Mouse SOD2 ELISA Kit

Enzyme Immunoassay for the quantification determination of Mouse SOD2 in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

Catalog number: ARG83818

Package: 96 wells

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INTRODUCTION

This gene is a member of the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein that forms a homotetramer and binds one manganese ion per subunit. This protein binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen. Mutations in this gene have been associated with idiopathic cardiomyopathy (IDC), premature aging, sporadic motor neuron disease, and cancer. Alternate transcriptional splice variants, encoding different isoforms, have been characterized. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for SOD2 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any SOD2 present is bound by the immobilized antibody. After washing away any unbound substances, a conjugated antibody specific for SOD2 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of SOD2 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm. The concentration of SOD2 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard (Lyophilized)	2 Vials	4°C
Standard/Sample Diluent Buffer	20 ml (Ready-to-use)	4°C
100X Antibody Conjugate	120 µL	4°C
Antibody Conjugate Diluent Buffer	12 ml	4°C
100X HRP-Streptavidin Concentrate	120 µL	4°C
HRP-Streptavidin Diluent Buffer	12 ml	4°C
25X Wash buffer	20 ml	4°C
TMB substrate	10 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	6 ml (Ready-to-use)	4°C
Plate Covers	2 pieces	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Automated microplate washer (optional).

TECHNICAL HINTS AND PRECAUTIONS

- If the kit is opened, Store the whole kit at 4°C. If the kit is not used up in 1 week. Store the Pre-Coated Microplate, Standard, Biotin Conjugate and HRP-Streptavidin at -20°C, the rest reagents at 4°C, please used up within 6 months.

  If the kit is not opened, store the whole kit: 4°C (short time storage); -20°C (long-term storage). Avoid repeated freeze-thaw cycles

  If the whole kit is stored at -20°C, place the kit at 4°C the day before the experiment.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Diluted Wash Buffer is stable for 4 weeks when stored at 2-8°C. We recommend consumption on the same day.
- Briefly spin down the antibody conjugate Mixture and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-25°C) prior to use.
- If crystals are observed in the 25X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).

2. Wash cells 3 times in pre-cooled PBS.

3. Then, resuspend the cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.

4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Saliva - Collect saliva using a collection device, centrifuge 1000 x g for 15 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freezethaw cycles. The collection device should not have protein binding or filtering features.

Urine – Collect the first urine of the day (mid-stream) by micturating directly into a sterile container. Remove impurities by centrifugation at 1000 x g for 15 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed with PBS, sonicated and centrifuged at 5000 x g for 10 minutes, where the supernatant was collected for testing.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 × g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Cerebrospinal fluid (CSF) - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 25X Wash buffer into distilled water to yield **1X Wash buffer**. The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody Conjugate**: 20 minutes before use, dilute 100X Antibody Conjugate with Antibody Conjugate Diluent Buffer to yield **1X Antibody Conjugate**.
- **1X HRP-Streptavidin Solution**: 20 minutes before use, dilute 100X HRP-Streptavidin concentrate solution with HRP-Streptavidin Diluent Buffer to yield **1X HRP-Streptavidin Solution**. Keep 1X HRP-Streptavidin Solution in dark before use.
- **Sample**: If the initial assay found samples contain Mouse SOD2 higher than the highest standard, the samples can be diluted with Standard/Sample diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
- **Standards:** Reconstitute the standard with 1 ml of Standard/Sample Diluent Buffer to yield a stock concentration of **2000 pg/L.** Keep the buffer in the vail for at least 15 min at RT to make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer

serves as zero standard (0 µmol/L), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 2000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL. DO NOT reuse the reconstituted standard.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Add **100 µl** of **standards** or **samples** into wells.
2. Cover the plate and incubate for **80 minutes** at **37°C**.
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 time washes**. Wash by filling each well with **1× Wash Buffer (200 µL)** using a squirt bottle, manifold dispenser, or autowasher, and let it sit for 1-2 minutes. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Antibody Conjugate Mixture** to each wells.
5. Cover the plate and incubate for **50 minutes** at **37°C**.
6. Aspirate each well and **wash as step 3.**
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well. Cover the plate and incubate for **50 minutes** at **37°C**.
8. Aspirate each well and Wash as step 3, but for total 5 washes.
9. Add **90 µL** of **TMB Substrate** to each well. Cover and incubate for **20 minutes** at **37°C** in the dark.
10. Immediately Add **50 µL** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
11. Read the OD with a microplate reader at **450 nm** immediately. It is

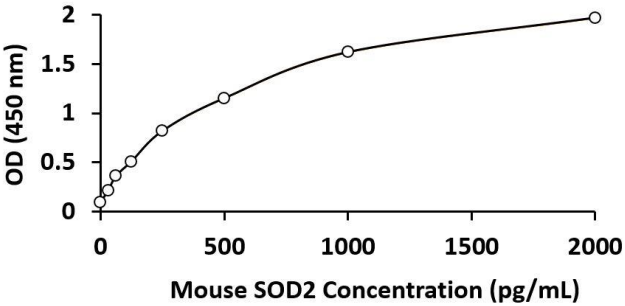
recommended reading the absorbance **within 30 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If these quality control criteria are not met the assay run is invalid and should be repeated.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

The standard range of Quinolones ranged from 31.25-2000 pg/mL.

The mean Limit of detection was 15.625 pg/ml.