



Cu/Zn-Superoxide Dismutase ELISA Kit

Manufactured by Bender MedSystems.

ALX-850-033-KI01

96 wells (~80 tests)

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For laboratory use only. Not for human or diagnostic use.

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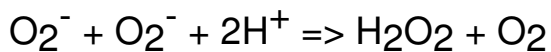
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1. INTENDED USE

The Cu/ZnSOD ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human copper zinc superoxide dismutase in cell culture supernatants, human serum, plasma, urine, amniotic fluid, fetal umbilical vein blood, or other body fluids. **The Cu/ZnSOD ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2. SUMMARY

Superoxide Dismutases (SODs) (E.C.1.15.1.1.) are a unique family of metalloproteins that catalyze the dismutation of superoxide anion radicals (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2)



SOD is ubiquitous in oxygen metabolizing cells protecting these cells against direct and indirect oxygen-mediated free radical damage. Four types of SOD have been defined on the basis of distinctions in their metal cofactors and distribution: Manganese (MnSOD) principally located in the matrix of mitochondria of all aerobes, copper/zinc (Cu/ZnSOD) mainly present in the cytoplasm of eukaryotic cells, iron (FeSOD), predominantly in the cytosol, chloroplasts or mitochondria of prokaryotes as well as extracellular (ECSOD), which is found in the extracellular fluids or membrane associated in mammals.

The properties of Cu/Zn superoxide dismutase are quite different from those of the manganese or iron enzymes. Sequence analysis has indicated a homology between Mn and Fe class enzymes but these have no homology with the Cu/Zn enzyme (15). The human Cu/Zn superoxide dismutase is a dimeric protein (3) composed of 2 subunits of 153 amino acid residues and a molecular weight of 16 kDa each. Dissociation of the subunits is facilitated by alkylation of the two sulfhydryl groups in the protein or by removal of the copper and zinc ions.

The human Cu/ZnSOD gene has been localized to chromosome 21q22.1 (13).

Cu/ZnSOD gene expression is induced by mediators of oxidative stress like sulfhydryl antioxidants (4, 12, 14), interleukin-1, tumor necrosis

factor (7). Constitutive expression of copper and zinc SOD mRNA is highest in dividing cells.

Induction of Cu/ZnSOD expression resulting in elevated levels of Cu/ZnSOD in human body fluids is of diagnostic value for measuring the activity of different diseases.

- nephropathies:

Cu/ZnSOD determination provides a tool for early diagnosis of nephropathies (8).

- monitoring of therapeutic treatments:

Cu/ZnSOD is a useful therapeutic tool in the treatment of chronic inflammation e.g. rheumatoid arthritis (2) or of the ischemic myocardium in the phase of reperfusion (6). Due to the short half-life of SOD injected into the blood circulation, a rapid assay is necessary for monitoring SOD levels.

- Trisomy 21 (Down's Syndrome):

In cases with Down's Syndrome an additional part of chromosome 21 is present in the genome of the patient as a structural chromosome aberration. The Cu/ZnSOD gene is localized on chromosome 21, closely associated with the gene complex responsible for the phenotype of Down's Syndrome. A gene-dosage effect for Cu/ZnSOD in Down's Syndrome providing a diagnostic marker for this syndrome has been described (13).

a) Patients with Down's Syndrome have significantly elevated serum and urine levels of Cu/ZnSOD (9).

b) Prenatal diagnosis of Down's Syndrome (10): Cu/ZnSOD levels are quantitated from erythrocytes of fetal umbilical vein blood and related to the number of cells, the content of haemoglobin and to the haematocrit. In case of Trisomy 21 the significantly elevated levels of Cu/ZnSOD are determined (5, 11).

