



ARBOR
ASSAYS

DetectX[®]
Catalase
Fluorescent Activity Kit

2 Plate Kit

Catalog Number K033-F1

SPECIES INDEPENDENT

Sample Types Validated:

**Serum, Plasma, Cells, Tissues
and Erythrocyte Lysates**

**Please read this insert completely prior to using the
product.**

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

www.ArborAssays.com

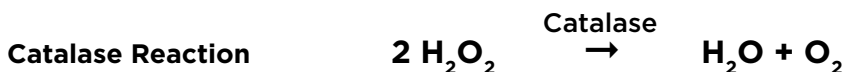
WEB INSERT
TABLE OF CONTENTS

120703

Background	3
Assay Principle	4
Related Products	4
Supplied Components	5
Storage Instructions	5
Other Materials Required	6
Precautions	6
Sample Types	6
Sample Preparation	7
Standard Preparation	8
Assay Protocol	9
Calculation of Results	10
Typical Data	10-11
Validation Data Sensitivity, Linearity, etc.	11-13
Sample Values	14
Warranty & Contact Information	15
Plate Layout Sheet	16



Hydrogen peroxide, H₂O₂ is one of the most frequently occurring reactive oxygen species. It is formed either in the environment or as a by-product of aerobic metabolism, superoxide formation and dismutation, or as a product of oxidase activity. Both excessive hydrogen peroxide and its decomposition product hydroxyl radical, formed in a Fenton-type reaction, are harmful for most cell components. Its rapid removal is essential for all aerobically living prokaryotic and eukaryotic cells^{1,2}. Hydrogen peroxide however can act as a second messenger in signal transduction pathways, in immune cell activation, inflammation processes, cell proliferation, and apoptosis³⁻⁵.



One of the most efficient ways of removing peroxide is through the enzyme catalase, which is encoded by a single gene, and is highly conserved among species⁶⁻⁸. Mammals, including humans and mice, express catalase in all tissues, and a high concentration of catalase can be found in the liver, kidneys and erythrocytes^{9,10}. The expression is regulated at transcription, post-transcription and post-translation levels^{6, 11}. High catalase activity is detected in peroxisomes¹². More recently, short wavelength UV radiation has been shown to produce reactive oxygen species (ROS) through the action of catalase¹³. This response is thought to act as a mechanism to protect DNA by converting damaging UV radiation into ROS species that can be metabolized and detoxified by cellular antioxidant enzymes.

1. Cabiscol E, Tamarit J, Ros J. "Oxidative stress in bacteria and protein damage by reactive oxygen species." *Int. Microbiol.*, 2000, 3:3-8.
2. Kirkman HN, Gaetani GF, "Mammalian catalase: a venerable enzyme with new mysteries." *Trends Biochem Sci* 2007, 32:44-50.
3. Peus D, , et al., "H₂O₂ is required for UVB-induced EGF receptor and downstream signaling pathway activation.", *Free Radic. Biol. Med.*, 1999, 27:1197-1202.
4. Rahman I, and Adcock IM., "Oxidative stress and redox regulation of lung inflammation in COPD.", *Eur. Respir. J.* 2006, 28:219-242.
5. Veal EA, Day AM, Morgan BA., "Hydrogen peroxide sensing and signaling." *Mol. Cell.* 2007, 26:1-14.
6. Reimer, DL, Bailey, J, Singh, SM., "Complete cDNA and 5' genomic sequences and multilevel regulation of the mouse catalase gene." *Genomics*, 1994, 21:325-336.
7. Quan, . Korneluk, RG Tropic, MB, Gravel, RA., "Isolation and characterization of the human catalase gene." *Nucleic Acids Res.*1986 14:5321-5335.
8. Nakashima, H., et al., "Isolation and characterization of the rat catalase-encoding gene, *Gene* 1989, 79:279-288.
9. Deisseroth, A, Dounce, AL., "Catalase: physical and chemical properties, mechanism of catalysis, and physiological role." *Physiol. Rev.* 1970, 50:319-375.
10. Schisler, NJ, Singh, SM., "Inheritance and expression of tissue-specific catalase activity during development and aging in mice." *Genome* 1987, 29:748-760.
11. Masters, C, Pegg, M, Crane, D., "On the multiplicity of the enzyme catalase in mammalian liver." *Mol. Cell Biochem.* 1986, 70:113-120.
12. Chance, B, Sies, H, Boveris, A., "Hydroperoxide metabolism in mammalian organs." *Physiol. Rev.* 1979, 59:527-605.
13. Heck, DE, Vetrano, AM, Mariano, TM & Laskin, JD, "UVB light stimulates production of reactive oxygen species: unexpected role for catalase." 2003, *J. Biol. Chem.* 278:22432-22436.

