ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

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

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BACKGROUND

Hydrogen peroxide, H_2O_2 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 apoptosis[3-5].

Catalase Reaction $\quad 2\,H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$

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 species[6-8]. 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 peroxisomes[12]. More recently, short wavelength UV radiation has been shown to produce reactive oxygen species (ROS) through the action of catalase[13]. 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.

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3. Peus, D., et al. (1999). H_2O_2 is required for UVB-induced EGF receptor and downstream signaling pathway activation. *Free Radical Biology and Medicine, 27*(11-12), 1197–1202.
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6. Reimer, D. L., et al. (1994). Complete cDNA and 5' genomic sequences and multilevel regulation of the mouse catalase gene. *Genomics, 21*(2), 325–336.
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8. Nakashima, H., et al. (1989). Isolation and characterization of the rat catalase-encoding gene. *Gene, 79*(2) 279–288.
9. Deisseroth, A. & Dounce, A. L. (1970). Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. *Physiological Reviews, 50*(3), 319–375.
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12. Chance, B., et al. (1979). Hydroperoxide metabolism in mammalian organs. *Physiological Reviews, 59*(3), 527–605.
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ASSAY PRINCIPLE

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 emission is at 590 nm with excitation at 570 nm. Increasing levels of catalase in the samples causes a decrease in H_2O_2 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

Kits	Catalog No.
Catalase Colorimetric Activity Kit	K033-H1
Hemoglobin High Sensitivity Colorimetric Detection Kit	K013-H1X/H5X
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione S-Transferase Fluorescent Activity Kit	K008-F1
Glutathione Reductase Fluorescent Activity Kit	K009-F1
Nitric Oxide Colorimetric Detection Kit	K023-H1
Superoxide Dismutase (SOD) Activity Kit	K028-H1

SUPPLIED COMPONENTS

Black Half Area 96-Well Plates

Corning Costar Plate 3694.

2 Plates — Catalog Number X037-2EA

Catalase Standard

100 Unit/mL of bovine catalase in a special solution.

90 µL — 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 µL — Catalog Number X107-60UL

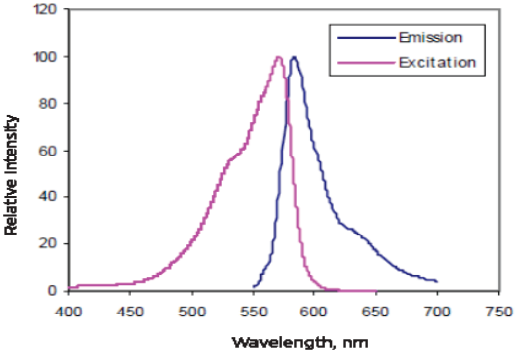
STORAGE INSTRUCTIONS

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 solution 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. 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.

SAMPLE PREPARATION

Cell Suspensions and Adherent Cells

1. Centrifuge > 1 x 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 ≤ -70°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 ≤ -70°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 ≤ -70°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 ≤ -70°C. Dilute in Assay Buffer ≥ 1:10 prior to measuring catalase activity.

REAGENT PREPARATION

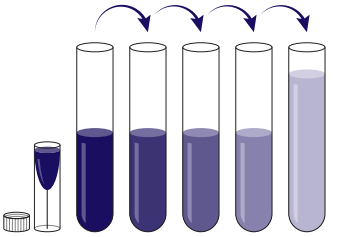
Allow the kit reagents to come to room temperature for 30 minutes. Ensure all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

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 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

Horseradish Peroxidase (HRP) Reagent Preparation

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

	1/2 Plate	1 Plate	1.5 Plates	2 Plates
Horseradish Peroxidase	15 µL	25 µL	38 µL	50 µL
Assay 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

The HRP Preparation will be stable for one day.

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Catalase activities.

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.

See: www.ArborAssays.com/resources/#general-info for plate dimension data.

