

Datasheet for ABIN955788

SOD1 ELISA Kit





Overview

| Quantity: | 96 tests |
|--------------------|---|
| Target: | SOD1 |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Application: | ELISA |
| Product Details | |
| Purpose: | The Human Cu/Zn SOD ELISA is an enzyme-linked immunosorbent assay for the quantitative |
| | determination of human Cu/Zn SOD in cell culture supernatants, serum, plasma, urine, amniotic |
| | fluid, fetal umbilical vein blood and other body fluids. |
| Sample Type: | Cell Culture Supernatant, Plasma, Serum, Urine |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | The interference of circulating factors of the immune system was evaluated by spiking these |
| | proteins at physiologically relevant concentrations into a human Cu/ZnSOD positive serum. |
| | There was no cross reactivity detected. |
| Sensitivity: | The limit of detection of Cu/Zn SOD defined as the analyte concentration resulting in an |
| | absorption significantly higher than that of the dilution medium (mean plus two standard |
| | deviations) was determined to be 0.04 ng/mL (mean of 6 independent assays). |
| Characteristics: | An anti-Cu/Zn SOD monoclonal coating antibody is adsorbed onto microwells. Cu/Zn SOD |
| | present in the sample or calibrator binds to antibody adsorbed to the microwells, a HRP- |

conjugated monoclonal anti- Cu/Zn SOD antibody is added and binds to Cu/Zn SOD captured by the first antibody. Following incubation unbound enzyme-conjugated anti-Cu/Zn SOD is removed during a wash step and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of Cu/Zn SOD present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A calibration curve is prepared from seven Cu/Zn SOD calibrator dilutions and Cu/Zn SOD sample concentration determined.

Components:

1 aluminum pouch with Microwell Plate (12 x 8-well strips) coated with monoclonal antibody (mouse) to human Cu/Zn SOD

2 vials (20 µL) HRP-Conjugate anti-Cu/Zn SOD monoclonal (mouse) antibody

2 vials (0.5 mL) Cu/Zn SOD Calibrator, 5 ng/mL

1 bottle (50 mL) Wash Buffer Concentrate 20X (PBS with 1% Tween 20)

1 vial (5 mL) Assay Buffer Concentrate 20X (PBS with 1% Tween 20 and protein stabilizer)

1 bottle (5 mL) Phosphate Buffered Saline Concentrate (PBS), 20X

1 vial (15 mL) Substrate Solution

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

2 vials (0.4 mL each), Blue Dye, Green Dye 2 adhesive Plate Seals.

Material not included:

5 mL and 10 mL graduated pipettes

 $5\,\mu\text{L}$ to 1,000 μL adjustable single channel micropipettes with disposable tips

 $50~\mu L$ to $300~\mu L$ adjustable multi-channel micropipette with disposable tips

Multi-channel micropipette reservoir

Beakers, flasks, cylinders necessary for preparation of reagents

Device for delivery of wash solution (multi-channel wash bottle or automatic wash system)

Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Distilled or de-ionized water

Statistical calculator with program to perform linear regression analysis.

Target Details

| Target: | SOD1 |
|-------------------|---|
| Alternative Name: | Cu/Zn SOD (SOD1 Products) |
| Background: | Superoxide dismutases (SODs) are a unique family of metalloproteins that catalyze the dismutation of superoxide anion radicals (O2 -) to oxygen (O2) and hydrogen peroxide (H2O2). 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 (Mn SOD) principally located in the matrix of mitochondria of all aerobes, copper/zinc (Cu/Zn SOD) mainly present in the cytoplasm of eukaryotic cells, iron (Fe SOD), predominantly in the cytosol, chloroplasts or mitochondria of prokaryotes as well as extracellular (EC SOD), 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 SOD gene has been localized to chromosome 21q22.1. Cu/Zn SOD 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 SOD expression resulting in elevated levels of Cu/Zn SOD in human body fluids is of value for measuring the activity of different diseases.

Pathways:

Sensory Perception of Sound, Transition Metal Ion Homeostasis

Application Details

| Plate: | Pre-coated | |
|-------------------------|---|--|
| Sample Preparation: | Cell culture supernatants, human serum, EDTA, citrate or heparinized plasma, urine, amniotic | |
| | fluid, fetal umbilical vein blood, or other body fluids will be suitable for use in the assay. Remove | |
| | 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 should be aliquoted and must be | |
| | stored frozen at -20°C to avoid loss of bioactive Cu/Zn SOD. If samples are to be run within 24 | |
| | hours, they may be stored at 4°C. Avoid repeated freeze-thaw cycles. Prior to assay, frozen | |
| | sample should be brought to room temperature slowly and mixed gently. | |
| Calculation of Results: | Calculate the mean absorbance values for each set of duplicate calibrators and samples. | |
| | Duplicates should be within 20 percent of the mean. | |
| | Create a calibration curve by plotting the mean absorbance for each calibrator concentration on | |
| | the ordinate against the Cu/Zn SOD concentration on the abscissa. Draw a best-fit curve | |
| | through the points of the graph (a 5-parameter curve fit is recommended). | |
| | To determine the concentration of circulating Cu/Zn SOD for each sample, first find the mean | |
| | | |

absorbance value on the ordinate and extend a horizontal line to the calibration curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Cu/Zn SOD concentration.

For samples which have been diluted according to the instructions given in this package insert (1:200), the concentration read from the calibration curve must be multiplied by the dilution factor (x 200).

Note: Calculation of samples with a concentration exceeding calibrator 1 may result in incorrect, low Cu/Zn SOD levels (Hook Effect). Such samples require further dilution with PBS (1X) in order to precisely quantitate the actual Cu/Zn SOD level.

It is suggested that each testing facility establishes a control sample of known Cu/Zn SOD concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

Restrictions:

For Research Use only

Handling

Handling Advice:

Since exact conditions may vary from assay to assay, a calibration curve must be established for every assay run.

Bacterial or fungal contamination of either 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 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 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 mouse monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to mouse immunoglobulins can still be analyzed in such assays when mouse immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Storage:

4°C

Storage Comment:

Store kit reagents at 4°C as indicated. Immediately after use remaining reagents should be returned to cold storage (4°C). Expiration date of the kit and reagents is stated on labels. The

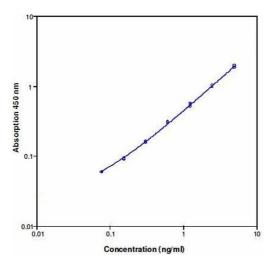
Handling

expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Expiry Date:

The expiry date is stated on the label.

Images



ELISA

Image 1.