The DetectX[®] Catalase Activity Kit is designed to quantitatively measure catalase activity in a variety of samples. Please read the complete kit insert before performing this assay. A bovine catalase standard is provided to generate a standard curve for the assay and all samples should be read off of the standard curve. Samples are diluted in the provided Assay Buffer and added to the wells of a half area black plate. Hydrogen peroxide is added to each well and the plate incubated at room temperature for 30 minutes. The supplied Fluorescent Detection Reagent is added, followed by diluted horseradish peroxidase and 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 is read at 590 nm with excitation at 570 nm. Increasing levels of catalase in the samples causes a decrease in H₂O₂ concentration and a reduction in fluorescent product. The activity of the catalase in the sample is calculated after making a suitable correction for any dilution, using software available with most plate readers. The results are expressed in terms of units of catalase activity per mL.

RELATED PRODUCTS

DetectX[®] Kits

Hemoglobin Dual Range Detection Kit	Catalog Number K013-H1
Glutathione Fluorescent Detection Kits	Catalog Number K006-F1/F5
Glutathione Colorimetric Detection Kit	Catalog Number K006-H1
Glutathione S-Transferase Fluorescent Activity Kit	Catalog Number K008-F1
Glutathione Reductase Fluorescent Activity Kit	Catalog Number K009-F1
Nitric Oxide Colorimetric Detection Kit	Catalog Number K023-H1
Superoxide Dismutase (SOD) Activity Kit	Catalog Number K028-H1



WEB INSERT
SUPPLIED COMPONENTS

120703

Black 96 well Half Area Plates	2 Plates	Catalog Number X037-2EA
Catalase Standard 100 Unit/mL of bovine catalase in a special solution.	90 μ L	Catalog Number C114-90UL
Assay Buffer Concentrate A 5X buffer concentrate containing detergents and stabilizers.	25 mL	Catalog Number X106-25ML
Hydrogen Peroxide Reagent Hydrogen peroxide solution containing stabilizers.	5 mL	Catalog Number C115-5ML
Fluorescent Detection Reagent A solution of the substrate in a special stabilizing buffer.	5 mL	Catalog Number C116-5ML
Horseradish Peroxidase Concentrate A 100X concentrated solution of HRP in a special stabilizing solution.	60 μ L	Catalog Number X107-60UL

STORAGE INSTRUCTIONS

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



WEB INSERT
OTHER MATERIALS REQUIRED

120703

Repeater pipet with disposable tips capable of dispensing 25 μ L.

96 well plate reader capable of reading fluorescence at 580-590 nm with excitation at 570-580 nm. Set plate parameters for a 96-well Corning Costar 3694 plate. See: <http://www.ArborAssays.com/resources/lit.asp> for plate dimension data.

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 solution 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. This assay has been validated for serum, plasma and erythrocyte lysates. Samples containing visible particulate should be centrifuged prior to using.

Process any cell pellet as described for Cell Lysates on page 7.



Cell Suspensions and Adherent Cells

1. Centrifuge $> 1 \times 10^6$ cells in suspension at 250 x g for 10 minutes at 4°C. Discard the supernatant. Adherent cells should be gently dislodged using a rubber policeman - do not use proteolytic enzymes.
2. Homogenize or sonicate the pellet in 1-2 mL of cold Assay Buffer per 100 mg of cells. Centrifuge at 10,000 x g for 15 minutes at 4°C.
3. Collect the supernatant and assay immediately, or store at $\leq -70^\circ\text{C}$. Dilute in Assay Buffer prior to measuring catalase activity.

Tissue Samples

1. Wash tissue thoroughly with ice cold PBS prior to processing to remove any red blood cells or clots.
2. Homogenize or sonicate the tissue in 0.5-1 mL of cold Assay Buffer per 100 mg of tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C.
3. Collect the supernatant and assay immediately, or store at $\leq -70^\circ\text{C}$. Dilute in Assay Buffer prior to measuring catalase activity.

Serum Samples

1. Collect serum in tubes without anticoagulant. Allow to clot for 30 minutes at room temperature. Centrifuge the sample at 2,000 x g for 15 minutes at 4°C. Aspirate off the pale yellow serum without disturbing the white buffy layer.
2. Assay immediately or freeze at $\leq -70^\circ\text{C}$.
3. Serum should be diluted at least 1:5 by taking one part of serum and adding 4 parts of Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

Plasma and RBC/Erythrocytes

1. Collect plasma in tubes with EDTA or heparin anticoagulant.
2. Centrifuge at 700-1,000 x g for 10 minutes at 4°C. Aspirate off the pale yellow plasma without disturbing the white buffy layer.
3. Remove the white buffy layer and discard.
4. Eyrthrocytes can be lysed by taking the pelleted RBCs and adding 4 volumes of ice cold deionized water.
5. Centrifuge at 10,000 x g for 15 minutes at 4°C to remove debris.
6. Collect the supernatant and assay immediately, or store at $\leq -70^\circ\text{C}$. Dilute in Assay Buffer $\geq 1:10$ prior to measuring catalase activity.

