ab119520 –Superoxide Dismutase 1 Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Cu/Zn Superoxide Dismutase concentrations in Cell culture supernatant, serum and plasma (EDTA, citrate, heparin), amniotic fluid, fetal umbilical vein blood and urine.

This product is for research use only and is not intended for diagnostic use.

Version 7d Last Updated 11 July 2023

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1. BACKGROUND

Abcam's Human Superoxide Dismutase 1 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate quantitative measurement of Human Cu/Zn Superoxide Dismutase concentrations in cell culture supernatant, serum and plasma (EDTA, citrate, heparin), amniotic fluid, fetal umbilical vein blood and urine.

Cu/Zn Superoxide Dismutase specific antibodies have been precoated onto 96-well plates. Standards and test samples are added to the wells along with a Cu/Zn Superoxide Dismutase HRP-conjugated detection antibody and the microplate is then incubated at room temperature. After the removal of unbound proteins by washing, TMB is added and catalyzed by HRP to produce a blue color product that changes to yellow after addition of an acidic stop solution. The density of yellow coloration is directly proportional to the Cu/Zn Superoxide Dismutase amount of sample captured in plate.

Superoxide Dismutases (SODs) (E.C.1.15.1.1.) are a unique family of metalloproteins that catalyze the dismutation of superoxide anion radicals (O2-) to oxygen (O2) and hydrogen peroxide (H2O2)

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

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/Zn Superoxide Dismutase) mainly present in the cytoplasm of eukaryotic cells, iron (FeSOD), predominantly in the cytosol, chloropasts 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. The Human Cu/Zn superoxide dismutase is a dimeric protein 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/Zn Superoxide Dismutase gene has been localized to chromosome 21q22.1.

Cu/Zn Superoxide Dismutase gene expression is induced by mediators of oxidative stress like sulfhydryl antioxidants, interleukin-1, tumor necrosis factor. Constitutive expression of copper and zinc SOD mRNA is highest in dividing cells.

Induction of Cu/Zn Superoxide Dismutase expression resulting in elevated levels of Cu/Zn Superoxide Dismutase in Human body fluids is of diagnostic value for measuring the activity of different diseases:

Nephropathies:

Cu/Zn Superoxide Dismutase determination provides a tool for early diagnosis of nephropathies.

Monitoring of therapeutic treatments:

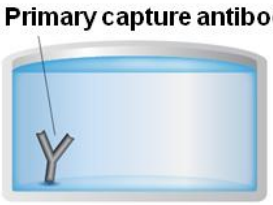
Cu/Zn Superoxide Dismutase is a useful therapeutic tool in the treatment of chronic inflammation e.g. rheumatoid arthritis or of the ischemic myocardium in the phase of reperfusion. Due to the short half-life of SOD injected into the blood circulation, a rapid assay is necessary for monitoring SOD levels.

Trisomy 21 (Down's Syndrome):

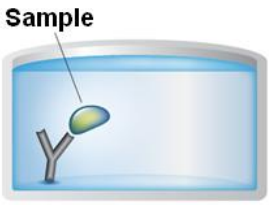
In cases with Down's Syndrome an additional part of chromosome 21 is present in the genome of the patient as a structural chromosome aberration. The Cu/Zn Superoxide Dismutase gene is localized on chromosome 21, closely associated with the gene complex responsible for the phenotype of Down's Syndrome.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

a) Patients with Down's Syndrome have significantly elevated serum and urine levels of Cu/Zn Superoxide Dismutase.
b) Prenatal diagnosis of Down's Syndrome: Cu/Zn Superoxide Dismutase 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/Zn Superoxide Dismutase are determined.