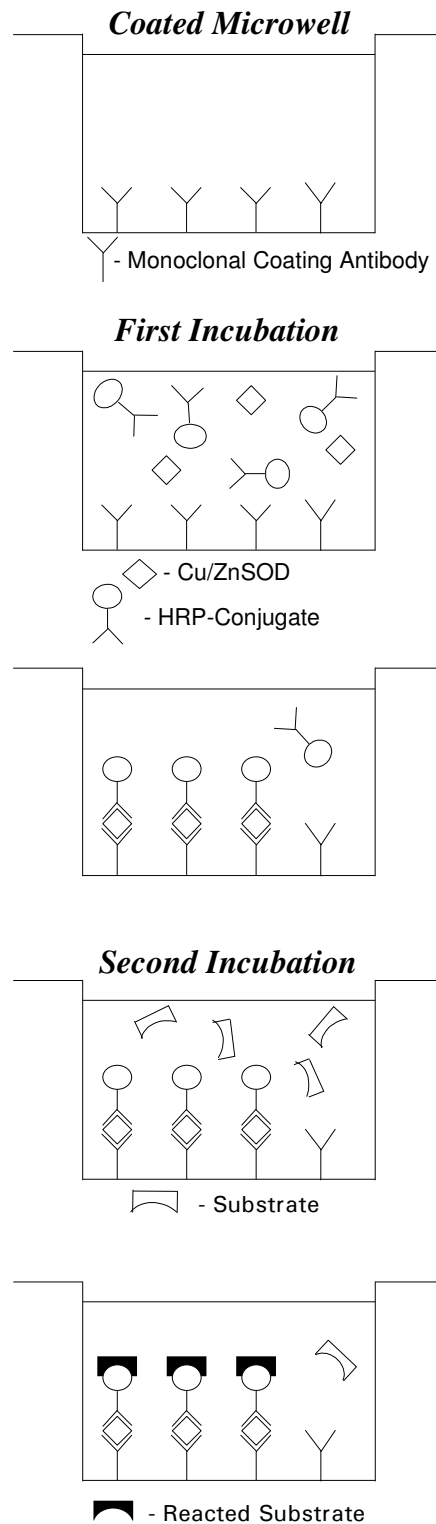
3. PRINCIPLES OF THE TEST

An anti-Cu/ZnSOD monoclonal coating antibody is adsorbed onto microwells.

Cu/ZnSOD present in the sample or standard binds to antibodies adsorbed to the microwells; a HRP-conjugated monoclonal anti-Cu/ZnSOD antibody is added and binds to Cu/ZnSOD captured by the first antibody.

Following incubation unbound enzyme conjugated anti-Cu/ZnSOD is removed during a wash step and substrate solution reactive with HRP is added to the wells.

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 450 nm. A standard curve is prepared from seven Cu/ZnSOD standard dilutions and Cu/ZnSOD sample concentration determined.



4. REAGENTS PROVIDED

- 1 aluminium pouch with a **Microwell Plate coated with Monoclonal Antibody** (murine) to human Cu/ZnSOD
- 2 vials (20 μ l) **HRP-Conjugate** anti-Cu/ZnSOD monoclonal (murine) antibody
- 2 vials (0.5 ml) 5 ng/ml **Cu/ZnSOD Standard**
- 1 bottle (50 ml) **Wash Buffer Concentrate** 20x (phosphate-buffered saline with 1% Tween 20)
- 1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)
- 1 vial (5 ml) **Phosphate Buffered Saline Concentrate** (PBS) 20x
- 1 vial (15 ml) **Substrate Solution**
- 1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)
- 1 vial (0.4 ml) **Blue-Dye**
- 1 vial (0.4 ml) **Green-Dye**
- 2 adhesive **Plate Covers**

Reagent Labels

5. STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

6. SPECIMEN COLLECTION

Cell culture supernatants, human serum, EDTA, citrate or heparinized plasma, urine, amniotic fluid, fetal umbilical vein blood, or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive Cu/ZnSOD. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, frozen sera or plasma should be brought to room temperature slowly and mixed gently and properly prediluted with PBS (1:20 - 1:40 see 10).

For sample stability refer to 13., page 27.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1000 μ l adjustable single channel micropipettes with disposable tips
- 50 μ l to 300 μ l adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis.

8. PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9. PREPARATION OF REAGENTS

Except for the HRP-Conjugate (reagent D., see page 11) the reagents should be prepared before starting with the test procedure.

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add contents of Assay Buffer Concentrate (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Phosphate buffered saline (PBS)

Mix the contents of the bottle well. Add contents of PBS concentrate (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the PBS is stable for 30 days. PBS may be prepared as needed according to the following table:

Number of Strips	PBS Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

D. Preparation of HRP-Conjugate

Dilute the HRP-Conjugate 1 : 5 just prior to use by adding 80 µl Assay Buffer (reagent B) to the tube containing the HRP-Conjugate concentrate. Mix the contents of the tube well.

