. Increasing levels of H_2O_2 cause a linear increase in color.

Sample Types

Sample Types Validated:
Fresh Urine, Buffers and TCM

Samples that need to be stored after collection should be stored at -70°C or lower, preferably after being frozen in liquid nitrogen. Urine samples can be used after being diluted ≥ 1:10. This assay has been validated for buffer and media samples.

Sample Preparation

Dilute samples ≥ 1:10 with Assay Buffer prior to running in the assay.

Reagent Preparation

Assay Buffer Preparation
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Horseradish Peroxidase (HRP) Preparation
Dilute the HRP Stock solution 1:50 with Assay Buffer using the table below:

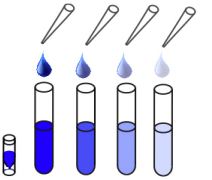
HRP Dilution Table:

	1/2 Plate	One Plate	Two Plates
HRP Stock	30 µL	60 µL	110 µL
Assay Buffer	1.47 mL	2.94 mL	5.39 mL
Total Volume	1.5 mL	3 mL	5.5 mL

Standard Preparation

Hydrogen Peroxide Standards are prepared by labeling six tubes as #1 through #6. Briefly vortex to mix the vial of H_2O_2 standard. Pipet 450 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #6. Carefully add 50 µL of the H_2O_2 Standard to tube #1 and vortex completely. Take 200 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #6. The concentration of H_2O_2 in tubes 1 through 6 will be 100, 50, 25, 12.5, 6.25, and 3.125 µM.

Use all Standards within 2 hours of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Assay Buffer (µL)	450	200	200	200	200	200
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Volume of Addition (µL)	50	200	200	200	200	200
Final Concentration (µM)	100	50	25	12.5	6.25	3.125

Assay Protocol

Use the plate layout sheet on page 17 to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3695 plate. Plate Dimensions (in mm): Well Depth: 10.54; Plate Length: 127.8; Plate Width: 85.5; A1 Row Offset: 11.2; A1 Column Offset: 14.3.

1. Pipet 50 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 50 µL of Assay Buffer into duplicate wells as the Zero standard.
3. Add 25 µL of Colorimetric Substrate to each well using a repeater pipet.
4. Initiate the reaction by adding 25 µL of the HRP Preparation to each well using a repeater pipet.
5. Incubate at room temperature for 15 minutes.
6. Read the plate at 560 nm (Acceptable Range 540-580 nm.).

ANALYSIS

Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean ODs for the Zero wells. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

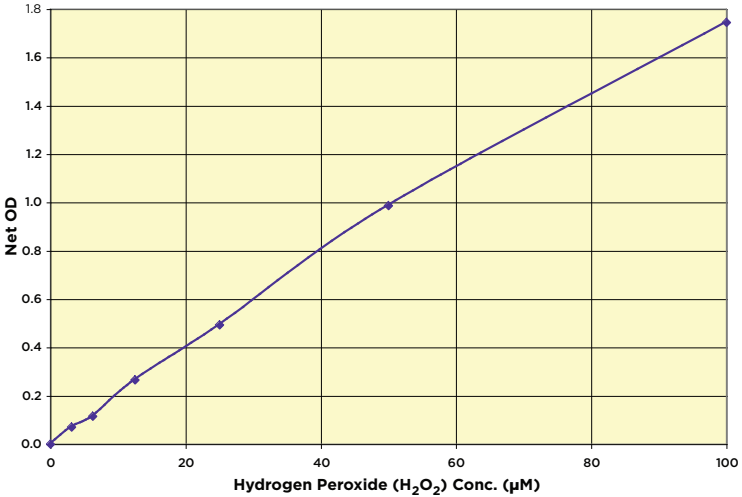
Sample	Mean OD	Net OD	H_2O_2 Concentration (µM)
Zero	0.075	0	0
Standard 1	1.820	1.745	100
Standard 2	1.062	0.987	50
Standard 3	0.569	0.494	25
Standard 4	0.341	0.266	12.5
Standard 5	0.190	0.115	6.25
Standard 6	0.146	0.071	3.125
Sample 1	1.453	1.378	76.7
Sample 2	0.434	0.359	18.3

Always run your own standard curves for calculation of results.

Do not use these data.

Conversion Factor: 100 µM of Hydrogen Peroxide is equivalent to 3.4 µg/mL.

Typical Standard Curve



Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 1.83 µM. This is equivalent to 91.3 pmol (3.10 ng) H_2O_2 per well

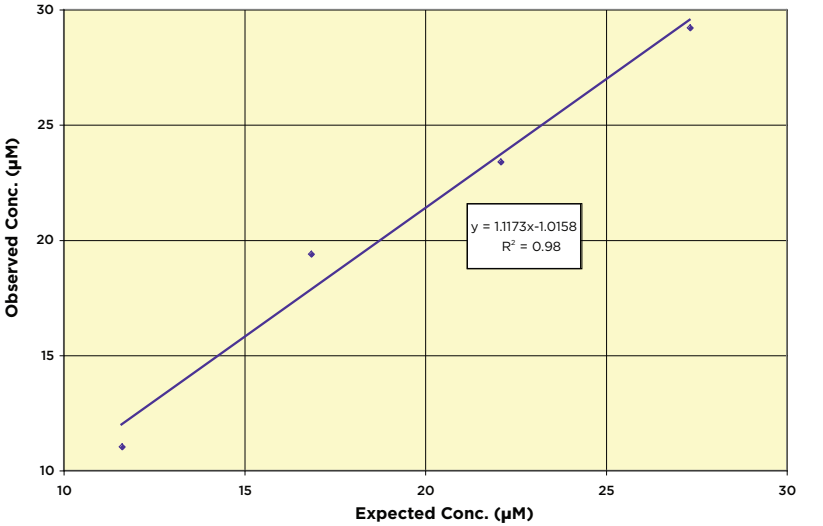
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human sample.

The Limit of Detection was determined as 1.96 µM. This is equivalent to 98.0 pmol (3.33 ng) H_2O_2 per well

Linearity

Linearity was determined by taking two diluted human urine samples with known H_2O_2 concentrations and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Observed Concentration (µM)	Expected Concentration (µM)	% Recovery
80%	20%	29.2	27.3	106.8
60%	40%	23.4	22.1	105.8
40%	60%	19.4	16.9	114.9
20%	80%	11.0	11.6	94.9
			Mean Recovery	105.6%



Intra Assay Precision

Three buffer samples were run in replicates of 20 in an assay. The mean and precision of the calculated concentrations were:

Sample	H_2O_2 Concentration (µM)	%CV
1	82.2	2.1
2	53.1	2.4
3	19.4	5.9

Inter Assay Precision

Three buffer samples were run in duplicate in twelve assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	H_2O_2 Concentration (µM)	%CV
1	79.9	3.7
2	49.5	4.5
3	18.4	4.3

References

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5. Imlay, JA., and Linn, S. "DNA damage and oxygen radical toxicity." 1988, Science 240:1302–1309.
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Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

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Hours: M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).