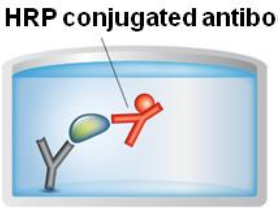
2. ASSAY SUMMARY



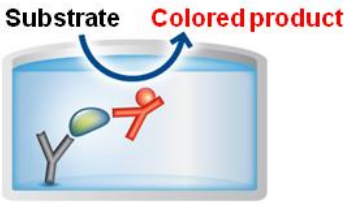
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



Add standard or sample to each well used.



Add prepared HRP labeled secondary detector antibody. Incubate at room temperature



Aspirate and wash each well. Add TMB Substrate Solution to each well. Incubate and then add stop solution. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9 Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Microplate coated with anti Human Cu/Zn SOD monoclonal antibody (12 x 8 wells)	96 wells	2-8 ºC
HRP Conjugated anti-Human Cu/Zn Superoxide Dismutase monoclonal antibody	2 x 20 µL	2-8 ºC
Cu/Zn Superoxide Dismutase Standard (5 ng/mL)	2 x 500 µL	2-8 ºC
20X Wash Buffer Concentrate	50 mL	2-8 ºC
20X Assay Buffer Concentrate	5 mL	2-8 ºC
Phosphate Buffered Saline Concentrate (PBS)	5 mL	2-8 ºC
TMB Substrate Solution	15 mL	2-8 ºC
Stop Solution (1 M Phosphoric Acid)	15 mL	2-8 ºC
Adhesive Films	1X 2 units	2-8 ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL 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)
- Microplate 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 regression analysis

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers
- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps.
- As exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, 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 radio immunotherapy has significantly increased the number of patients with Human anti-mouse IgG antibodies (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 sample.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

9.1 1X Wash Buffer

Prepare 1X Wash Buffer by diluting the 20X Wash Buffer Concentrate with distilled or deionized water. To make 500 mL 1X Wash Buffer, combine 25 mL 20X Wash Buffer Concentrate with 475 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

Note: The 1X Wash Buffer should be stored at 2-8 ºC and is stable for 30 days.

9.2 1X Assay Buffer

Prepare 1X Assay Buffer by diluting the 20X Assay Buffer Concentrate with distilled or deionized water. To make 50 mL 1X Assay Buffer, combine 2.5 mL 20X Assay Buffer Concentrate with 47.5 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

Note: The 1X Assay Buffer should be stored at 2-8 ºC and is stable for 30 days.

9.3 1X Phosphate Buffered Saline (PBS)

Prepare the 1X Phosphate Buffered Saline (PBS) by diluting the 20X Phosphate Buffered Saline with distilled or deionized water. To make 100 mL 1X PBS, combine 5 mL 20X PBS concentrate with 95 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

Note: The 1X Phosphate Buffer Saline should be stored at 2-8 ºC and is stable for 30 days.

9.4 **1X HRP-Conjugated Antibody**

Dilute the HRP-Conjugate anti-Human Cu/Zn Superoxide Dismutase by adding 80 µL 1X Assay Buffer. Mix thoroughly and gently.

Make a further 1:100 dilution with 1X Assay Buffer in a clean plastic tube or reagent reservoir as needed using the following table to prepare as much 1X HRP-Conjugated Antibody as required:

Number of strips	Volume of HRP-conjugate solution Concentrate (µL)	Volume of 1X Assay Buffer (mL)
1 - 6	30	2.97
7 - 12	60	5.94

Note: The 1X HRP conjugate should be used within 30 minutes after dilution.

- All other solutions are supplied ready to use

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every experiment.