Make a further 1 : 100 dilution with Assay Buffer (reagent B) in a clean plastic tube.

Please note that the HRP-Conjugate should be used within 30 minutes after dilution. The second dilution (1 : 100) of the HRP-Conjugate may be prepared as needed according to the following table:

Number of Strips	Prediluted (1:5) HRP-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

E. Addition of colour-giving reagents: **Blue-Dye, Green-Dye**

In order to help our customers to avoid any mistakes in pipetting the Bender MedSystems ELISAs, Bender MedSystems now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (**Blue-Dye, Green-Dye**) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the **Blue-Dye** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of **Blue-Dye**, proceed according to the instruction booklet.

5 ml Diluent	20 µl Blue-Dye
12 ml Diluent	48 µl Blue-Dye
50 ml Diluent	200 µl Blue-Dye
60 ml Diluent	240 µl Blue-Dye

2. HRP-Conjugate: Before dilution of the concentrated conjugate, add the **Green-Dye** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of **Green-Dye** according to the instruction booklet, preparation of HRP-conjugate.

3 ml Assay Buffer	30 μ l Green-Dye
6 ml Assay Buffer	60 μ l Green-Dye
12 ml Assay Buffer	120 μ l Green-Dye

10. TEST PROTOCOL

- a. Mix all reagents thoroughly without foaming before use.
- b. Predilute serum or plasma samples 1:20 with **PBS** according to the following dilution scheme:

10 μ l **Sample** + 190 μ l **PBS**

For fetal umbilical vein blood first adjust samples to 2×10^7 erythrocytes/ml. (For sample preparation details please refer to 14, references 5, 10, 11). Then proceed as above.

- c. 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. Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to human Cu/ZnSOD from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
- d. Wash the microwell strips twice with approximately 300 μ l **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

- e. Add 100 μl of **PBS**, in duplicate, to the standard wells, leaving the first wells (5 ng/ml) empty. Prepare standard dilutions by pipetting 200 μl of **Cu/ZnSOD Standard**, in duplicate, into well A1 and A2 (see Figure 1 and 2). Transfer 100 μl to wells B1 and B2 respectively. Mix the contents by repeated aspiration and ejection and transfer 100 μl to well C1 and C2 respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure four times, creating two rows of Cu/ZnSOD standard dilutions ranging from 5 to 0.08 ng/ml. Discard 100 μl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of Cu/ZnSOD standard dilutions:

