Catalase (CAT)

Fluorometric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-33102-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Erythrocytes, Cell Lysates and Tissue Samples

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

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Introduction

Background

Catalase (EC 1.11.1.6), is a ubiquitous antioxidant, heme-containing peroxisomal homo-tetrameric enzyme that has a detoxification role by catalyzing the decomposition of the toxic cellular by-product hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in eukaryotic cells as a by-product of various oxidase and superoxide dismutase reactions. Hydrogen peroxide is metabolized by Catalase and also Glutathione peroxidase. Hydrogen peroxide is highly deleterious to cells, and its accumulation causes oxidation of cellular components such as DNA, proteins, and lipids leading to mutagenesis and cell death. Therefore removal of the hydrogen peroxide from cells by Catalase provides protection against oxidative damage. The highest levels of Catalase in humans are found in the liver, kidney and erythrocytes. Defects in the *Catalase* gene are the cause of acatalasia (ACATLAS); also known as a atalasemia. This disease is characterized by the absence of Catalase activity in red blood cells and is often associated with ulcerating oral lesions.

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

Assay principle

The ZellX® Catalase Activity assay is designed to quantitatively measure catalase activity in a variety of samples. The horseradish peroxidase (HRP), provided in the kit, uses H_2O_2 to oxidize a non-fluorescent substrate into a fluorescent product, which can be measured at 590 nm with the excitation at 570 nm. Increasing levels of Catalase in the samples causes a decrease in H_2O_2 concentration and a reduction in the fluorescent product. A Bovine Catalase Standard is provided to create a standard curve for the assay, and the results are expressed in terms of units of activity per mL (U/mL).

General information

Materials supplied in the Kit

Component	*Quantity*
Catalase Standard (100 U/mL)	45 µL
Hydrogen Peroxidase Reagent	2.5 mL
Assay Buffer Concentrate (5 X)	12.5 mL
Fluorescent Detection Reagent	2.5 mL
HRP Concentrate (100 X)	30 µL
Black Half Area 96 Well Plate	1 plate

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Storage instruction

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

Materials required but not supplied

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate reader capable of reading fluorescent at 590 nm with excitation at 570 nm

Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

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 H_2O_2 in supplied hydrogen peroxide solution is much diluted.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for 3 month.

ii. **HRP Reagent:** Vortex the suspension of HRP Concentrate prior to pipetting and then add 25 µL of HRP Concentrate to 2.475 mL of Assay Buffer and mix well. HRP Reagent can be stored for 1 day.

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Sample preparation

After collecting the sample, extraction should be immediately carried out in accordance with related instruction. After extraction, experiment should be conducted immediately, otherwise, keep the sample at -70 or -80˚C. Avoid repeated freeze-thaw cycles.

All samples and standards must be used within 2 hours of dilution.

I. **Serum:**

- Collect serum in tubes without anticoagulant.
- Allow to clot for 30 minutes at room temperature.
- Centrifuge at 2000 g for 15 min at 4˚C.
- Aspirate off the pale yellow serum without disturbing the white buffy layer.
- Serum should be diluted ≥ 1:5 by taking one part of serum and adding 4 or more parts of Assay Buffer prior to conducting assay.

II. **Plasma and RBC/Erythrocytes:**

- Collect plasma with heparin or EDTA.
- Centrifuge at 700-1000 g for 10 min at 4˚C.
- Aspirate off the pale yellow serum without disturbing the white buffy layer
- Remove the white buffy layer and discard.
- Erythrocytes can be lysed by taking the pelleted RBCs and adding 4 volumes of ice cold deionized water.
- Centrifuge at 10000 g for 15 minutes at 4°C to remove debris.
- Serum should be diluted ≥ 1:10 by taking one part of serum and adding 9 or more parts of Assay Buffer prior to conducting assay.

III. **Cell lysate:**

- Centrifuge > 1 x 10^6 cells in suspension at 250 g for 10 minutes at 4°C and Discard the supernatant.
- Adherent cells should be gently dislodged using a cell scrapper or a rubber “policeman”. **do not use proteolytic enzymes.**
- Homogenize or sonicate the pellet in 1-2 mL of cold Assay Buffer per 100 mg of cells.
- Centrifuge at 10000 g at 4°C for 15 minutes and collect supernatant.