10.1 Label eight tubes with numbers 1 - 8.

10.2 Pipette 450 µL of the undiluted Standard into tube 1.

10.3 Add 225 µL 1X PBS into tubes number 2 - 8.

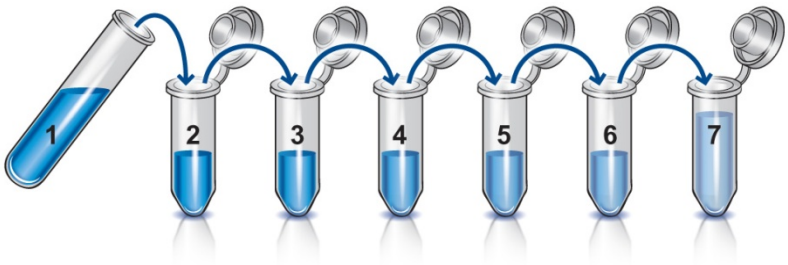
10.4 Prepare **Standard 2** by transferring 225 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.5 Prepare **Standard 3** by transferring 225 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.6 Using the table below as a guide, repeat for tubes number 4 through to 7.

10.7 **Standard 8** contains no protein and is the Blank control

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of 1X PBS (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	450	-	5.00	5.00
2	Standard 1	225	225	5.00	2.50
3	Standard 2	225	225	2.50	1.25
4	Standard 3	225	225	1.25	0.63
5	Standard 4	225	225	0.63	0.31
6	Standard 5	225	225	0.31	0.16
7	Standard 6	225	225	0.16	0.08
8	None	-	225	-	0



11. SAMPLE COLLECTION AND STORAGE

- Cell culture supernatant, serum, plasma (EDTA, citrate, heparin), amniotic fluid, urine and fetal umbilical vein blood were tested with this assay. We have tested the kit with standard protein spiked into cell culture medium. Other biological samples might be suitable for use in the assay. Remove serum from the clot or cells 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 should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Human Cu/Zn Superoxide Dismutase. 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, the frozen sample should be brought to room temperature slowly and mixed gently and properly diluted with 1X Sample Diluent.

- Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed several times, and the Human Cu/Zn Superoxide Dismutase levels determined. There was no significant loss of Human Cu/Zn Superoxide Dismutase immunoreactivity detected by freezing and thawing.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use.
- Unused well strips should be returned to the plate packet and stored at 2-8°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections. Determine the number of microplate strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Predilute serum and plasma samples 1:20 with 1X PBS (10 µL sample to 190 µL 1X PBS).

13.2. Wash the microplate twice with approximately 400 µL 1X Wash Buffer per well with thorough aspiration of microplate contents between washes. Allow the 1X Wash Buffer to remain in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microplate.

13.3. After the last wash step, empty wells and tap microplate on absorbent pad or paper towel to remove excess 1X Wash Buffer. Use the microplate strips immediately after washing. Alternatively the microplate strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

13.4. Add 100 µL of prepared standards (including the standard blank control) to the appropriate wells.

13.5. Add 90 µL 1X PBS to wells to which samples will be added.

13.6. Add 10 µL of samples into the appropriate wells.

13.7. Add 50 µL of HRP conjugate to all wells.

13.8. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 3 hours (microplate can be incubated on a shaker set at 400 rpm).

13.9. Remove adhesive film and empty wells. Wash microplate strips 3 times according to step 13.2. Proceed immediately to step 13.10.

13.10. Add 100 µL of TMB Substrate Solution to all wells.

13.11. Incubate the microplate strips at room temperature (18 to 25°C) for 10 minutes. Avoid direct exposure to intense light.

Note: The color development on the plate should be monitored and the substrate reaction stopped (see step 13.12) before the signal in the positive wells becomes saturated. Determination of the ideal time period for color development should to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 - 0.95.

13.12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well.

Note: It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microplate strips are stored at 2 - 8°C in the dark.

13.13. Read absorbance of each microplate on a spectrophotometer 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 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.