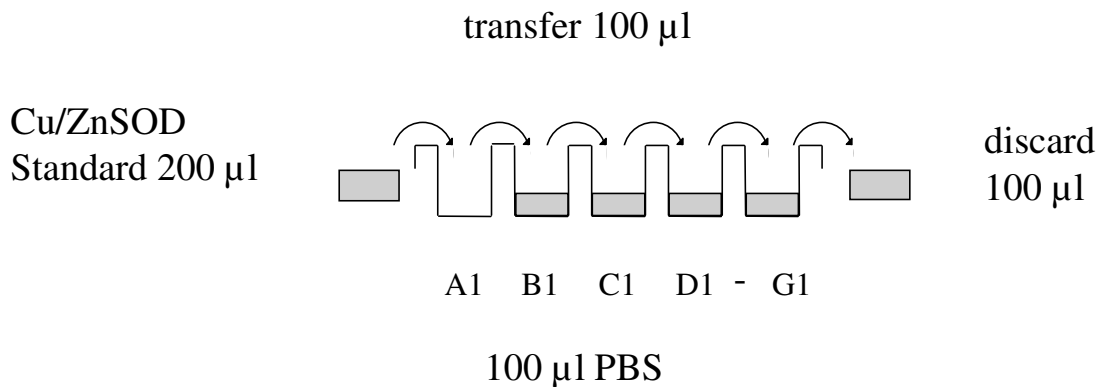


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (5 ng/ml)	Standard 1 (5 ng/ml)	Sample 1	Sample 1
B	Standard 2 (2.5 ng/ml)	Standard 2 (2.5 ng/ml)	Sample 2	Sample 2
C	Standard 3 (1.25 ng/ml)	Standard 3 (1.25 ng/ml)	Sample 3	Sample 3
D	Standard 4 (0.63ng/ml)	Standard 4 (0.63ng/ml)	Sample 4	Sample 4
E	Standard 5 (0.32ng/ml)	Standard 5 (0.32ng/ml)	Sample 5	Sample 5
F	Standard 6 (0.16 ng/ml)	Standard 6 (0.16 ng/ml)	Sample 6	Sample 6
G	Standard 7 (0.08 ng/ml)	Standard 7 (0.08 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- f. Add 100 μ l of **PBS**, in duplicate, to the blank wells.
- g. Add 90 μ l of **PBS** to all wells designated for samples.
- h. Add 10 μ l of each prediluted **Sample**, in duplicate, to the designated wells.
- i. Prepare **HRP-Conjugate**. (Refer to preparation of reagents 9.D.)
- j. Add 50 μ l of diluted **HRP-Conjugate** to all wells.
- k. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour, if available on a rotator set at 100 rpm.

- l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point d. of the test protocol. Proceed immediately to the next step.
- m. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- n. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point o. of this protocol) before positive wells are no longer properly recordable.
It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.
- o. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
- p. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the Cu/ZnSOD standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

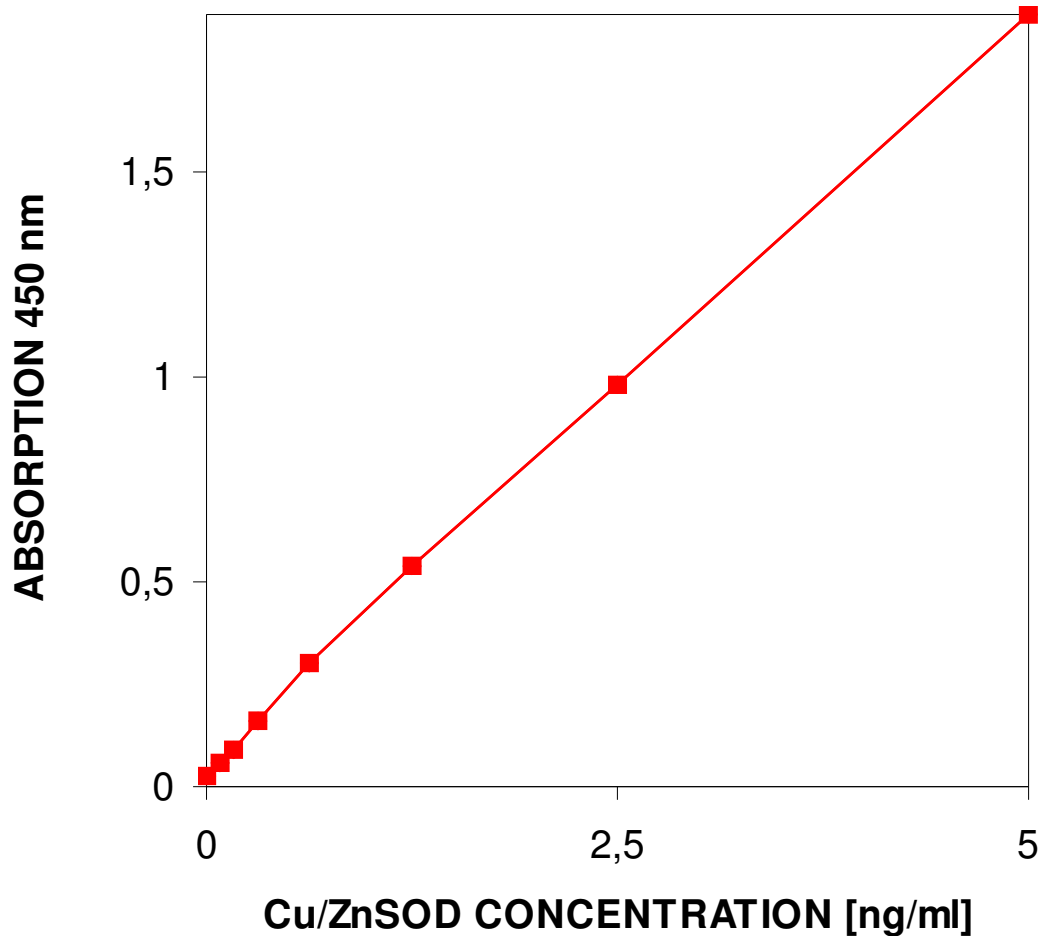
11. CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the Cu/ZnSOD concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating Cu/ZnSOD for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Cu/ZnSOD concentration.
- **For samples which have been diluted according the instructions given in this manual 1 : 200 the concentration read from the standard curve must be multiplied by the dilution factor (x 200).**

