Hydrogen Peroxide Colorimetric Detection Kit

Catalog Number KA1017

96 assays

Version: 05

Intended for research use only

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Introduction

Background

Hydrogen Peroxide (H_20_2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states.[1,2] Functioning through NF kappa-B and other factors, hydroperoxide-mediated pathways have been linked to asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's Syndrome.[3-11] Perhaps the most intriguing aspect of H_20_2 biology is the recent report that antibodies have the capacity to convert molecular oxygen into hydrogen peroxide to contribute to the normal recognition and destruction processes of the immune system.[12,13] Measurement of this reactive species will help to determine how oxidative stress modulates varied intracellular pathways

Principle of the Assay

The Colorimetric Hydrogen Peroxide kit is a complete kit for the quantitative determination of Hydrogen Peroxide in biological fluids and tissue culture media. Please read the complete kit insert before performing this assay. The kit is designed to measure low concentrations of H_20_2 in biological matrices. The kit has a color reagent that contains a dye, xylenol orange, in an acidic solution with sorbitol and ammonium iron sulfate that reacts to produce a purple color proportional to the concentration of H_20_2 in the sample. The exact mechanism of the color reaction is not known, but probably involves coordinated iron reacting with H_20_2 and the dye molecule.

General Information

Materials Supplied

List of component

Component	Description	Amount
Half-Area Microtiter Plate	The plate is ready to use.	1 each
Hydrogen Peroxide Standard	A solution of Hydrogen Peroxide at 100,000 ng/mL in water with preservatives.	0.5 mL
Hydrogen Peroxide Color Reagent	A solution of colorimetric substrate in dilute acid.	11 mL
Plate Sealer		2 each

Storage Instruction

All components of this kit, except the Hydrogen Peroxide Color Reagent, are stable at 4℃ until the kit's expiration date. The Hydrogen Peroxide Color Reagent must be stored at -20℃.

Materials Required but Not Supplied

- ✓ Recommended sample diluent, 50 mM Phosphate, pH 6.0.
- ✓ Precision pipets for volumes between 34 µL and 1,000µL.
- ✓ Repeater pipet for dispensing 100µL.
- ✓ Microplate reader capable of reading between 540 and 570 nm, ideally 550 nm.
- ✓ Graph paper for plotting the standard curve.

Precautions for Use

- Precautions

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- ✓ Dispose of the contents of the plate with care. Attention should be taken in handling because of unknown effects of the contents.
- ✓ We test this kit's performance in a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
- ✓ The standard is light-sensitive and should be protected from direct light for prolonged periods of time.

- Procedure Note:

✓ Do not mix components from different lot numbers or use reagents beyond the expiration date.

✓ Allow all reagents, with the exception of the Hydrogen Peroxide Color Reagent, to warm to room temperature for at least 30 minutes before opening.

✓ The Hydrogen Peroxide Color Reagent must be kept at 4℃ during use.

✓ Standards can be made up in either glass or plastic tubes.

✓ Pre-rinse the pipet tip with the reagent, use fresh pipet tips for each sample, standard and reagent.

✓ Pipet standards and samples to the bottom of the wells.

✓ Add the reagent to the side of the well to avoid contamination.

Assay Protocol

Reagent Preparation

- Hydrogen Peroxide Standard(s)

Allow the 100,000 ng/mL Hydrogen Peroxide standard solution to warm to room temperature.
Label six 12 x 75 mm tubes #1 through #6.

Pipet 966 µL of sample diluent (buffer or Tissue Culture Media) into tube #1. Pipet 500µL of Diluent into tubes #2 - #6.

Add 34 µL of the 100,000 ng/mL standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #6.

The concentration of Hydrogen Peroxide in tubes #1 through #6 will be 3,400, 1,700, 850, 425, 212.5 and 106.25 ng/mL respectively. This converts to 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM and 3.125 µM respectively.

