

ARBOR ASSAYS™  
Interactive Assay Solutions™



**DetectX<sup>®</sup>**

## **Hydrogen Peroxide Colorimetric Detection Kit**

2 Plate Kit Catalog Number K034-H1

**Species Independent**

**Sample Types Validated:**

**Fresh Urine, Buffers and TCM**

Please read this insert completely prior to using the product.  
For research use only. Not for use in diagnostic procedures.

[www.ArborAssays.com](http://www.ArborAssays.com)   

WEB K034-H 240214

# TABLE OF CONTENTS

Background	3
Assay Principle	4
Related Products	4
Supplied Components	4
Storage Instructions	4
Other Materials Required	5
Precautions	5
Sample Types and Preparation	5
Reagent Preparation	6
Assay Protocol	7
Calculation of Results	7
Typical Data	8
Validation Data Sensitivity, Linearity, etc.	8-10
Warranty & Contact Information	11
Plate Layout Sheet	12



## 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 ( $O_2^{\cdot-}$ ), which is spontaneously or enzymatically dismutated by superoxide dismutase to  $H_2O_2$ . Many cells produce low levels of  $O_2^{\cdot-}$  and  $H_2O_2$  in response to a variety of extracellular stimuli, such as cytokines (TGF- $\beta$ 1, TNF- $\alpha$ , 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<sup>1</sup>. 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<sup>2</sup> described the oxidation of tartaric acid by  $Fe^{2+}$  and  $H_2O_2$ .  $H_2O_2$  and  $O_2$  may participate in the production of singlet oxygen and peroxyxynitrite 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<sup>3,4</sup>.

A substantial portion of  $H_2O_2$  lethality involves DNA damage by oxidants generated from iron-mediated Fenton reactions<sup>5,6</sup>. 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<sup>7,8</sup>.

1. Rhee SG, Bae YS, Lee SR, Kwon J., "Hydrogen peroxide: A key messenger that modulates protein phosphorylation through cysteine oxidation." 2000, Science's stke. Available at: <http://stke.sciencemag.org/cgi/content/abstract/sigtrans;2000/53/pe1>
2. Fenton, HJH. J. Chem. Soc. (Lond.) 1894, 65:899–910.
3. Sies, H. Mutat. Res., 1993, 299:183–191.
4. Squadrito, GL., and Pryor, WA. "The formation of peroxyxynitrite *in vivo* from nitric oxide and superoxide.", 1995, Chem. Biol. Interact. 96:203–206.
5. Imlay, JA., and Linn, S. "DNA damage and oxygen radical toxicity." 1988, Science 240:1302–1309.
6. Mello-Filho, AC., Meneghini, R. "Iron is the intracellular metal involved in the production of DNA damage by oxygen radicals". 1991, Mutat. Res., 251:109–113.
7. von Sonntag, C., In: "The Chemical Basis of Radiation Biology" 1987, pp. 238–249, Taylor and Francis, New York.
8. Henle, ES., Roots, R., Holley, WR., and Chatterjee, A., "DNA strand breakage is correlated with unaltered base release after gamma irradiation ". 1995, Radiat. Res. 143:144–150.

## ASSAY PRINCIPLE

The DetectX<sup>®</sup> Hydrogen Peroxide Colorimetric Detection Kit is designed to quantitatively measure H<sub>2</sub>O<sub>2</sub> 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 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<sub>2</sub>O<sub>2</sub> cause a linear increase in color.

## RELATED PRODUCTS

Kits	Catalog No.
<b>Catalase Fluorescent Activity Kit</b>	K033-F1
<b>Glutathione Colorimetric Detection Kit</b>	K006-H1
<b>Glutathione Fluorescent Detection Kits</b>	K006-F1/F5
<b>Superoxide Dismutase (SOD) Activity Kit</b>	K028-H1

## SUPPLIED COMPONENTS

### Clear Half Area 96 well Plates

Corning Costar Plate 3695.