1. Pipet 25 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 25 µL of Assay Buffer into duplicate wells as the Zero standard.
3. Add 25 µL of the supplied Hydrogen Peroxide Reagent to each well using a repeater pipet.
4. Incubate at room temperature for 30 minutes.
5. Add 25 µL of the supplied Fluorescent Detection Reagent to each well using a repeater pipet.
6. Initiate the reaction by adding 25 µL of the prepared HRP Reagent to each well using a repeater pipet.
7. Incubate at room temperature for 15 minutes.
8. 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 the 4PLC fitting routine on the plate reader. The sample activity obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-catalase-fluorescent-activity-kit-k033-f.assay

TYPICAL DATA

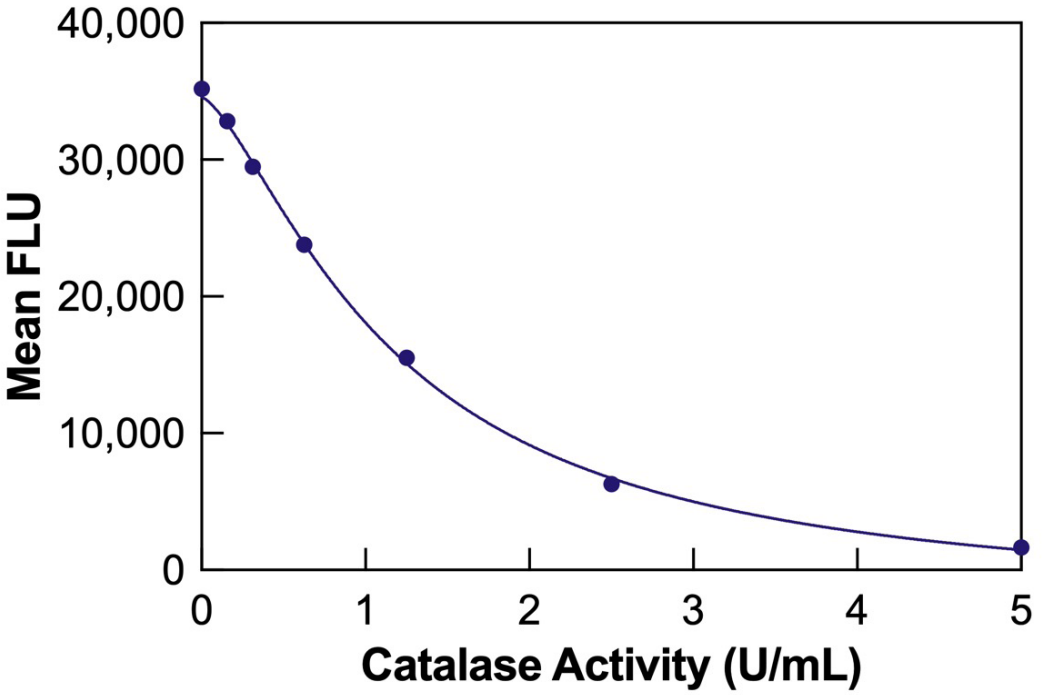
Sample	Mean FLU	Catalase Activity (U/mL)
Standard 1	1,658	5.0
Standard 2	6,276	2.5
Standard 3	15,513	1.25
Standard 4	23,794	0.625
Standard 5	29,495	0.313
Standard 6	32,824	0.156
Zero	35,198	0
Sample 1	5,440	2.88
Sample 2	25,092	0.55

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

Catalase Unit Definition

One Unit of Catalase decomposes one micromole of H_2O_2 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.**

