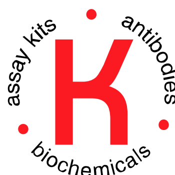

KAMIYA BIOMEDICAL COMPANY

Catalase Colorimetric Activity Kit

For the quantitative determination of catalase activity in serum, plasma, cells, tissues and erythrocyte lysates

Cat. No. KT-711

For Research Use Only.

PRODUCT INFORMATION

Catalase Colorimetric Activity Kit

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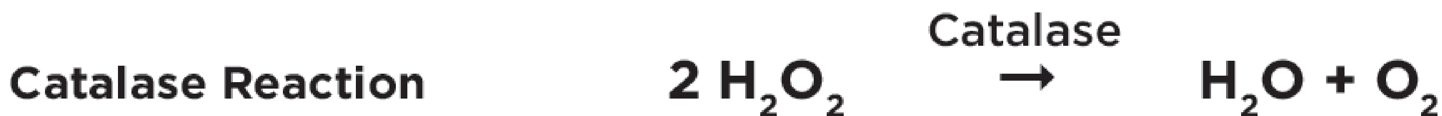
BACKGROUND
Hydrogen peroxide, H_2O_2 is one of the most frequently occurring reactive oxygen species. It is formed either in the environment or as a by-product of aerobic metabolism, superoxide formation and dismutation, or as a product of oxidase activity. Both excessive hydrogen peroxide and its decomposition 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. Hydrogen peroxide however can act as a second messenger in signal transduction pathways, in immune cell activation, inflammation processes, cell proliferation, and apoptosis.



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. 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. The expression is regulated at transcription, post-transcription and post-translation levels. High catalase activity is detected in peroxisomes. More recently, short wavelength UV radiation has been shown to produce reactive oxygen species (ROS) through the action of catalase. 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.

PRINCIPLE
The 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 calibrator is provided to generate a calibration curve for the assay and all samples should be read off of the calibration curve. Samples are diluted in the provided Assay Buffer and added to the wells of a half area clear plate. Hydrogen peroxide is added to each well and the plate incubated at room temperature for 30 minutes. The supplied Colorimetric 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 pink-colored product. The colored product is read at 560 nm. Increasing levels of catalase in the samples causes a decrease in H_2O_2 concentration and a reduction in pink 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.

COMPONENTS
Clear 96 well Half Area Plates 2 Plates
Catalase Calibrator 90 µL
100 Unit/mL of bovine catalase in a special solution.
Assay Buffer Concentrate 25 mL
A 5X buffer concentrate containing detergents and stabilizers.
Hydrogen Peroxide Reagent 5 mL
Hydrogen peroxide solution containing stabilizers.
Colorimetric Detection Reagent 5 mL
A solution of the substrate in a special stabilizing buffer.
Horseradish Peroxidase Concentrate 120 µL
A 50X concentrated solution of HRP in a special stabilizing solution.

STORAGE
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 optical density at 560 nm (Acceptable Range 540-580 nm.).

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 below.

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.

CALIBRATOR PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. We recommend that all calibrators 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.

Calibrator Preparation
Calibrators 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 0.156 U/mL.

Use all Calibrators 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	27 µL	50 µL	76 µL	100 µL
Assay Buffer	1.323 mL	2.45 mL	3.724 mL	4.9 mL
Final Mixture	1.35 mL	2.5 mL	3.8 mL	5 mL

The HRP Preparation will be stable for one day.

ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and calibrator identification. Set plate parameters for a 96-well Corning Costar 3695 plate.

1. Pipet 25 µL of samples or appropriate calibrators into duplicate wells in the plate.
2. Pipet 25 µL of Assay Buffer into duplicate wells as the Zero calibrator.
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 Colorimetric 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 optical density at (Acceptable Range 540-580 nm.).

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration 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.

