**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-SOD1 (Human) mAb

Code No.	Clone	Subclass	Quantity	Form
M062-3	1G2	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Cu/Zn-superoxide dismutases (Cu/Zn-SOD, SOD1) are metalloenzymes involved in the mechanisms of cellular defense against oxidative damage. They have been found in the cytoplasm of all the eukaryotic cells and in the periplasm of several bacterial species. Human SOD1 (approx. MW=19 kDa) is homodimers that contain one atom of zinc and one atom of copper per subunit and catalyze the dismutation of the superoxide anion ($O_2^{-}\bullet$) quite efficiently at a rate close to the diffusion limit, over the entire pH range from 5 to 10.:

$$2O_2^{-}\bullet + 2H^{+} \xrightarrow{\text{(SOD1)}} O_2 + H_2O_2$$

SOD1 possess a very compact and stable structure that is highly resistant to urea, SDS and proteolytic enzymes. SOD1 homodimer consist of two pieces of eight-stranded β-barrel structure monomer closely packaged on each hydrophobic interface stabilized by intrasubunit disulfide bond. SOD1 catalyzes the disproportion of superoxide anions via the alternate reduction and reoxidation of the copper ion at the active site of the enzyme. Recent studies in yeast have identified a protein termed the copper chaperone for superoxide dismutase (CCS) as the factor responsible for copper incorporation into SOD1 through direct protein-protein interaction. SOD1's endogenous role remains poorly understood. Mice lacking this enzyme were apparently healthy and displayed no increased sensitivity to hyperoxia. However, they exhibited a pronounced susceptibility to paraquat which induces acute oxidative stress in mice. As a relation to disease, elevated level of SOD1 is reported in Down syndrome patients as a result of increase of genedosage (trisomy). Moreover, linkage studies have revealed that mutations in SOD1 are responsible for 10-15% of case of the fetal motor neuron disease familial amyotrophic lateral sclerosis (FALS). Evidence from transgenic mice expressing FALS associated SOD1 mutations, as well as mice homozygous for a deletion of the SOD1 gene, indicates that this neuronal degeneration arises from a gain-of-function associated with the SOD1 mutations rather than loss-of-function. In addition to the legitimate dismutation reaction, SOD1 is able to catalyze surrogate reactions such as the production of •OH using anionic scavengers and H_2O_2 and nitration of the tyrosine residues of proteins by peroxynitrate. These reactions might be the causes of cytotoxicity.

SOURCE: This antibody was purified from hybridoma (clone 1G2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with Balb/c mouse splenocyte immunized with the recombinant full-length human SOD1.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody detects ~20 kDa of human SOD1 on Western blotting with total cell lysate from human cell lines.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1) and 2).
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOL**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, HeLa, Raji, HL-60	NIH/3T3, WR19L, BaF/3	PC12, Rat-1
Reactivity on WB	+	-	-

INTENDED USE:

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REFERENCES:

1) Tokuda, E., *et al., Mol. Neurodegener.* **12**, 2 (2017) [IHC]
2) Morisaki, Y., *et al., Sci. Rep.* **6**, 27354 (2016) [IHC]
3) Hineno, A., *et al., J. Neurol. Sci.* **319**, 63-74 (2012) [WB]
4) Ren, G., *et al., Mol. Neurodegener.* **6**, 29 (2011) [WB]
5) Doi, K., *et al., Neuropathology* **28**, 269-276 (2008) [WB]
6) Nawa, M., *et al., Cell Signal.* **20**, 493-505 (2008) [WB]
7) Schonhoff, C. M., *et al., PNAS* **103**, 2404-2409 (2006) [WB]
8) Kanekura, K., *et al., J. Biol. Chem.* **279**, 19247-19256 (2004) [WB]
9) Hashimoto, Y., *et al., PNAS* **98**, 6336-6341 (2001) [WB]
10) Casareno, R.L.B., *et al., J. Biol. Chem.* **273**, 23625-28 (1998)
11) Przedborski, S., *et al., Nat. Genet.* **18**, 99-100 (1998)
12) Fridovich, I., *et al., J. Biol. Chem.* **272**, 18515-17 (1997)
13) Kondo, T., *et al., J. Neurosci.* **17**, 4180-89 (1997)

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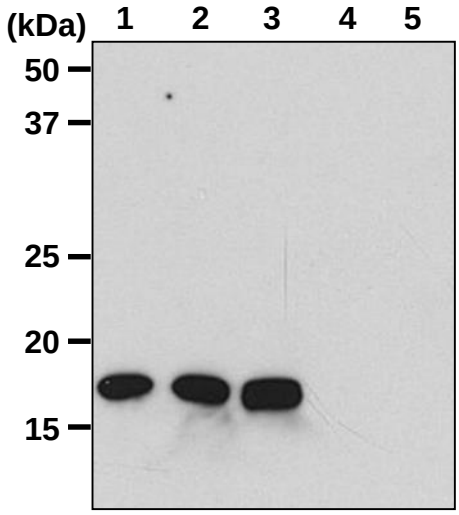
14) Yim, M. B., *et al., PNAS.* **93**, 5709-14 (1996)
15) Reaume, A. G., *et al., Nat. Genet.* **13**, 43-47 (1996)
16) Brown, R. H., Jr., *et al., Cell* **80**, 687-692 (1995)
17) Fridovich, L., *et al., Annu. Rev. Biochem.* **64**, 97-112 (1995)
18) Rosen, D. R., *et al., Nature* **362**, 59-62 (1993)
19) Deng, H. X., *et al., Science* **261**, 1047-1051 (1993)
20) Badway, J. A., *et al., Annu. Rev. Biochem.* **49**, 695-726 (1980)

This antibody is used in reference number 1) - 9).



Western blot analysis of SOD1

Lane 1: Jurkat
Lane 2: Raji
Lane 3: HeLa
Lane 4: WR19L
Lane 5: PC12

PROTOCOL:

SDS-PAGE & Western Blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.)
2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
3) Boil the samples for 2 minutes. Load 5 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
8) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
11) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 1 minute.
13) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji and HeLa)