2 Plates

Catalog Number X018-2EA

### Hydrogen Peroxide Standard

Hydrogen Peroxide at 1,000 µM in a special stabilizing solution.

220 µL

Catalog Number C130-220UL

### Assay Buffer Concentrate

A 5X buffer concentrate containing detergents and stabilizers.

25 mL

Catalog Number X106-25ML

### Substrate

A solution of the substrate in a special stabilizing buffer.

5 mL

Catalog Number C129-5ML

### Horseradish Peroxidase Concentrate

A 50X concentrated solution of HRP in a special stabilizing solution.

120 µL

Catalog Number X113-120UL

## STORAGE INSTRUCTIONS

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



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4

WEB K034-H 240214

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## OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 25  $\mu$ L.

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. See: [www.ArborAssays.com/resources/#general-info](http://www.ArborAssays.com/resources/#general-info) for plate dimension data.

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.

## 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  $H_2O_2$ .

## SAMPLE TYPES AND PREPARATION

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

## SAMPLE PREPARATION

Dilute samples  $\geq 1:10$  with Assay Buffer prior to running in the assay.

## REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

### 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

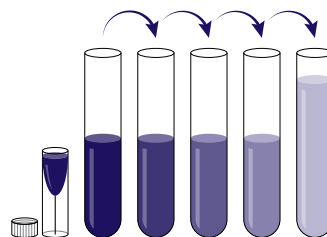
Dilute the HRP Stock solution 1:50 with Assay Buffer using the table below:

#### HRP Dilution Table

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

### Standard Preparation

Hydrogen Peroxide Standards are prepared by labeling tubes as #1 through #6. Briefly vortex to mix the vial of H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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.**

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
<b>Assay Buffer (µL)</b>	<b>450</b>	200	200	200	200	200
<b>Addition</b>	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
<b>Vol of Addition (µL)</b>	<b>50</b>	200	200	200	200	200
<b>Final Conc (µM)</b>	100	50	25	12.5	6.25	3.125



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6

WEB K034-H 240214

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## ASSAY PROTOCOL

We recommend all standards and samples be run in duplicate to allow the end user to accurately determine H<sub>2</sub>O<sub>2</sub> concentration.

Use the plate layout sheet on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3695 plate. See: [www.ArborAssays.com/resources/#general-info](http://www.ArborAssays.com/resources/#general-info) for plate dimension data.

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 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.).

## 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. Or use the online tool from MyAssays to calculate the data: [www.myassays.com/arbor-assays-hydrogen-peroxide-colorimetric-detection-kit.assay](http://www.myassays.com/arbor-assays-hydrogen-peroxide-colorimetric-detection-kit.assay)