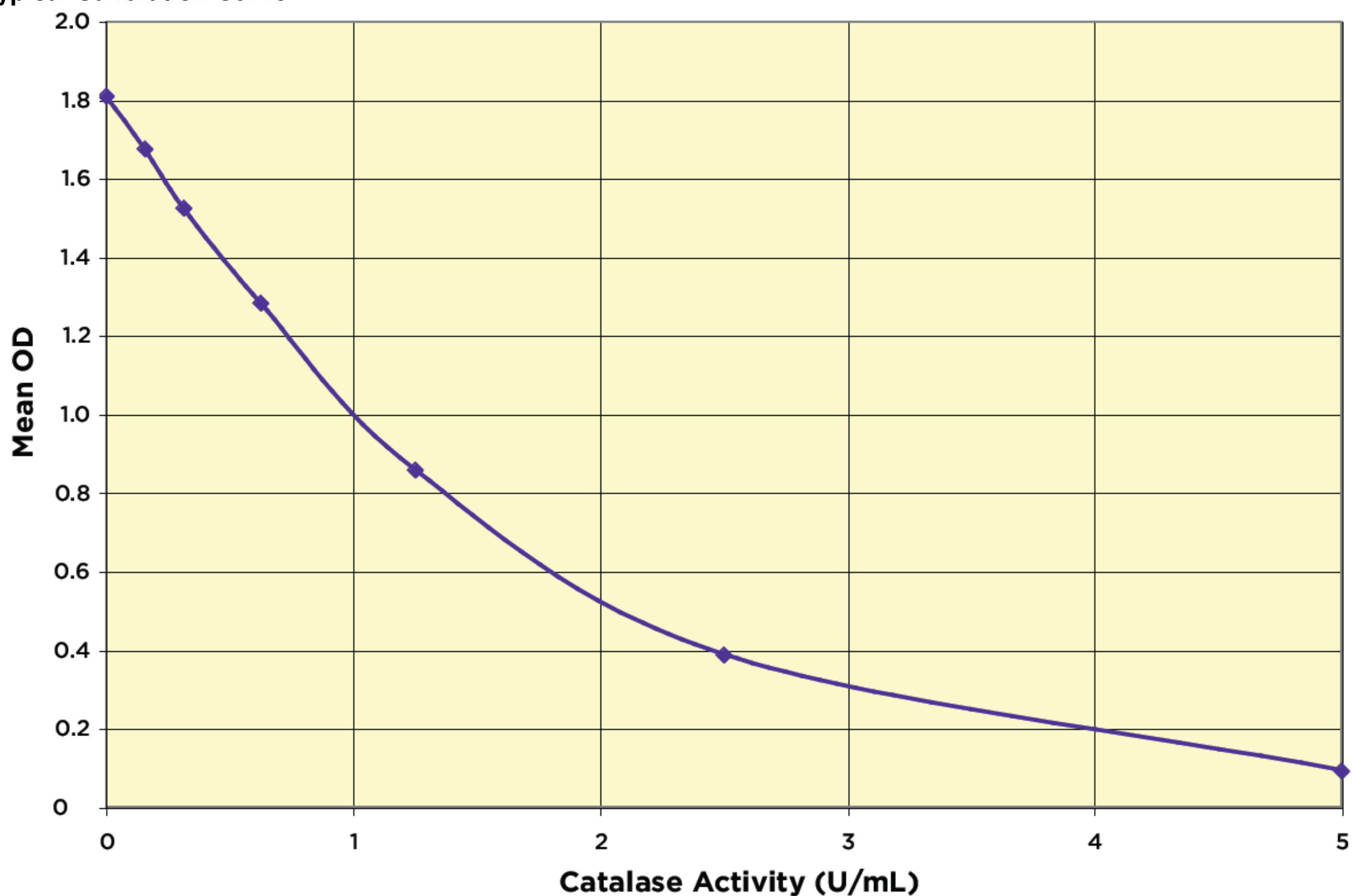
TYPICAL DATA

Sample	Mean OD	Catalase Activity (U/mL)
Standard 1	0.092	5.0
Standard 2	0.388	2.5
Standard 3	0.858	1.25
Standard 4	1.283	0.625
Standard 5	1.525	0.313
Standard 6	1.676	0.156
Zero	1.811	0
Sample 1	0.626	1.76
Sample 2	1.387	0.48

Always run your own calibration curve 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℃ and pH 7.0.

Typical Calibration Curve



Always run your own calibration curve for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and calibrator #6. The detection limit was determined at two (2) standard deviations from the zero along the calibration curve.

Sensitivity was determined as 0.052 U/mL.