IV. **Tissue sample:**

- Wash fresh tissue with cold PBS to remove red blood cells and clot.
- Incise sample and weigh up.

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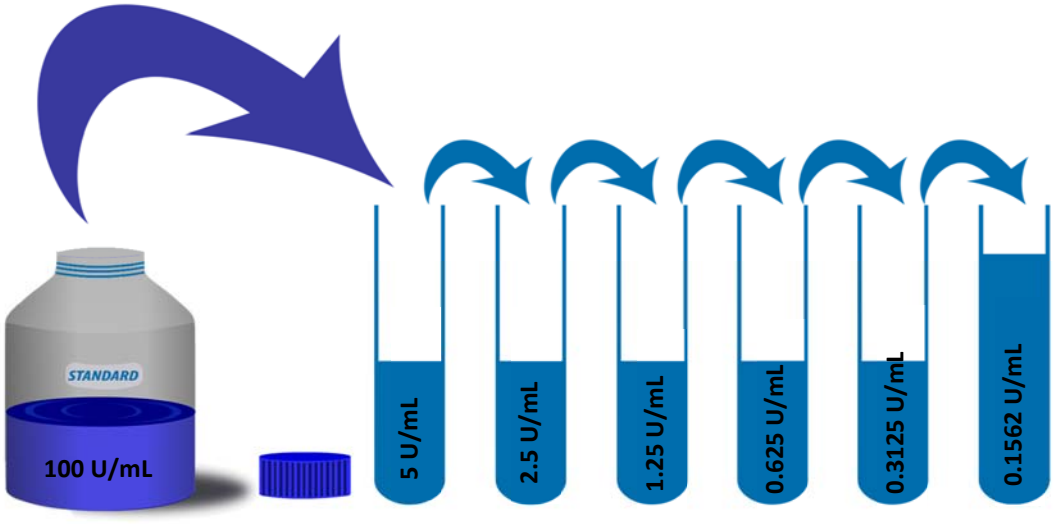
- Homogenize or sonicate in 100 mg/0.5-1 mL in cold PBS.
- Centrifuge at 10000 g for 15 minutes at 4°C and collect the supernatant.

All the samples must be used within 2 hours of dilution

Standard preparation

- Prepare a 1:20 dilution of Catalase Standard with Assay Buffer (mix 10 µL of standard with 190 µL of Assay Buffer), and label as the Standard No.6 (5 U/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 U/mL standard.

No.	*Concentration*	*Material needed*
Standard No.6	5 U/mL	10 µL CAT Standard + 190 µL Assay Buffer
Standard No.5	2.5 U/mL	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.4	1.25 U/mL	100 µL Standard No.5 + 100 µL Assay Buffer
Standard No.3	0.625 U/mL	100 µL Standard No.4 + 100 µL Assay Buffer
Standard No.2	0.3125 U/mL	100 µL Standard No.3 + 100 µL Assay Buffer
Standard No.1	0.1562 U/mL	100 µL Standard No.2 + 100 µL Assay Buffer
Standard No.0	0 U/mL	100 µL Assay Buffer