WEB INSERT STANDARD PREPARATION

120703

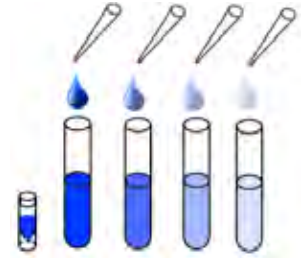
Allow the kit reagents to come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Catalase activities. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to 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.

Standard Preparation

Standards are prepared by labeling six tubes as #1 through #6. Add 190 μL of Assay Buffer to tube #1. Pipet 100 μL of Assay Buffer into tubes #2 to #6. Carefully add 10 μL of the Catalase Stock from the vial to tube #1 and vortex completely. Take 100 μL of the catalase solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The catalase activity in tubes 1 through 6 will be 5, 2.5, 1.25, 0.625, 0.313 and 0.1563 U/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer Vol (μL)	190	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (μL)	10	100	100	100	100	100
Final Activity (U/mL)	5.0	2.5	1.25	0.625	0.313	0.156

HRP Reagent Preparation

Vortex the suspension of HRP prior to pipetting. Pipet from the base of the tube.

	1/2 Plate	1 Plate	1.5 Plates	2 Plates
Horseradish Peroxidase	15 μL	25 μL	38 μL	50 μL
Assay Buffer Buffer	1.485 mL	2.475 mL	3.762 mL	4.95 mL
Final Mixture	1.5 mL	2.5 mL	3.8 mL	5 mL



WEB INSERT ASSAY PROTOCOL

120703

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 3694 plate. See: <http://www.ArborAssays.com/resources/lit.asp> for plate dimension data.

1. Pipet 25 μ L of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 25 μ L of Assay Buffer into duplicate wells as the Zero standard.
3. Add 25 μ L of the supplied Hydrogen Peroxide Reagent to each well using a repeater pipet.
4. Incubate at room temperature for 30 minutes.
5. Add 25 μ L of the supplied Fluorescent Detection Reagent to each well using a repeater pipet.
6. Initiate the reaction by adding 25 μ L of the prepared HRP Reagent to each well using a repeater pipet.
7. Incubate at room temperature for 15 minutes.
8. Set plate parameters for a 96-well Corning Costar 3694 plate. See: <http://www.ArborAssays.com/resources/lit.asp> for plate dimension data. Read the fluorescent emission at 585 ± 5 nm with excitation at 575 ± 5 nm. Please contact your plate reader manufacturer for suitable filter sets.



WEB INSERT
CALCULATION OF RESULTS

120703

Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader. The sample activity obtained should be multiplied by the dilution factor to obtain neat sample values.

TYPICAL DATA

Sample	Mean FLU	Catalase Activity (U/mL)
Standard 1	1,658	5.0
Standard 2	6,276	2.5
Standard 3	15,513	1.25
Standard 4	23,794	0.625
Standard 5	29,495	0.313
Standard 6	32,824	0.156
Zero	35,198	0
Sample 1	5,440	2.88
Sample 2	25,092	0.55