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect low Cu/ZnSOD levels. Such samples require further dilution of 1:400 - 1:800 with PBS in order to precisely quantitate the actual Cu/ZnSOD level.

- It is suggested that each testing facility establishes a control sample of known Cu/ZnSOD concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for Cu/ZnSOD ELISA. Recombinant Cu/ZnSOD was diluted in serial two-fold steps in PBS; symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the Cu/ZnSOD ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Cu/ZnSOD Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	5	1.942	1.886	4.2
	5	1.829		
2	2.5	1.006	0.981	3.6
	2.5	0.956		
3	1.25	0.568	0.542	6.8
	1.25	0.516		
4	0.63	0.309	0.299	4.7
	0.63	0.289		
5	0.32	0.160	0.158	2.2
	0.32	0.155		
6	0.16	0.091	0.091	0.8
	0.16	0.090		
7	0.08	0.058	0.059	1.2
	0.08	0.059		
Blank	0	0.022	0.024	
	0	0.026		

12. LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergent before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the PBS.

13. PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection for Cu/ZnSOD, defined as the analyte concentration resulting in an absorption significantly higher than the absorption of the dilution medium (mean plus two standard deviations) was determined to be 0.04 ng/ml (mean of 10 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of Cu/ZnSOD. Two standard curves were run on each plate. Data below show the mean Cu/ZnSOD concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 5.1 %.

Positive Sample	Experiment	Cu/ZnSOD Concentration (ng/ml)	Coefficient of Variation (%)
1	1	110.1	4.7
	2	99.2	6.1
	3	93.9	7.0
2	1	194.9	2.2
	2	185.1	1.0
	3	179.4	3.6
3	1	129.4	4.2
	2	123.7	6.1
	3	124.8	3.4
4	1	48.1	1.8
	2	45.6	10.7
	3	38.0	8.2
5	1	149.2	1.4
	2	145.5	4.7
	3	150.1	1.8
6	1	64.2	7.8
	2	53.3	7.8
	3	58.5	7.0
7	1	133.5	7.5
	2	122.5	9.5
	3	121.0	2.5
8	1	42.8	5.9
	2	40.1	4.6
	3	42.1	2.1

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of Cu/ZnSOD. Two standard curves were run on each plate. Data below show the mean Cu/ZnSOD concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 5.8 %.

Sample	Cu/ZnSOD Concentration (ng/ml)	Coefficient of Variation (%)
1	101.1	8.2
2	186.5	4.2
3	126.0	2.4
4	43.9	11.9
5	148.3	1.6
6	58.7	9.3
7	125.7	5.4
8	41.6	3.4

C. Recovery Studies

Spiked samples were prepared by adding four different levels of Cu/ZnSOD to 2 human serum samples with different base levels of Cu/ZnSOD. As shown below, recoveries were determined in two independent experiments ranging from 89 % to 108 % with an overall mean recovery of 98 %.

Experiment 1

Cu/ZnSOD Base level (ng/ml)	Recovery (%) Cu/ZnSOD Spike			
	4 ng	2 ng	1 ng	0.5 ng
42.9	91	92	100	103

Experiment 2

Cu/ZnSOD Base level (ng/ml)	Recovery (%) Cu/ZnSOD Spike			
	4 ng	2 ng	1 ng	0.5 ng
88.4	89	93	106	108

D. Dilution Parallelism

Four serum samples with different levels of Cu/ZnSOD were assayed at four serial two-fold dilutions (1:200 - 1:1600) covering the working range of the standard curve. In the table below the percent recovery of expected values is listed. Recoveries ranged from 80 % to 107 % with an overall mean recovery of 90 %.

