APA596Hu01 100µg Active Superoxide Dismutase Copper Chaperone Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Gln23~Gln269 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.0 Predicted Molecular Mass: 29.9kDa Accurate Molecular Mass: 33kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

QSCVDAVR KSLQGVAGVQ DVEVHLEDQM VLVHTTLPSQ EVQALLEGTG RQAVLKGMGS GQLQNLGAAV AILGGPGTVQ GVVRFLQLTP ERCLIEGTID GLEPGLHGLH VHQYGDLTNN CNSCGNHFNP DGASHGGPQD SDRHRGDLGN VRADADGRAI FRMEDEQLKV WDVIGRSLII DEGEDDLGRG GHPLSKITGN SGERLACGII ARSAGLFQNP KQICSCDGLT IWEERGRPIA GKGRKESAQ

[ACTIVITY]

Superoxide dismutase copper chaperone (SOD4) is an enzyme that in humans is encoded by the CCS gene. This gene encodes a member of the superoxide dismutase (SOD) protein family. SODs are antioxidant enzymes that catalyze the dismutation of two superoxide radicals into hydrogen peroxide and oxygen. Acroding to the report, in a weakly alkaline buffer solution (pH=8.2) with N-tris(hydroxymethyl) amino methane-HCL, pyrogallol can occur autoxidation in the air, then SOD can inhibit this reaction. Thus, we use this way to measued the activity of recombinant human SOD4. The reaction was performed in adding 5 µI 5 mmol/L pyrogallol to 200 $\,\mu$ I 50mmol/L Tris-HCl, rapidly mixing at 25 $\,^{\circ}$ C, then read at 325 nm (using 50 mmol/L Tris-HCl as blank control) in kinetic mode for 3 minutes using a microplate reader controlling the pyrogallol autoxidation rate at 0.70 OD/min. Different concentrations of recombinant human SOD4 were added into 200 μ I 50 mmol/L Tris-HCI, incubated for 20min at 25 °C, then adding 5 μ I 5 mmol/L pyrogallol to each well, rapidly mixing and read at 325 nm in kinetic mode for 3 minutes. Under these conditions, the enzyme amount of 50% inhibition of pyrogallol autooxidation per minute is defined as a unit. The specific activity of recombinant human SOD4 is 97 U/mg.

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Calculation

SOD4 activity (U/mg) =
$$\frac{\frac{0.070 - A325 / \text{min}}{0.070} \times 100\%}{50\%} / M$$

Where:

0.070=pyrogallol autoxidation rate A325/min= inhibition pyrogallol autoxidation rate of SOD4 M=mass of enzyme

[IDENTIFICATION]

	kDa 70
	44
-	33
	26
	22
	18
	14
	10

Figure 1. SDS-PAGE

Sample: Active recombinant SOD4, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.