Catalog Number	Size
EA100096	96 Tests

Human Cu/ZnSOD ELISA KIT

For the quantitative determination of human Cu/ZnSOD concentrations in cell culture supernates, serum, and plasma.This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

Superoxide Dismutases (SODs), originally identified as Indophenoloxidase (IPO), are enzymes that catalyze the conversion of naturallyoccuring but harmful superoxide radicals into molecular oxygen and hydrogen peroxide. Superoxide Dismutases 1, SOD1, also known as Cu/Zn SOD, soluble SOD and IPOA, is a soluble, cytoplasmic 16 kDa homodimer. Each SOD1 monomer binds one Cu2+ and one Zn2+ ion. Three isozymes of SOD have been identified and are functionally related but have very modest sequence homology. SOD1 shares 23% and 27% sequence identity with SOD2 and SOD3, respectively. Mutations in SOD1 have been implicated as causes of familial amyotrophic lateral sclerosis (ALS). The ALScausing mutations of SOD1 are scattered throughout the protein and provide no clear functional or structural clues to the underlying disease mechanism. The oligomerization hypothesis suggests that mutant SOD1 proteins become misfolded and consequently oligomerize into high molecular weight aggregates that result in the death of motor neurons. The oxidative damage hypothesis suggests that loss of function mutation in SOD1 protein results in the accumulation of cellular superoxide radical, leading to free radicalmediated damage, the release of cytochrome c, and apoptosis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Cu/ZnSOD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Cu/ZnSOD present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for Cu/ZnSOD is added to the wells and binds to the combination of capture antibody- Cu/ZnSOD in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of Cu/ZnSOD present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven Cu/ZnSOD standard dilutions and Cu/ZnSOD sample concentration determined.

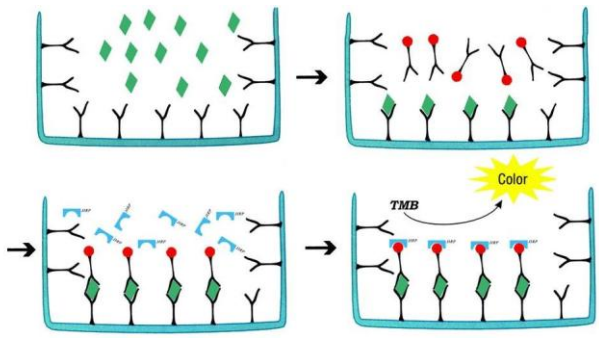

Figure 1:Schematic diagram of the assay

REAGENTS

1. Aluminium pouches with a Microwell Plate coated with antibody to human Cu/ZnSOD (8X12)
2. 2 vials human Cu/ZnSOD Standard lyophilized, 2500 pg/ml upon reconstitution
3. 2 vials HRP-Conjugate solution
4. 4 bottle Standard /sample Diluent
5. 1 bottle HRP-Conjugate Diluent
6. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
7. 1 vial Substrate Solution
8. 1 vial Stop Solution
9. 4 pieces Adhesive Films
10. Package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="6">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>HRP-Conjugate solution</td></tr>
<tr><td>HRP-Conjugate Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20℃.
Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**</td></tr>
</table>

**Provided this is within the expiration date of the kit

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃ and 8℃. After use all reagents should be immediately returned to cold storage (2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.

5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 0.5mL of Standard /sample Diluent. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 250µL of Standard/sample Diluent into the 2500 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 5000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

   Dilute serum and plasma samples 1:20 with Standard/sample Diluent according to the following scheme: 10 µl sample + 190 µl Standard/sample Diluent.
   For fetal umbilical vein blood first adjust samples to $2x10^7$ erythrocytes/ml.
4. Working solution of HRP-Conjugate: Make a 1:100 dilution of the concentrated HRP-Conjugate solution with the HRP-Conjugate Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

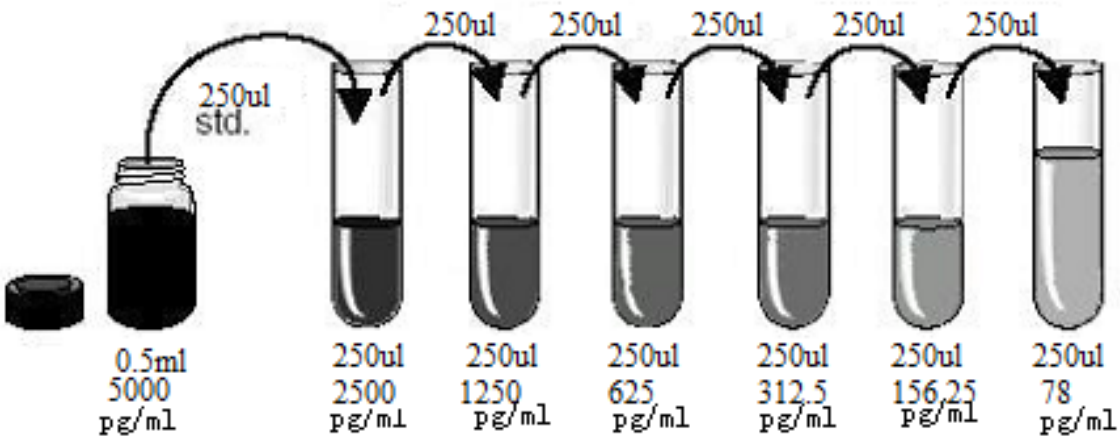

Figure 2: Preparation of Cu/ZnSOD standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100µL of Standard, control, or sample, per well, then add 50µL of the working solution of HRP-Conjugate to each well. Cover with the adhesive strip provided and incubate 1 hour at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser or auto-washer. 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 100µL of Substrate Solution to each well. Incubate for 10-20 minutes at RT. Avoid placing the plate in direct light.
6. Add 100µL of Stop Solution to each well. Gently tap the plate to ensure

thorough mixing.