Sample Preparation

The Hydrogen Peroxide kit is compatible with samples in a wide range of matrices. Samples diluted sufficiently into the recommended Sample Diluent of 50 mM Phosphate, pH 6.0 can be read directly from the standard curve. If the end user chooses to use a different buffer as the Sample Diluent, it is up to the end user to determine if the buffer works in the assay, the appropriate dilution of samples, and assay validation.
Samples in Tissue Culture Media can also be read in the assay provided the standards have been diluted into the Tissue Culture Media. There will be a small change in the standard curve associated with running the standards and samples in media. The presence of pH indicator will not affect relative assay detection as long as standards and samples are in the same media.

Assay Procedure

All standards and samples should be run in duplicate.
All samples should be allowed to warm to room temperature for at least 30 minutes prior to use.

1. Determine the number of wells to be used. Cover unused wells tightly with a plate sealer. DO NOT REUSE WELLS!
2. Pipet 50 µL of sample diluent (buffer or Tissue Culture Media) into duplicate Blank (Zero Standard) wells.

3. Pipet 50 µL of Standards #1 through #6 into duplicate wells.
4. Pipet 50 µL of Samples into duplicate wells.
5. Pipet 100 µL of Color Reagent into the Blank, Standards and Sample wells.
6. Mix well by shaking or tapping the side of the plate for 10 seconds.
7. Incubate for 30 minutes at room temperature.
8. Blank the plate reader against the blank wells, read the optical density between 540 and 570 nm, preferably at 550 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the blank wells from all readings.

Data Analysis

Calculation of Results

Several options are available for the calculation of the concentration of Hydrogen Peroxide in the samples. We recommend that the data be handled by a software package utilizing a 4 parameter logistic curve fitting program. If this type of data reduction software is not readily available, the concentration of Hydrogen Peroxide can be calculated as follows:

✓ Calculate the average net Optical Density (OD) for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample:

Average Net OD = Average OD - Average Blank OD

✓ Using Log-it log paper, plot the Average Net OD for each Standard versus Hydrogen Peroxide Concentration. Approximate a straight line through the points. The concentration of Hydrogen Peroxide in the unknowns can be determined by interpolation.

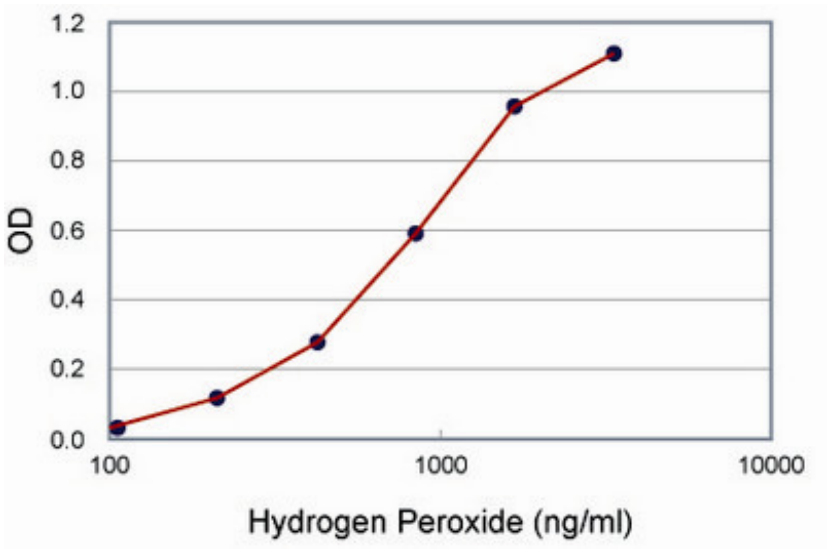
- **Typical Results**

  The results shown below are for illustration only and should not be used to calculate results from another assay.

			Hydrogen Peroxide	
Sample	Average OD	Net OD	(ng/mL)	μM
Blank OD	0.306			
S1	1.412	1.106	3,400	100
S2	1.262	0.956	1,700	50
S3	0.899	0.593	850	25
S4	0.585	0.279	425	12.5
S5	0.420	0.115	212.5	6.25
S6	0.341	0.035	106.25	3.125
Unknown 1	0.838	0.532	767.6	22.58
Unknown 2	1.200	0.894	1,555.1	45.74

- **Typical Standard Curve**

  Typical standard curves are shown below. The curves must not be used to calculate Hydrogen Peroxide concentrations; each user must run a standard curve for each assay.

