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ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
MOST SENSITIVE

DetectX®

HYDROGEN PEROXIDE
Fluorescent Activity Kit

2 Plate Kit Catalog Number K034-F1

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.

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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-β1, TNF-α, 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 Fe^{2+} 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].

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 peroxynitrite *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® Hydrogen Peroxide Fluorescent 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 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 fluorescent product. The fluorescent product emission is at 590 nm with excitation at 570 nm. Increasing levels of H_2O_2 cause a linear increase in fluorescent product.

RELATED PRODUCTS

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

SUPPLIED COMPONENTS

Black Half Area 96-Well Plates
Corning Costar Plate 3694.
2 Plates — Catalog Number X037-2EA

Hydrogen Peroxide Standard
Hydrogen Peroxide at 100 µM in a special stabilizing solution.
220 µL — Catalog Number C117-220UL

Assay Buffer Concentrate
A 5X concentrate containing detergents and stabilizers that should be diluted with deionized or distilled water.
25 mL — Catalog Number X106-25ML

Fluorescent Detection Reagent
A solution of the substrate in a special stabilizing buffer.
5 mL — Catalog Number C116-5ML

Horseradish Peroxidase Concentrate
A 100X concentrate in a special stabilizing solution.
60 µL — Catalog Number X107-60UL

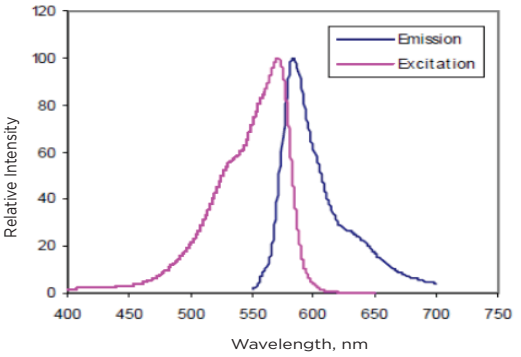
STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 25 µL.

96 well plate reader capable of reading fluorescence. Optimal signal will be obtained with emission at 590 nm and exitation at 570 nm, however other combinations of excitation and emission wavelengths should be considered. Please see the figures below for the excitation and emission data. The output of signal changes based on filters selected, but the ratio of signal is similar.



Ex/Em Wavelength	High FLU	%High/Low Signal
520/590	27,410	22%
530/520	9,350	23%
544/620	9,870	22%
584/620	7,120	21%
584/665	502	20%

Example of high signal and signal ratio across various wavlength combinations.

Note: Filter bandwidth must be considered when selecting em/ex wavelength

Software for converting fluorescent 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°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.

REAGENT PREPARATION

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

Assay Buffer

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.

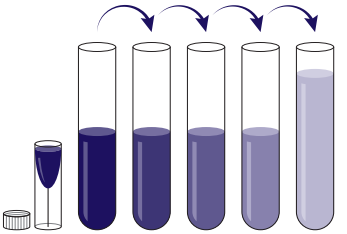
Horseradish Peroxidase (HRP) Preparation

Dilute the HRP Stock solution 1:100 with Assay Buffer using the table below:

	1/2 Plate	One Plate	Two Plates
HRP Stock	15 µL	30 µL	55 µL
Assay Buffer	1.485 mL	2.97 mL	5.445 mL
Total Volume	1.5 mL	3 mL	5.5 mL

Standard Preparation

Hydrogen Peroxide Standards are prepared by labeling tubes as #1 through #7. 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 #7. 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 #7. The concentration of H_2O_2 in tubes 1 through 7 will be 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 µM.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	**450**	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**50**	200	200	200	200	200	200
Final Conc (µM)	10	5	2.5	1.25	0.625	0.313	0.1569

ASSAY PROTOCOL

We reommend all standards and samples be run in duplicate to allow to the end user to accurately determine H_2O_2 concentrations.

Use the plate layout on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3694 plate. See: 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 fluorescent emission with proper wavelength excitation. See page 5 for emission and excitation spectra. We recommend emission at 590 nm with excitation at 520 nm. Please contact your plate reader manufacturer for suitable filter sets.