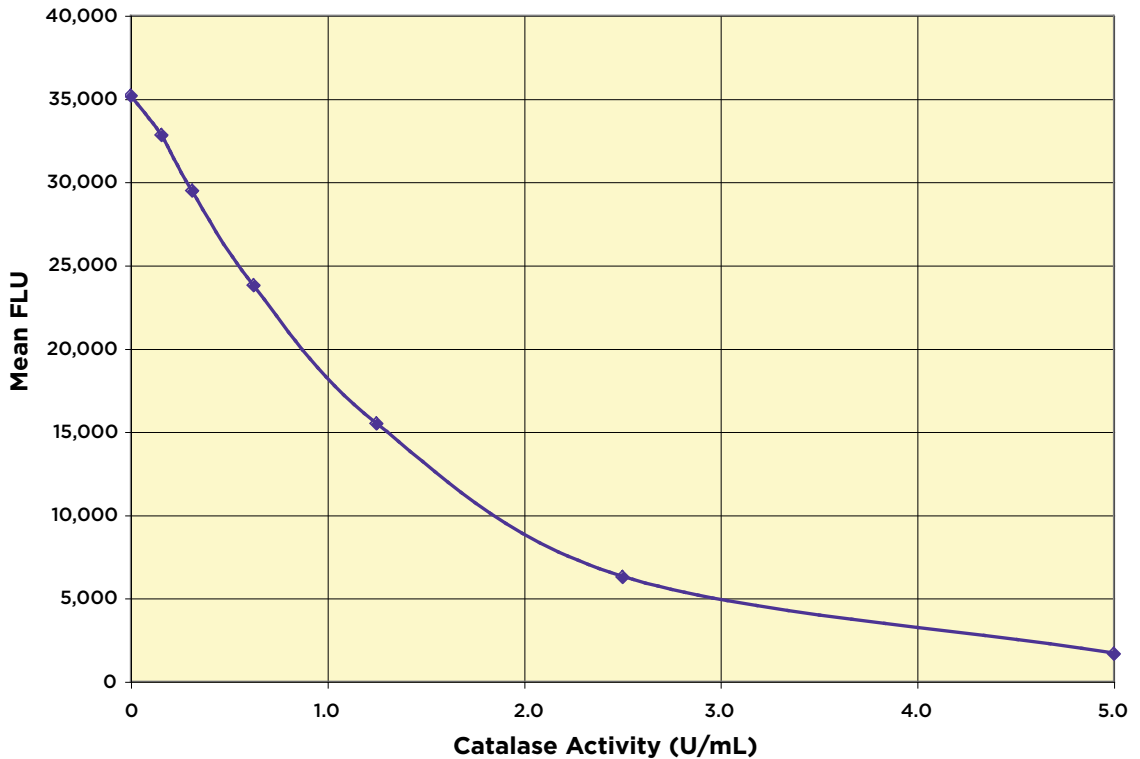
**Always run your own standard curves for calculation of results.
Do not use these data.**

Catalase Unit Definition

One Unit of Catalase decomposes one micromole of H₂O₂ per minute at 25°C and pH 7.0.



Typical Standard Curve



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

VALIDATION DATA

Sensitivity

Sensitivity was calculated by comparing the FLUs 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 0.073 U/mL. This is equivalent to 1.83 mU/well.

Limit of Detection

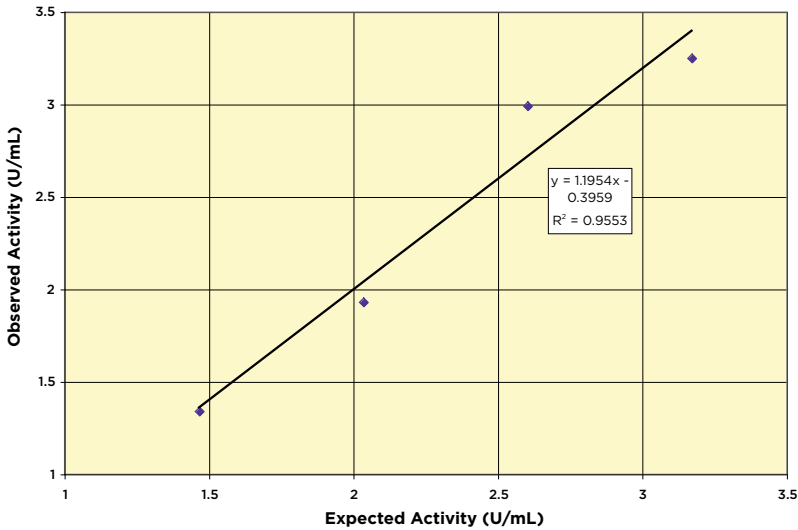
The Limit of Detection for the assay was determined in a similar manner by comparing the FLU's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 0.112 U/mL. This is equivalent to 2.80 mU/well.

Linearity

Linearity was determined by taking two samples, one with a high known catalase activity of 3.74 U/mL and the other with a lower catalase activity of 0.90 U/mL and mixing them in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

High Sample	Low Sample	Observed Activity (U/mL)	Expected Activity (U/mL)	% Recovery
80%	20%	3.25	3.17	102.5
60%	40%	2.99	2.60	114.8
40%	60%	1.93	2.04	94.8
20%	80%	1.34	1.47	91.3
			Mean Recovery	100.8%



Intra Assay Precision

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

Sample	Catalase Activity (U/mL)	%CV
1	2.89	3.2
2	1.15	4.6
3	0.69	5.9

Inter Assay Precision

Three samples diluted in Assay Buffer were run in duplicates in twelve assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	Catalase Activity (U/mL)	%CV
1	2.85	7.3
2	1.10	7.8
3	0.63	7.4



WEB INSERT
SAMPLE VALUES

120703

Seven random adult human serum samples were diluted in Assay Buffer between 1:10 and 1:40 fold and run in the assay. The serum samples ranged from 32.1 to 190.5 U/mL with an average of 62.9 U/mL after adjusting for dilution.



WEB INSERT
LIMITED WARRANTY

120703

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

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

E Mail Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

DetectX®, ThioStar® and the Arbor Assays logo are all registered trademarks.



	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