- 

Performance Characteristics

- **Sensitivity**

  Hydrogen Peroxide sensitivity in 50mM Phosphate, pH 6.0 was calculated by determining the average OD bound for sixteen (16) wells run as the zero standard, and comparing to the average OD for sixteen (16) wells run with Standard #6. The detection limit was determined as the concentration of Hydrogen Peroxide measured at two (2) standard deviations from the zero along the standard curve.

  OD for Zero Standard = 0.277 ± 0.020 (7.3%)
  OD for Standard #6 = 0.362 ± 0.051 (14.0%)
  Delta OD (106.25-0 ng/mL) = 0.362-0.277 = 0.085
  2 SD's of Zero Standard = 0.041
  Sensitivity = 0.041/0.085 x 106.25 ng/mL = 51.25 ng/mL

- **Linearity**

  A sample containing 2,880 ng/mL Hydrogen Peroxide was serially diluted 5 times 1:2 in the recommended sample diluent and measured in the assay. The data was plotted graphically as actual Hydrogen Peroxide concentration versus measured Hydrogen Peroxide concentration. The line obtained had a slope of 0.9342 with a correlation coefficient of 0.9983.

- **Precision**

  Intra-assay precision was determined by taking samples containing low, medium and high concentrations of Hydrogen Peroxide and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of Hydrogen Peroxide in multiple assays (n=8). The precision numbers listed below represent the percent

coefficient of variation for the concentrations of Hydrogen Peroxide determined in these assays as calculated by a curve fitting program.

	Hydrogen Peroxide (ng/ml)	Intra-assay %CV	Inter-assay %CV
Low	314.2		10.0
Medium	772.1		3.0
High	1,606.2		4.0
Low	326.46	2.2	
Medium	768.21	1.7	
High	1,732.93	5.7	

- **Sample Recoveries**

  Hydrogen Peroxide concentrations were measured in horse heparinized plasma, human serum, human urine and tissue culture media. Hydrogen Peroxide was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

Sample	**%Recovery**	**Dilations**
Horse heparin Plasma	94.5	≤1:64
Human Serum	94.7	≤1:64
Human Urine	90.6	≤1:64
Tissue Culture Media	105.6	none

* See Sample Handling instructions for details.

Resources

References

1. Davies KJ. IUBMB Life (1999) 48(1):41-7.
2. Zhang J et al. Antioxid. Redox Signal (2001) Jun;3(3):493-504.
3. Li, N & M. Karin Is NF-kB the sensor of oxidative stress? FASEB J. 1999 13:1137-43. Review.
4. Kim DK, et al. (2001) J. Cell Sci.. 114:4329-34.
5. Emelyanov A., et al. (Chest (2001) 120:1136-9.
6. Uesuge M., et al. J. Immunol. (2000) 165:6532-7.
7. Okuda M. et al. Artherioscler. Thromb. Vasc. Biol. (2001) Sep;21(9):1483-7.
8. Peiro C. et al. Br. J. Pharmacol. (2001) Aug;133(7):967-74.
9. Mody N. et al. Free Radic. Biol. Med. (2001) Aug165;31(4):509-19.
10. Halliwell B. Drugs Aging (2001) 18(9):685-716.
11. Sanji E. et al. Biochem. Biophys. Res. Commun. (2001) Oct 5;287(4):1003-8.
12. Wentworth Jr. P. et al. Science (2001) 293:1806-11.
13. Wentworth, AD. Et al. PNAS (2000) 97(20):10930-5.
14. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, 1989, NCCLS, Villanova, PA, 19085.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std4										
B	Blank	Std4										
C	Std1	Std5										
D	Std1	Std5										
E	Std2	Std6										
F	Std2	Std6										
G	Std3											
H	Std3											