14. CALCULATIONS

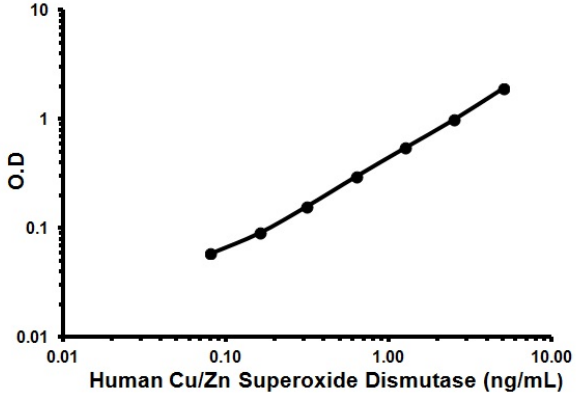
Average the duplicate readings for each standard, sample and control blank. Subtract the no protein control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A five parameter algorithm (5PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 5-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

If instructions in this protocol have been followed samples have been diluted either 1:200 in the case of plasma and serum samples (1:20 external predilution as step 13.1, 1:10 dilution on the plate as step 13.7), or 1:10 for other samples (as step 13.6). Therefore the concentration read from the standard curve must be multiplied by the dilution factor (either x 200 or x 10).

Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low Human Cu/Zn Superoxide Dismutase levels (Hook Effect). Such samples require further external predilution according to expected Human Cu/Zn Superoxide Dismutase values with Sample Diluent in order to precisely quantitate the actual Human Cu/Zn Superoxide Dismutase level.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed. The standard curve is expected to be sigmoidal.



Standard Curve Measurements			
Conc.	**O.D. 450 nm**		**Mean**
(ng/mL)	**1**	**2**	**O.D.**
0.00	0.022	0.026	0.024
0.08	0.058	0.059	0.059
0.16	0.091	0.090	0.091
0.31	0.160	0.155	0.158
0.63	0.309	0.289	0.299
1.25	0.568	0.516	0.542
2.50	1.006	0.956	0.981
5.00	1.942	1.829	1.886

Figure 1. Example of Human and the Human Cu/Zn Superoxide Dismutase standard protein standard curve.

16. TYPICAL SAMPLE VALUES

EXPECTED VALUES –

Panels of 40 serum as well as EDTA, citrate and heparin plasma samples from randomly selected apparently healthy donors (males and females) were tested for Human Cu/Zn Superoxide Dismutase. Measurement of Human Cu/Zn Superoxide Dismutase from erythrocytes of fetal umbilical vein blood ranged from 11.0 to 16.0 ng SOD/106 fetal erythrocytes for normals and > 20.0 ng SOD/106 fetal erythrocytes for fetuses with Down's syndrome. For detected Human Cu/Zn Superoxide Dismutase see Table below. The levels measured may vary with the sample collection used.

Sample Matrix	Number of Samples Evaluated	Range (ng/mL)	% Detectable	Mean of Detectable (ng/mL)
Serum	40	nd *- 35.2	17.5	22.6
Plasma (EDTA)	40	nd *- 59.7	20	15.3
Plasma (Citrate)	40	nd *- 104.7	97.5	42.4
Plasma (Heparin)	40	nd *- 50.7	57.5	26.7

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

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 Human Cu/Zn Superoxide Dismutase determinations. It is nevertheless highly recommended to assure the uniformity of blood preparations used in one assay.

SENSITIVITY –

The limit of detection for Cu/Zn Superoxide Dismutase 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 6 independent assays).

RECOVERY –

Spiked samples were prepared by adding four different levels of recombinant Cu/Zn Superoxide Dismutase into serum. Recoveries were determined in 2 normal pooled serum samples. The unspiked serum was used as blank in these experiments. The recovery ranged from 89% to 108% with an overall mean recovery of 98%.

DILUTION PARALLELISM –

A serum sample was assayed at four two-fold dilutions covering the working range of the standard curve. The recovery ranged from 80% to 107% with an overall recovery of 90%.

PRECISION –

Intra- and Inter-assay reproducibility was determined by measuring samples containing different concentrations of Human CD137.

	Intra-Assay	Inter-Assay
n=	8	8
%CV	5.1	5.8

17. ASSAY SPECIFICITY

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a Cu/Zn Superoxide Dismutase positive serum. There was no detectable cross reactivity detected.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)