7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (Optionally 650nm as the reference wave length; 610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

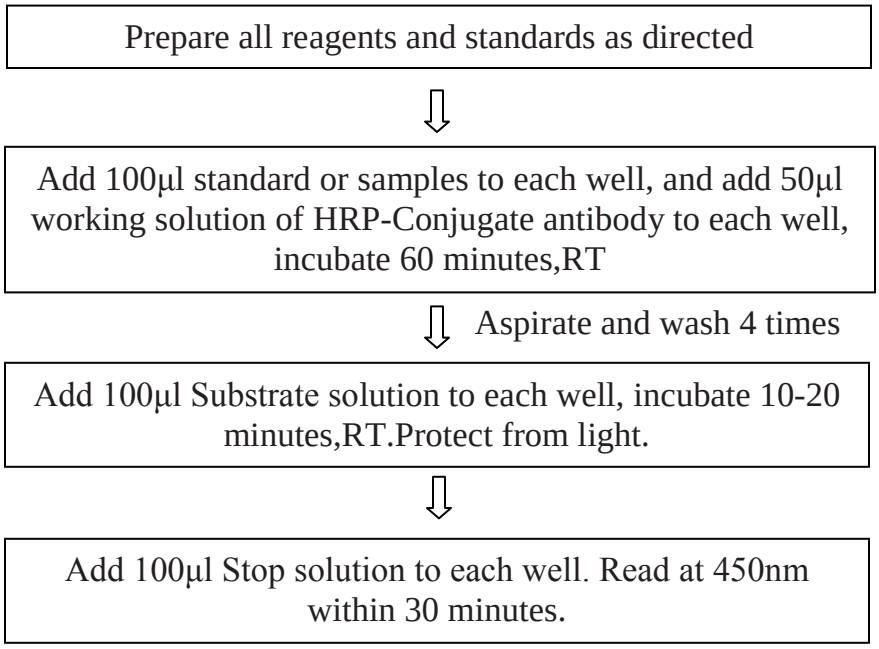

Figure 3: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. 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.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. 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 y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the Cu/ZnSOD 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.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2:Typical data using the Cu/ZnSOD ELISA (Measuring wavelength: 450nm, Reference wavelength: 650nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.008	0.006	0.007	——
78	0.088	0.084	0.086	0.079
156	0.173	0.168	0.171	0.164
312.5	0.344	0.340	0.342	0.335
625	0.629	0.621	0.625	0.618
1250	1.174	1.167	1.171	1.164
2500	2.020	2.012	2.016	2.009
5000	2.727	2.697	2.712	2.705

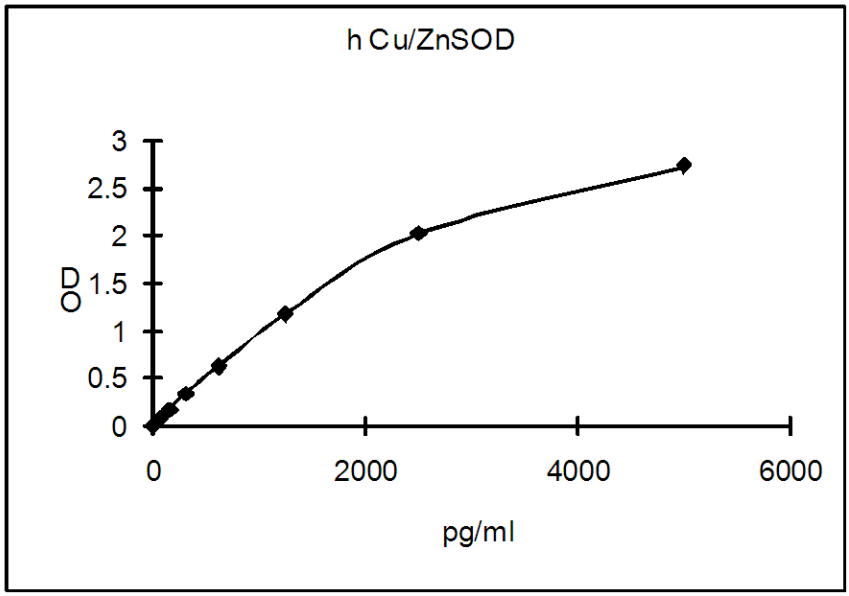

Figure 4:Representative standard curve for Cu/ZnSOD ELISA. Cu/ZnSOD was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 40 pg/mL.

REFERENCES

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If you have any questions, please tell us!