Rabbit superoxide dismutase [Mn], mitochondrial (SOD2) ELISA Kit

Catalog No. CSB-EL022398RB

(96 tests)

- This immunoassay kit allows for the in vitro rapid detection of **rabbit SOD2** concentrations in **serum, plasma, tissue homogenates.**
- **Expiration date** six months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to SOD2 has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with HRP-conjugated SOD2 and incubated. A competitive inhibition reaction is launched between SOD2 (Standards or samples) and HRP-conjugated SOD2 with the pre-coated antibody specific for SOD2. The more amount of SOD2 in samples, the less pre-coated antibody specific for SOD2 bound by HRP-conjugated SOD2. Then the substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of SOD2 in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

The standard curve concentrations used for the ELISA's were 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml.

SPECIFICITY

This assay recognizes rabbit SOD2. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of rabbit SOD2 is typically less than 31.2 pg/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard	1 x 200µl (A solution of 10000 pg/ml)
HRP-conjugate	1 x 60µl
HRP-conjugate Diluent	1 x 10 ml
Sample Diluent	2 x 20 ml
Wash Buffer	1 x 20 ml (25×concentrate)
TM Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. Bring all reagents and plate to room temperature for at least 30 minutes before use. Unused wells need store at 2-8℃and avoid sunlight.
2. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.
3. **HRP-conjugate** Centrifuge the vial before opening. Dilute to the working concentration using **HRP-conjugate Diluent(1:100)**, respectively.
4. **Standard**

- Centrifuge the vial before opening. Allow the 10000 pg/ml standard solution to warm to room temperature .Label six tubes#1 through #6.
- Pipette 270µl Sample Diluent into tube**#**1. Add 30µl of the 10000 pg/ml standard to tube #1. Vortex thoroughly. The 1000 pg/ml standard**(tube #1)**serves as the high standard.
- Pipette 150µl Sample Diluent into tubes**#**2-6. Add 150µl of tube **#**1 to tube #2 and vortex thoroughly ,Add 150µl of tube **#**2 to tube **#**3 and vortex thoroughly,Continue this for tubes #3 through #5. Mix each tube thoroughly before the next transfer. Sample Diluent serves as the zero standard (0 pg/ml) **(tube #6)**.

The concentration of rabbit SOD2 in tubes #1through #6 will be 1000, 500 ,250 ,125 , 62.5 and 0 pg/ml ,respectively.

Diluted standards should be used within 30 minutes of preparation.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE PREPARTION

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates** 100mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARTION

Recommend to dilute the serum or plasma samples with Sample Diluent(1:100) before test. The suggested 100-fold dilution can be achieved by adding 10μl sample to 40μl of Sample Diluent. Complete the 100-fold dilution by adding 15μl of this solution to 285μl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Set a **Blank** well without any solution.
2. Add 50μl of Standard or Sample per well. Standard need test in duplicate.
3. Add 50μl of **HRP-conjugate working solution** to each well , Mix well and then incubate for 40 minutes at 37°C.
4. Fill each well with Wash Buffer (about 200μl), stay for 10 seconds and Spinning. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 90μl of **TMB Substrate** to each well, mix well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50μl of **Stop Solution** to each well.
7. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the rabbit SOD2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.