StressXpress®

Catalase Activity Kit

Catalog# SKT-215 (2 Plate kit)

Quantitative colorimetric measurement of catalase activity

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Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-215A	Clear 96 well Half Area Plates	2 Plates	-
SKC-215B	Catalase Standard	90 µL	100 Unit/mL of bovine catalase in a special solution.
SKC-215C	Assay Buffer Concentrate	25 mL	A 5X buffer concentrate containing detergents and stabilizers.
SKC-215D	Hydrogen Peroxide Reagent	5 mL	Hydrogen peroxide solution containing stabilizers.
SKC-215E	Colorimetric Detection Reagent	5 mL	A solution of the substrate in a special stabilizing buffer.
SKC-215F	Horseradish Peroxidase Concentrate	120 µL	A 50X concentrated solution of HRP in a special stabilizing solution.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

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.

Storage

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

Materials Needed But Not Supplied

- 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.

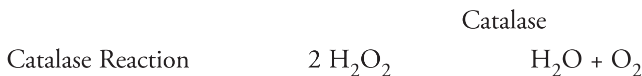
Please read this insert completely prior to using the product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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).



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 levels6, (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.

Assay Overview

The Catalase *StressXpress*® Colorimetric 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 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.

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Serum, Plasma, Cells, Tissues and Erythrocyte Lysates

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 106 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.

Standard Preparation

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.

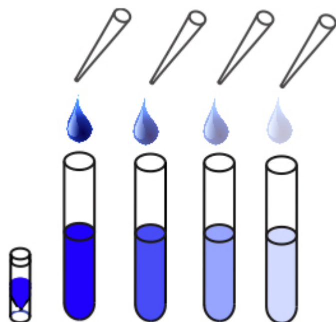
Assay Buffer

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 0.156 U/mL.

Use all Standards within 2 hours of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Assay Buffer Volume(μL)	190	100	100	100	100	100
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Volume 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 standard identification. Set plate parameters for a 96-well Corning Costar 3695 plate. Plate Dimensions (in mm): Well Depth: 10.54; Plate Length: 127.8; Plate Width: 85.5; A1 Row Offset: 11.2; A1 Column Offset: 14.3.

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 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 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 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 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 OD's 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 0.052 U/mL.

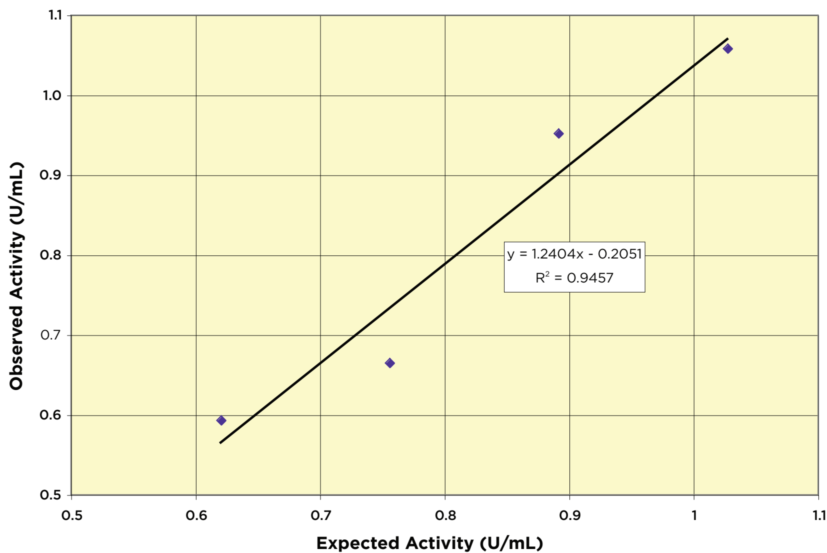
Limit of Detection

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 standard 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%


1.1
1.0
0.9
0.8
0.7
0.6
0.5
Observed Activity (U/mL)
y = 1.2404x - 0.2051
R² = 0.9457
0.5
0.6
0.7
0.8
0.9
1
1.1
Expected Activity (U/mL)

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.

References

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Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

Technical Service Contact Information
Phone: 250-294-9065
Fax: 250-294-9025
E-Mail: techsupport@stressmarq.com
Hours: M-F 9:00 AM to 5:00 PM PST
In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

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B												
C												
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