Limit of Detection

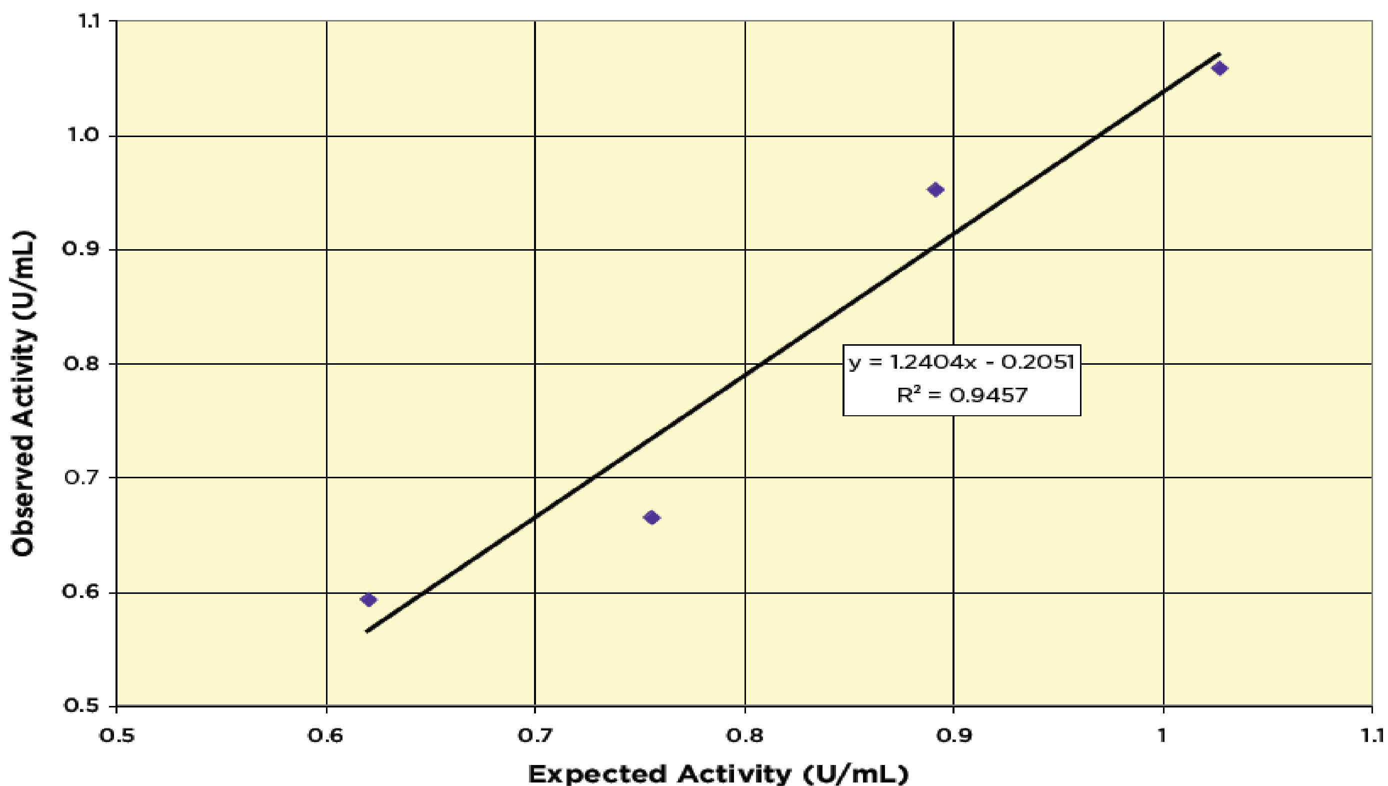
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero calibrator and a low concentration human sample.

Limit of Detection was determined as 0.062 U/mL.

Linearity

Linearity was determined by taking two serum samples, one with a high known catalase activity of 1.163 U/mL and the other with a lower catalase activity of 0.485 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%	1.058	1.027	103.0
60%	40%	0.952	0.892	106.8
40%	60%	0.665	0.756	87.9
20%	80%	0.593	0.621	95.6
			Mean Recovery	**98.3%**



Intra Assay Precision
Three human serum 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	1.71	3.5
2	0.84	4.0
3	0.48	4.8

Inter Assay Precision
Three human serum samples diluted in Assay Buffer were run in duplicates in twenty-one assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	Catalase Activity (U/mL)	%CV
1	1.79	11.9
2	0.94	9.8
3	0.53	12.3

SAMPLE VALUES

Five random adult human serum samples and five adult human plasma samples were diluted in Assay Buffer between 1:10 and 1:80 and run in the assay.

The serum samples ranged from 6.04 to 128.3 U/mL with an average of 36.9 U/mL after adjusting for dilution. The plasma samples ranged from 11.4 to 157.1 U/mL with an average of 70.2 U/mL after adjusting for dilution.

FOR RESEARCH USE ONLY

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