CALCULATION OF RESULTS

Average the duplicate FLU 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 FLUs 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-fluorescent-detection-kit.assay

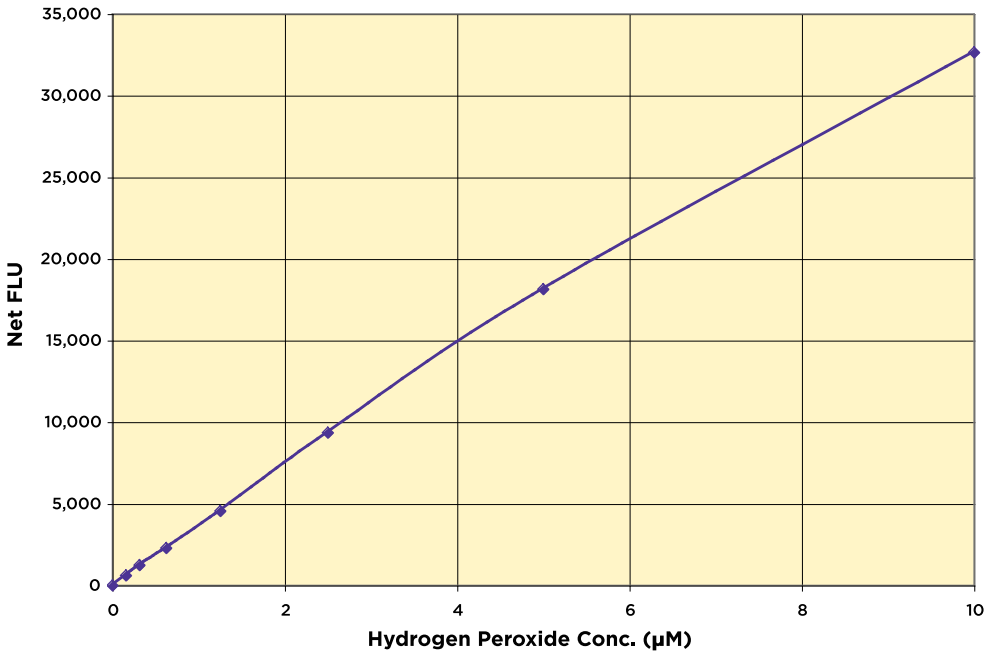
TYPICAL DATA

Sample	Mean FLU	Net FLU	H_2O_2 Conc. (µM)
Zero	3,782	0	0
Standard 1	36,417	32,635	10
Standard 2	21,919	18,137	5
Standard 3	13,134	9,352	2.5
Standard 4	8,333	4,551	1.25
Standard 5	6,072	2,290	0.625
Standard 6	5,031	1,249	0.313
Standard 7	4,398	616	0.156
Sample 1	6,578	2,796	0.76
Sample 2	24,680	20,898	5.85

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

Conversion Factor: 100 nM of Hydrogen Peroxide is equivalent to 3.4 ng/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 FLUs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.038 µM. This is equivalent to 1.9 pmol (64.6 pg) H_2O_2 per well

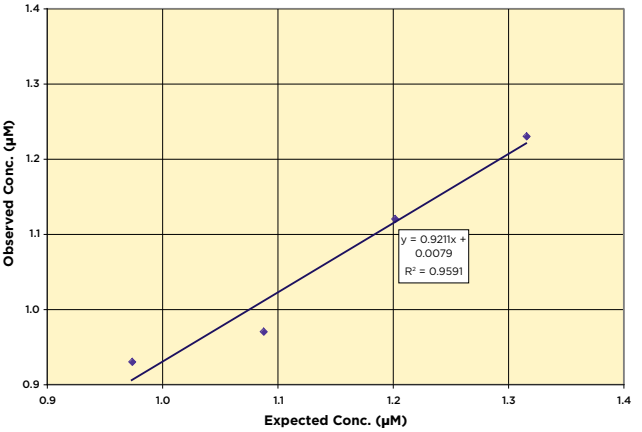
The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low concentration human sample.

The Limit of Detection was determined as 0.052 µM. This is equivalent to 2.6 pmol (88.4 pg) H_2O_2 per well.

Linearity

Linearity was determined by taking two RPMI-1640 media 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 RPMI	Low RPMI	Expected Conc. (µM)	Observed Conc. (µM)	% Recovery
80%	20%	1.32	1.23	93.5
60%	40%	1.20	1.12	93.2
40%	60%	1.09	0.97	89.2
20%	80%	0.97	0.93	95.5
			Mean Recovery	**92.8%**



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 Conc. (µM)	%CV
1	6.27	3.6
2	3.21	3.8
3	0.98	5.7

Inter Assay Precision

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

Sample	H_2O_2 Conc. (µM)	%CV
1	5.86	4.3
2	3.00	7.0
3	0.88	12.1

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 ELISA kits for wildlife conservation research.

ASSAYS™
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