Super Oxide Dismutase (SOD) Assay kit (96/48 Tests)

ZellBio GmbH (Germany)

CAT No. ZB-SOD-96A

CAT No. ZB-SOD-48A

Sample Types Validated

Serum, Plasma, Saliva, 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 SOD activity on the basis of colorimetric (**420 nm**) method. **ZellBio GmbH** microplate format kit is for research only and is not for use in diagnostic procedures.

Intended Use

ZellBio GmbH Superoxide Dismutase (SOD) assay kit is a simple, reproducible, and standardized tool for assessment of SOD activity in biological samples, e.g. **plasma, serum, saliva, tissue homogenates, cell lysates, and other related biological liquid.**

Test principle

ZellBio GmbH assay kit uses the superoxide anion for conversion to hydrogen peroxide and oxygen under enzymatic reaction conditions. Finally the product made a Chromogen to a colour which is measured colorimetrically at 420 nm.

$$2O^{2-} + 2H^{+} \longrightarrow O_2 + H_2O_2$$

Materials supplied in the SOD Test Kit

	Materials	96Tests	48Tests
1	Reagent 1 (2X)	Concentrated Buffer 13mL (2Vials)	Concentrated Buffer 13mL (1Vial)
2	Reagent 2 Ready to Use	2.2 mL	1.2 mL
3	Reagent 3	Powder	Powder
4	Reagent 4 Chromogen Diluent	2.2 mL	1.2 mL
5	Microplate 96 wells.	2	1

Materials required but not supplied

1. Microplate/ELISA reader
2. Precision pipettes and Disposable pipette tip
3. Double distilled water
4. Disposable tubes for sample preparation

Important Notes

1. Before using the kit, keep it at RT 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. The reading of Microplate/ELISA reader must be set as the reading at appropriate wavelength of determining the experiment result.

4. Pipette tips 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.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human SOD were tested 20 times on a microplate, respectively.

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

CV (%) = (SD/mean) ×100

Intra-Assay: CV 5.8%

Inter-Assay: CV 7.2%

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

1. Preparation of 1X R1: Dissolve 13 mI R1 with 13mL double distilled water.
2. Chromogen preparation: Dissolve R3 total powder into a total R4 reagent.

Assay Protocol

In this assay, each unknown sample used as its blank too.

1. All reagents and samples must be equilibrated to RT before the test. Shake the unknown sample for homogenation well.

	Measured Sample	Blank Sample
Sample	10µL	10µL
Diluted R1 (1X)	250µL	250µL
R2	10µL	10µL
dd-Water	10µL	10µL
Chromogen	20µL	-
dd-Water	-	20µL

2. Mix well and read the wells absorbance at times 0 and 2min with microplate reader/ELISA reader at 420nm.
3. Calculate SOD activity in unknown samples based on below formula:

$$SOD\ activity(U/mL) = (V_P - V_C)/(V_P) \times 60$$

$$V_P = OD_{smaple\ 2min} - OD_{blank\ 2min} \qquad V_C = OD_{smaple\ 0min} - OD_{blank\ 0min}$$

$$e.g. V_P = 0.0759 \ \text{ and } \ V_C = 0.0183$$

$$SOD\ activity(U/mL) = (0.0759 - 0.0183)/(0.0759) \times 60 = 45.52$$

Assay range

ZellBio GmbH SOD assay kit can be used for SOD activity determination in the range of **5-100U/mL**.

Sensitivity

ZellBio GmbH SOD activity assay kit can determine SOD activity in biological samples with **1U/mL** sensitivity (1KU/L). In this assay, SOD activity unit was considered as the amount of the sample that will catalyze decomposition of 1 µmole of O^{2-} to H_2O_2 and O_2 in one minute.

References

1. Bolann, B., and Ulvik, R.: Improvement of a Direct Spectrophotometric Assay for Routine Determination of Superoxide Dismutase Activity , Clin Chem 37, 1992, 1991.
2. Beauchamp, C., and Fridovich, I.: Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels , Anal Biochem 44, 276, 1971.
3. Heikkila, R., and Cabbat, F.: A Sensitive Assay for Superoxide Dismutase Based on the Autoxidation of 6-Hydroxydopamine , Anal Biochem 75, 356, 1976.
4. Kuthan, H., Haussmann, H., and Werringloer, J.: A Spectrophotometric Assay for Superoxide Dismutase Activities in Crude Tissue Fractions , Biochem J237, 175, 1986.

ZellBio GmbH
Veltinerweg 29, D-89075, Ulm, Germany
Tel: +49(0)731-37855302-3
Fax: +49(0)731-37855309
Mail: info@zellbio.com
Web: www.zellbio.com