Catalase activity (CAT) Assay kit

(96/48 Tests)

ZellBio GmbH (Germany)

CAT No. ZB-CAT-96A

CAT No. ZB-CAT-48A

Sample Types Validated

Serum, Plasma, Saliva, Semen, Cell Culture Supernatant, Tissue Homogenate and Other Related Biological Liquid

Please read this insert completely prior to using the product.

For Research Use Only. Not For in vitro Diagnostic use

Related Products

	ZellBio Antioxidant	Cat No.
1	Total Antioxidant Capacity (TAC)	ZB-TAC-48A/ ZB-TAC-96A
2	Glutathione Reductase (GR)	ZB-GR-48A/ ZB-GR-96A
3	Malondialdehyde (MDA)	ZB-MDA-48A/ ZB-MDA-96A
4	Catalase (CAT)	ZB-CAT-48A/ ZB-CAT-96A
5	Superoxide Dismutase (SOD)	ZB-SOD-48A/ ZB-SOD-96A
6	Glutathion (GSH)	ZB-GSH-48A/ ZB-GSH-96A
7	Glutathione peroxidase (GPX)	ZB-GPX-48A/ ZB-GPX-96A
8	Nitric Oxide (NO)	ZB-NO-48A/ ZB-NO-96A
9	Hydrogen Peroxide (H_2O_2)	ZB-HPO-48A/ ZB-HPO-96A
10	Vitamin C (Vit C)	ZB-VITC-48A/ ZB-VITC-96A
11	Vitamin E (Vit E)	ZB-VITE-48A/ ZB-VITE-96A
12	Paraoxonase (POX)	ZB-POX-48A/ ZB-POX-96A
13	Xanthine Oxidase (XOX)	ZB-XOX-48A/ ZB-XOX-96A
14	Total Oxidant Status (TOS)	ZB-TOS-48A/ ZB-TOS-96A
15	Total Polyphenol Content (TPC)	ZB-TPC-48A/ ZB-TPC-96A

ZellBio GmbH assay kit is used to **quantitative** assay Human/Animal/Biological samples CAT on the basis of colorimetric (405nm) method. **ZellBio GmbH** microplate format kit is for research only and is not for use in diagnostic procedures.

Intended Use

ZellBio GmbH (**CAT**) assay kit is a simple, reproducible, and standardized tool for assessment of lipid peroxidation in biological samples, e.g. **plasma, serum, saliva, Semen, tissue homogenates, cell lysates, and other related biological liquid.** Catalase is a heme containing peroxisomal homo-tetrameric enzyme that has a detoxification role by catalyzing the decomposition of the toxic cellular byproduct hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cell 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 the cell and its accumulation causes oxidation of cellular targets 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 (CAT) are the cause of acatalasia (ACATLAS); also known as acatalasemia. This disease is

characterized by absence of catalase activity in red cells and is often associated with ulcerating oral lesions.

Test principle

ZellBio GmbH assay kit uses Catalase Activity Assay involves two reactions. The first reaction is the catalase induced decomposition of hydrogen peroxide H_2O_2 into water and oxygen. The rate of disintegration of hydrogen peroxide into water and oxygen is proportional to the concentration of catalase. A catalase-containing sample can be incubated in a known amount of hydrogen peroxide. The reaction proceeds for exactly one minute, at which time the catalase is quenched with quencher and then a Chromogen react with remain hydrogen peroxide.

Catalase (EC 1.11.1.6), is a ubiquitous antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen.

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

By preventing excessive H_2O_2 build up, catalase allows important cellular processes which produce H_2O_2 as a byproduct to occur safely.

Materials supplied in the Kit

	Kit Contents	96 Tests	48 Tests
1	Reagent 1, Assay Buffer. Ready to use	12mL	6mL
2	Reagent 2, Peroxide solution. Ready to use	1.2mL	0.6mL
3	Reagent 3, Chromogen powder.	1 Vial	1 Vial
4	Reagent 4, Quencher. Ready to use	1.2mL	0.6mL
5	Microplate 96 wells	1	1
6	User Manual	1	1

Materials required but not supplied

1. 37 ˚C Incubator
2. Standard microplate reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water

Important Notes

1. Please read these instructions carefully before beginning this assay. Before opening the kit kept at the temperature of 2-8˚C, it takes at least 30 minutes to increase naturally to room temperature.
2. When adding samples, sample injector must be used for each time and should also be frequently checked for its precision to avoid individual error.
3. The instruction must be strictly followed while the reading of ELISA

reader must be set as the standard of determining the experiment result.

4. Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid cross contamination.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Hydrogen peroxide is corrosive and is harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 min. Keep away from combustible.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human CAT were tested 12 times on a microplate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human CAT were tested on 3 different plates, 8 replicates in each plate.

$$CV\ (\%) = (SD/mean) \times 100$$

Intra-Assay: CV 6.3%

Inter-Assay: CV 7.9%

Specimen requirements

1. After collecting the sample, extraction should be immediately carried out in accordance with related documents. After extraction, experiment should be conducted immediately as well. Otherwise, keep the sample at -20˚C or lower temperature. Avoid repeated freeze-thaw cycles.

2. **Serum:** Allow the serum to clot for 5-10 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

3. **Plasma:** In accordance with the requirements of sample collection, EDTA / sodium citrate / Heparin can be used as anti-coagulation. Add EDTA or sodium citrate and mix them for a minutes. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

4. **Cell Culture Supernatant:** Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated

freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

5. **Tissue sample**: Incise sample and weigh up. Add a certain amount of PBS (100mM, pH 7.4) for homogenization or freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8˚C and add a certain amount of PBS (pH 7.4) and then homogenize the sample (~100 mg tissue per 1 mL PBS buffer) thoroughly by hand or homogenizer. Centrifuge (at 4000-6000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use. Alternative method for tissue preparation is: Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4, containing 0.16 mg/ml heparin to remove any red blood cells and clots. Homogenize the tissue in 5-10 ml of cold buffer (i.e., 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT) per gram tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Reagent preparation

Preparation of R3 Reagent:

For 96 tests add 12mL dd- water to the Chromogen powder (R3) before use.

For 48 tests add 6mL dd-water to the Chromogen powder (R3) before use.

Assay procedure

1. All reagents and samples must be equilibrated to RT before test. Preferably R1 and R2 warm at 37ºC at first. Shake the unknown sample for homogenation well.
2. Add 10µL samples to related microwells and 10µL normal saline as blank.
3. Add 100µL R1 reagent to the all microwells.
4. Add 10µL R2 reagent to the all microwells.
5. Mix well and incubate the reaction for 1min (exact 60sec) (preferably at 37ºC).
6. Add 100µL R3 reagent to the all microwells.
7. Add 10µL R4 reagent to the all microwells.
8. Mix well and read the absorbance with microplate reader/ELISA reader at 405nm.
9. Calculate CAT activity in unknown samples based on below formula:

Calculation

$$Catalase\ activity\ \left(\frac{U}{mL}\right) = (\mathrm{OD}_{blank} - \mathrm{OD}_{sample}) \times 271 \times (\frac{1}{60} \times \text{Sample Volume})$$

$$\text{e.g.} \quad \mathrm{OD}_{blank} = 0.720 \ \ and\ \mathrm{OD}_{sample} = 0.675$$

$$Catalase\ activity\ \left(\frac{U}{mL}\right) = (0.720 - 0.675) \times 271 \times \left(\frac{1}{60} \times 10\right) = 2.03\mathrm{U/mL}$$

Assay range

ZellBio GmbH CAT assay kit can be used for CAT determination in the range of **1-100U/ml.**

Sensitivity

ZellBio GmbH CAT activity assay kit can determine CAT in biological samples with 0.5U/mL sensitivity (0.5KU/L). In this assay, CAT activity unit was considered as the amount of the sample that will catalyze decomposition of 1 µmole of H_2O_2 to water and O_2 in one minute.

Validity & Storage

Two years at 2-8˚C for intact kit and 12 months at 2-8˚C for opened and used kit. See label on the outer box for the specific date.

Summary

Prepare reagents, samples and standards.

Preferably R1 and R2 warm at 37ºC at first.

Add 10µL samples and normal saline (Blank) to related microwells

Add 100µL R1 reagent and 10 µL R2 reagent to the all microwells

Mix well and incubate the reaction for 1min (exact 60sec)
(preferably at 37ºC)

Add 100µL R3 reagent and 10µL R4 reagent to the all microwells

Mix well and read the absorbance with microplate reader/ELISA reader
at 405nm

Calculate CAT activity in unknown samples based on formula

References

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