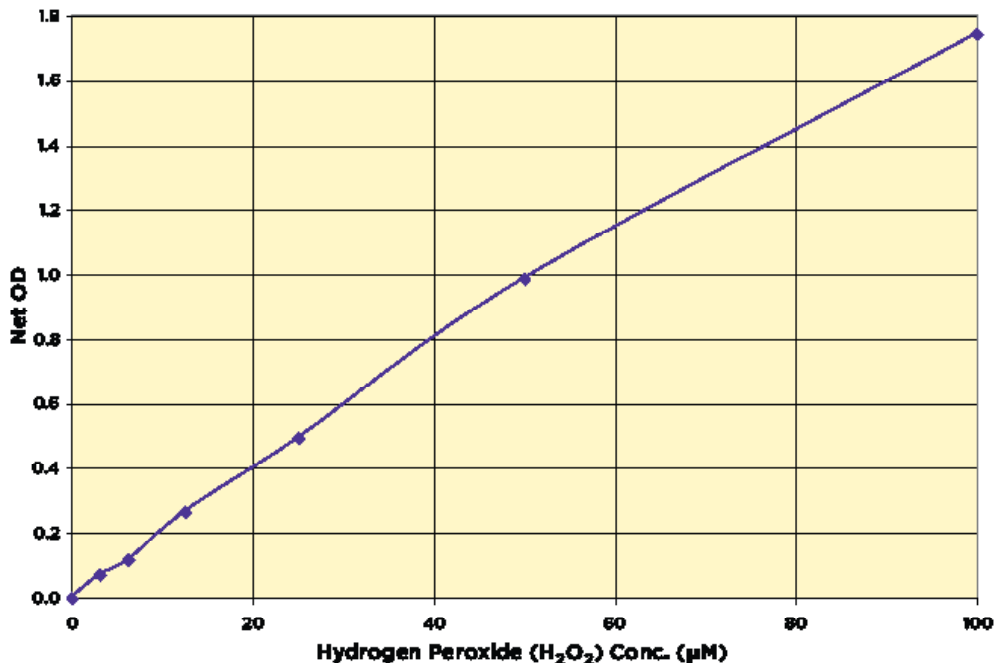
## TYPICAL DATA

Sample	Mean OD	Net OD	H <sub>2</sub> O <sub>2</sub> Conc. (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



Always run your own standard curves for calculation of results. Do not use these data.

## 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> per well.**

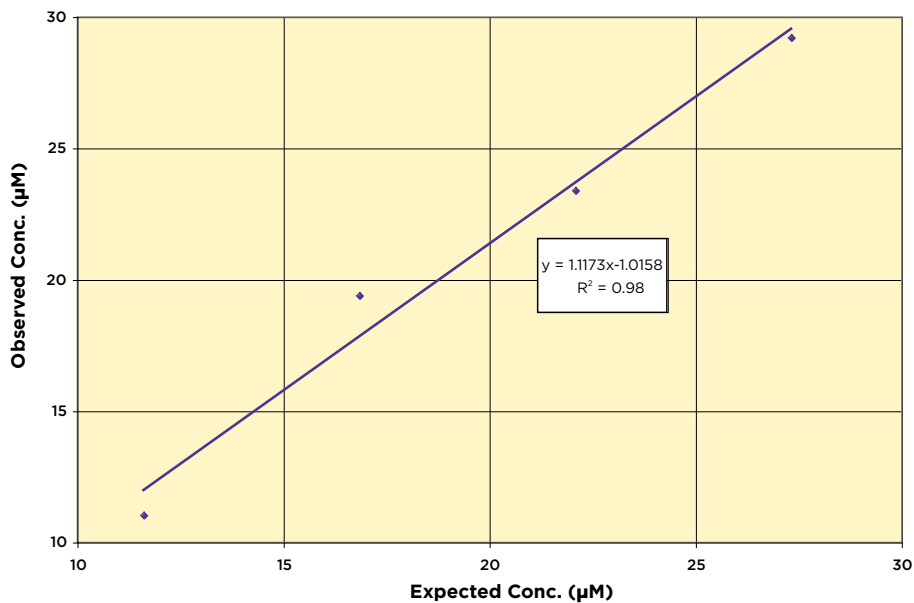




## Linearity

Linearity was determined by taking two diluted human urine samples with known H<sub>2</sub>O<sub>2</sub> 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	Expected Conc. (µM)	Observed Conc. (µM)	% Recovery
80%	20%	27.3	29.2	106.8
60%	40%	22.1	23.4	105.8
40%	60%	16.9	19.4	114.9
20%	80%	11.6	11.0	94.9
<b>Mean Recovery</b>				<b>105.6%</b>



### 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 <sub>2</sub> O <sub>2</sub> Conc. (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 <sub>2</sub> O <sub>2</sub> Conc. (μM)	%CV
1	79.9	3.7
2	49.5	4.5
3	18.4	4.3

## LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

## CONTACT INFORMATION

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## OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

*DetectX<sup>®</sup>, ThioStar<sup>®</sup> and the Arbor Assays logo are all registered trademarks.*

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