All standard must be used within 2 hours of preparation

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Assay Procedure

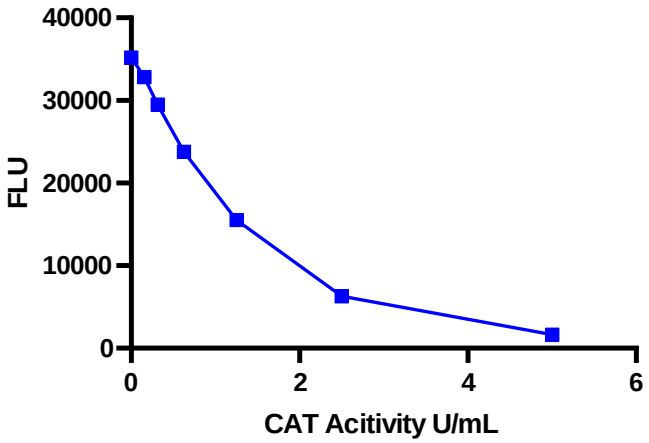
1. Pipette 25 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 25 µL of Assay Buffer as the Zero standard.
3. Add 25 µL of the Hydrogen Peroxide Reagent to each well using a multichannel/repeater pipette.
4. Incubate at room temperature for 30 minutes.
5. Add 25 µL of the Fluorescent Detection Reagent to each well using a multichannel/repeater pipette.
6. Add 25 µL of the HRP Reagent to each well using a multichannel/repeater pipet.
7. Incubate at room temperature for 15 minutes.
8. Read the fluorescent intensity at 590 nm with the excitation at 570 nm.

Calculation

- Average the duplicate Fluorescent Unit (FLU) readings for each standard and sample.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted FLU values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Catalase Unit definition:

One Unit of Catalase decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.



A typical standard curve of ZellX® CAT Assay kit

Run your own standard curves for calculation of results

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Assay range

The limit of detection of ZellX® CAT assay was determined as 0.112 U/mL.

Sensitivity

The sensitivity of the ZellX® CAT assay was determined as 0.073 U/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 samples were tested in duplicate on 12 different assays over multiple days.

Item	*%CV*
Intra assay	3.2, 4.6, 5.9
Inter assay	7.3, 7.8, 7.4

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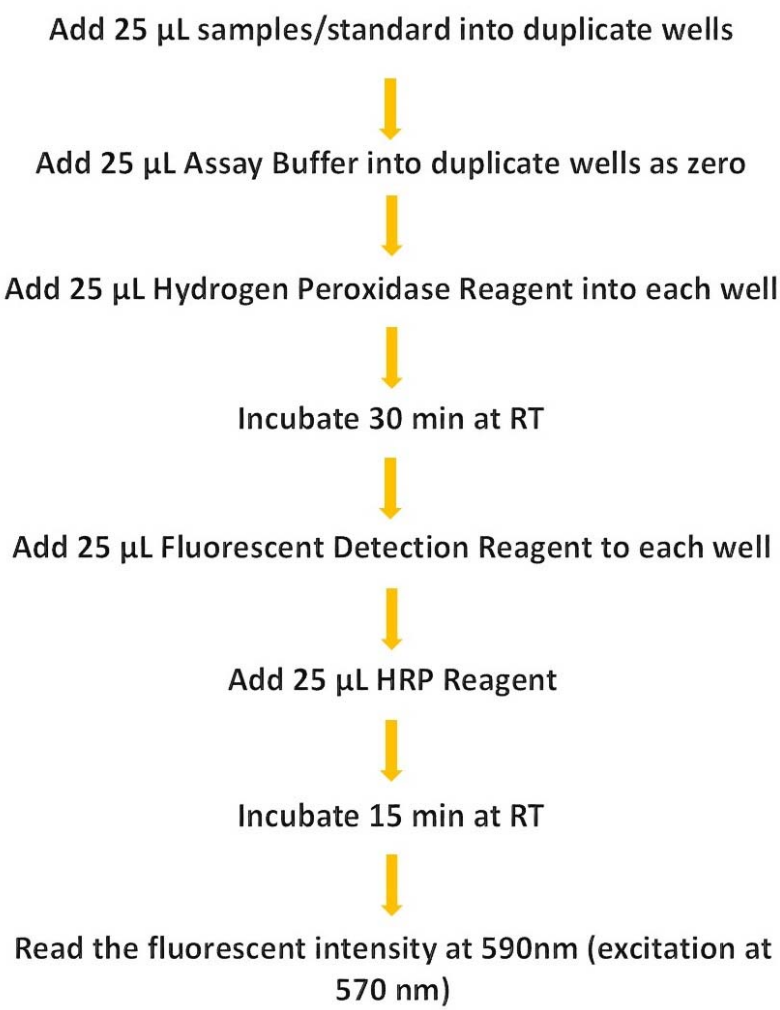
Protocol summary



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References

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