Sample	Dilution	Cu/ZnSOD Concentration (ng/ml)		
		Expected Value	Observed Value	% Recovery of Exp. Value
1	1:200	--	216.3	--
	1:400	108.2	96.1	89
	1:800	54.1	42.7	79
	1:1600	27.1	24.0	89
2	1:200	--	123.8	--
	1:400	61.9	52.8	85
	1:800	30.9	30.1	97
	1:1600	15.5	14.2	92
3	1:200	--	146.1	--
	1:400	73.1	63.7	87
	1:800	36.5	29.2	80
	1:1600	18.3	19.6	107
4	1:200	--	53.0	--
	1:400	26.5	25.9	98
	1:800	13.3	11.1	83
	1:1600	6.6	6.5	98

E. Expected Values

a) A panel of 22 sera from apparently healthy blood donors (male and female) was tested for Cu/ZnSOD. The detected Cu/ZnSOD levels ranged between 22.5 and 102.9 ng/ml with a mean level of 56.5 ng/ml and a standard deviation of 20.0 ng/ml.

Normal Cu/ZnSOD levels may vary depending on the serum collective used.

- b) Measurement of Cu/ZnSOD from erythrocytes of fetal umbilical vein blood resulted in Cu/ZnSOD levels of 11-16 ng SOD/10⁶ fetal erythrocytes for normals and > 20 ng SOD/10⁶ fetal erythrocytes for fetuses with Down's Syndrome (5, 10, 11).

F. Sample Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked with Cu/ZnSOD) were stored at -20°C and thawed several times, and the Cu/ZnSOD level determined. There was no significant loss of Cu/ZnSOD concentrations between 0 and 5 freeze-thaw cycles.

G. Sample Storage Stability

Aliquots of a serum sample (unspiked or spiked with Cu/ZnSOD) were stored at -20°C, 2-8°C, room temperature and at 37°C and the Cu/ZnSOD level determined after 24, 48 and 96 hours. There was no significant loss of Cu/ZnSOD immunoreactivity during storage under above conditions.

H. Comparison of Serum and Plasma

From 22 individuals, serum as well as EDTA, citrate and heparin plasma obtained at the same time point were evaluated. All these blood preparations are suitable for Cu/ZnSOD determinations. It is nevertheless highly recommended to assure the uniformity of blood preparations used in one assay.

I. Specificity

The interference of human MnSOD was evaluated by spiking this protein at physiologically relevant concentrations into a Cu/ZnSOD positive serum. There was no detectable cross reactivity.

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15. ORDERING INFORMATION

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16. REAGENT PREPARATION SUMMARY

A. Wash Buffer Add Wash Buffer Concentrate 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer	Number of Strips	Assay Buffer Concentr. (ml)	Distilled Water (ml)
	1 - 6	2.5	47.5
	1 - 12	5.0	95.0

C. PBS	Number of Strips	PBS Concentr. (ml)	Distilled Water (ml)
	1 - 6	2.5	47.5
	1 - 12	5.0	95.0

D. HRP-Conjugate Add 80 μ l Assay Buffer to the tube containing HRP-Conjugate concentrate. Mix. Make further dilution according to table.

Number of Strips	Prediluted (1:5) HRP-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

17. TEST PROTOCOL SUMMARY

- Predilute serum or plasma samples with **PBS** 1:20
- Wash Microwell Strips twice with **Wash Buffer**
- Add 100 µl **PBS**, in duplicate, to standard wells except the first wells (5ng/ml)
- Pipette 200 µl **Cu/ZnSOD Standard** into the first standard wells and create standard dilutions ranging from 5 to 0.08 ng/ml by transferring 100 µl from well to well; Discard 100µl from the last wells
- Add 100 µl **PBS**, in duplicate, to the blank wells
- Add 90 µl **PBS** to the sample wells
- Add 10 µl 1:20 prediluted **Sample**, in duplicate, to designated wells
- Prepare **HRP-Conjugate**
- Add 50 µl diluted **HRP-Conjugate** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C)
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 10 to 20 minutes at room temperature (18° to 25°C)
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: For samples which have been diluted according to the instructions given in this manual 1:200 the concentration read from the standard curve must be multiplied by the dilution factor (x200). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect low Cu/ZnSOD levels. Such samples require further dilution of 1:400 - 1:800 with PBS in order to precisely quantitate the actual Cu/ZnSOD level.