

ORDERING INFORMATION

Catalog Number: MAB3420

Clone: 350701

Lot Number: WJL01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Specificity: human/mouse/rat SOD3

Immunogen: *E. coli*-derived recombinant human SOD3 (aa 19 - 240)

Ig class: mouse IgG_{2b}

Recommended Application:
Western blot

Background

Superoxide Dismutases (SODs), originally identified as Indophenoloxidase (IPO), are enzymes that catalyze the conversion of naturally-occurring but harmful superoxide radicals into molecular oxygen and hydrogen peroxide. Superoxide Dismutases 3, SOD3, also known as extracellular (EC) SOD, is tetrameric glycoprotein with an apparent subunit molecular weight of about 30kDa. Like SOD1, SOD3 binds one Cu²⁺ and Zn²⁺ ions per subunit but differs in sequence and tissue distribution. Three isoenzymes of SOD have been identified and are functionally related but have very modest sequence homology. SOD3 shares 23% and 17% sequence identity with SOD1 and SOD2, respectively. SOD3 shares ~64% sequence homology with mouse and rat SOD3. SOD3 is a secretory protein and is synthesized with a putative 18-amino acid signal peptide preceding the 222 amino acids in the mature SOD3. SOD3 is found in plasma, lymph, and synovial fluid as well as in tissues. SOD3 binds on the surface of endothelial cells through the heparan sulfate proteoglycan and eliminates the oxygen radicals from the NADP-dependent oxidative system of neutrophils.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived recombinant human Superoxide Dismutase 3 (rhSOD3; aa 19 - 240; Accession # NP_003093). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

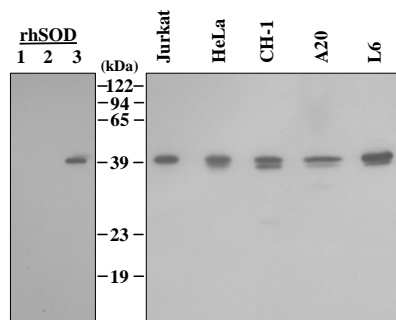
Specificity

The antibody is known to react with endogenous human, mouse, and rat SOD3 on Western blots. This antibody did not cross-react with recombinant human SOD1 or SOD2 on Western blot.

Applications

Western Blot - An antibody concentration of 0.5 µg/mL is recommended.

Optimal dilutions should be determined by each laboratory for each application.



Detection of SOD3 with MAB3420. Recombinant human SOD1, SOD2, and SOD3 (1 ng) and lysates from human Jurkat and HeLa, mouse CH-1 and A20, and rat L6 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.5 µg/mL mouse anti-SOD3 as described in *Protocols for Immunoblotting*. A 10 second exposure to film is shown.

Protocols for Immunoblotting

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	2% nonfat dry milk in Blotting Buffer
0.15 M NaCl		
0.1% Tween [®] 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.5 µg/mL anti-human/mouse/rat SOD3.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-mouse IgG-HRP (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

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