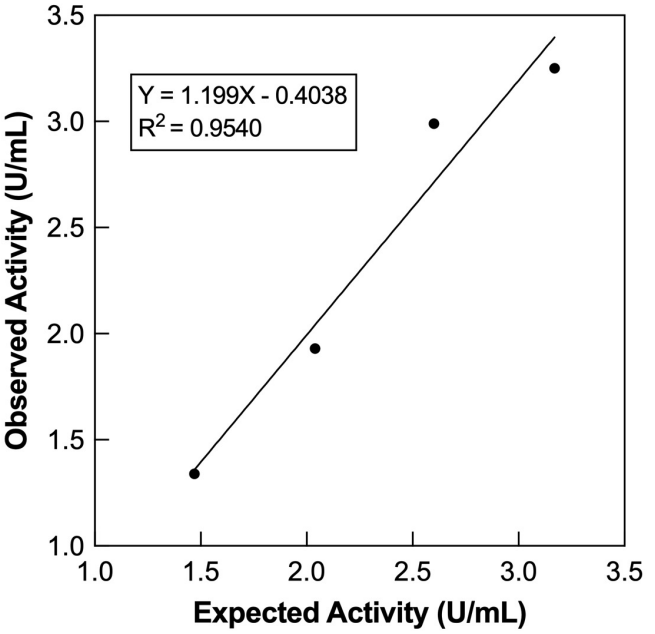
Limit of Detection

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

SAMPLE VALUES

Seven random adult human serum samples were diluted in Assay Buffer between 1:10 and 1:40 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.

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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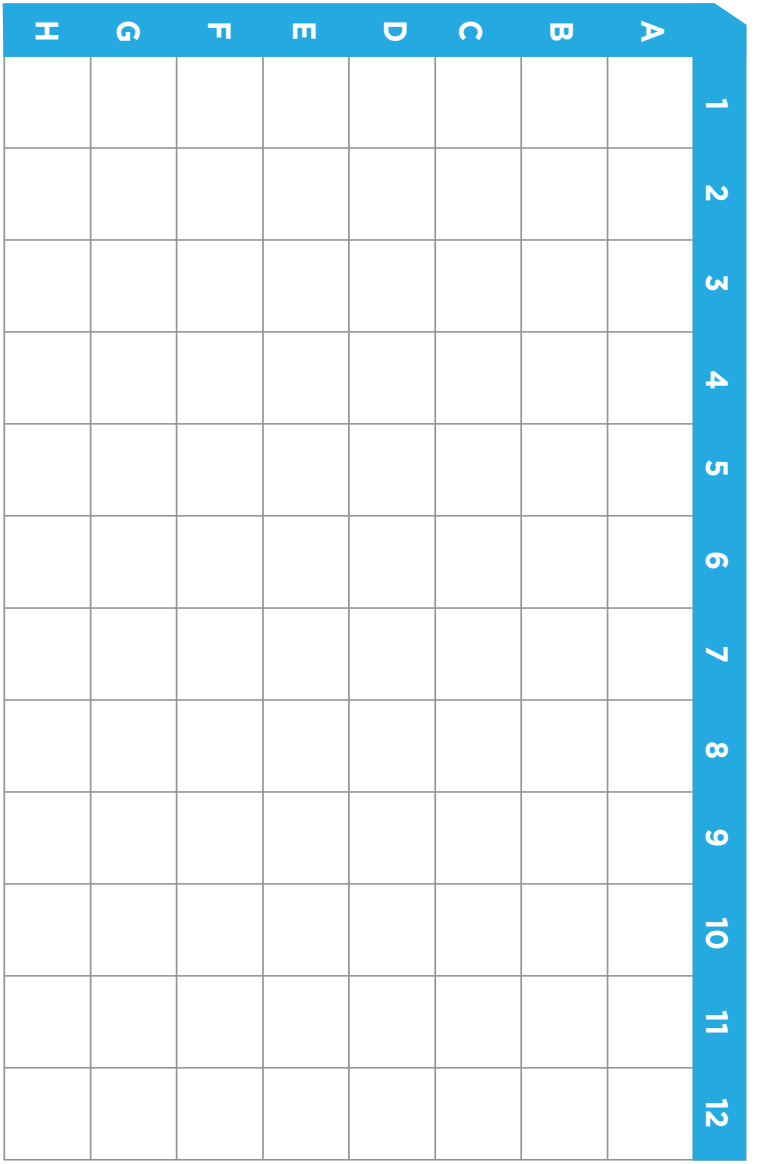
OFFICIAL SUPPLIER TO ISWE

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.



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