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Product no AS04 054

AOX1/2 | Plant alternative oxidase 1 and 2

Product information

Background	Alternative oxidases (**AOX**) are quinol oxidases located in the inner mitochondrial membrane of plants. They function as terminal oxidases in the alternate electron transport pathway, oxidizing ubiquinone to reduce oxygen to water.
Immunogen	KLH-conjugated synthetic peptide derived from fully conserved C-terminal consensus motif from plant AOX isoforms including *Arabidopsis thaliana* AOX1A. UniProt: Q39219, TAIR: At3g22370, AOX1B UniProt: O23913, TAIR:AT3G22360, AOX1C UniProt: O22048, TAIR: AT3G27620, *and* AOX2, UniProt: O22049, TAIR: AT5G64210, *Solanum lycopersicum* UniProt: Q7XBG9, *Oryza sativa* UniProt: Q7XT33, AOX1D, TAIR: AT1G32350
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water.
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.
Tested applications	Immunolocalization (IL), Western blot (WB)
Related products	AS06 152 Anti-AOX1 | alternative oxidase from *Chlamydomonas reinhardtii,* rabbit antibod*ies* AS04 054PRE | AOX1/2 | plant alternative oxidase 1 and 2, pre-immune serum AS04 054S | AOX | AOX positive control/quantitation standard Plant protein extraction buffer Secondary antibodies
Additional information	Mitochondrion inner membrane marker. Possibly in the inner surface of the inner mitochondrial membrane. Protocol for a plant mitochondria preparation can be found here. In protein samples which are older than few months AOX enzyme can undergo intensive dimerization. Such preparations should not be used to work with this antibody.

Application information

Recommended dilution	1 : 750 (IL), 1 : 1000 for 10-20 µg of mitochondrial protein/lane detection (WB)
Expected | apparent MW	36-40 | 36-40 for *Arabidopsis thaliana*
Confirmed reactivity	*Arabidopsis thaliana, Betula nana, Beta vulgaris, Brassica napus, Brassica oleracea, Kandelia candel, Eriphorum vaginatum, Hordeum vulgare, Lupinus luteus, Nicotiana tabacum, Oryza sativa, Picea abies, Pisum sativum, Poa annua, Robinia pseudoacacia, Solanum lycopersicum, Solanum tuberosum, Symplocarpus renifolius, Physcomitrella patens, Tigriopus californicus, Triticum aestivum*
Predicted reactivity	*Aegilops tauschii, Brachypodium distachyon, Capsella rubella, Citrus sinensis, Citrus clementina, Corylus heterophylla, Crocus sativus, Cucumis sativus, Daucus carota, Glycine max, Hypericum perforatum, Lotus japonicus, Malus x domestica, Medicago* *Species of your interest not listed?* Contact us *truncatula, Medicago sativa, Naegleria gruberi* (amoeba), *Nelumbo nucifera, Nicotiana benthamiana, Oryza brachyantha, Populus tremula, Picea sitchensis, Saccharum officinarum, Sauromatum venosum, Sorghum bicolor, Selaginella moellendorffii, Tetrahymena thermophila, Zea mays, Vigna*

radiata, Vigna unguiculata, Vitis vinifera

Not reactive in *Candidia albicans, Chlamydomonas reinhardtii* (use an antibody to algal AOX1, AS06 152)

Additional information According to Konert et al. (2015) AOX antibody is recognizing AOX1A and AOX1D.

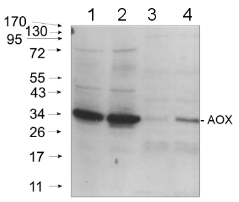
This product can be sold containing ProClin if requested.

For high resolution images, please visit the specific product page at www.agrisera.com

Selected references

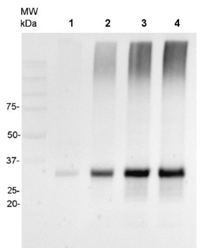
Makino et al. (2020). Induction of Terminal Oxidases of Electron Transport Chain in Broccoli Heads Under Controlled Atmosphere Storage. Foods, 9 (4)
Marchetti et al. (2020). Mitochondrial Pentatricopeptide Repeat Protein, EMB2794, Plays a Pivotal Role in NADH Dehydrogenase Subunit nad2 mRNA Maturation in Arabidopsis Thaliana. Plant Cell Physiol DOI: 10.1093/pcp/pcaa028
Garmash et al. (2020). Altered levels of AOX1a expression result in changes in metabolic pathways in Arabidopsis thaliana plants acclimated to low dose rates of ultraviolet B radiation. Plant Sci. 2020 Feb;291:110332. doi: 10.1016/j.plantsci.2019.110332.
Kuang et al. (2019). Quantitative Proteome Analysis Reveals Changes in the Protein Landscape During Grape Berry Development With a Focus on Vacuolar Transport Proteins. Front Plant Sci. 2019 May 15;10:641. doi: 10.3389/fpls.2019.00641. eCollection 2019.
Tward et al. (2019). Identification of the alternative oxidase gene and its expression in the copepod Tigriopus californicus. Comp Biochem Physiol B Biochem Mol Biol. 2019 Feb;228:41-50. doi: 10.1016/j.cbpb.2018.11.003.
Réthoré et al. (2019). Arabidopsis seedlings display a remarkable resilience under severe mineral starvation using their metabolic plasticity to remain self-sufficient for weeks. Plant J. 2019 Mar 22. doi: 10.1111/tpj.14325.
Luévano-Martínez et al. (2019). Mitochondrial alternative oxidase is determinant for growth and sporulation in the early diverging fungus Blastocladiella emersonii. Fungal Biology, Vol 123, Issue 1, 59-65.
Córdoba et al. (2019). Different Types of CA Domains Are Present in Complex I from Immature Seeds and Adult Plants in Arabidopsis thaliana. Plant Cell Physiol. 2019 Jan 22. doi: 10.1093/pcp/pcz011.
Czobor et al. (2019). Comparison of the response of alternative oxidase and uncoupling proteins to bacterial elicitor induced oxidative burst. PLoS One. 2019 Jan 10;14(1):e0210592. doi: 10.1371/journal.pone.0210592.
Hu et al. (2018). OsNDUFA9 encoding a mitochondrial complex I subunit is essential for embryo development and starch synthesis in rice. Plant Cell Rep. 2018 Dec;37(12):1667-1679. doi: 10.1007/s00299-018-2338-x.
Borovik and Grabelnych (2018). Mitochondrial alternative cyanide-resistant oxidase is involved in an increase of heat stress tolerance in spring wheat. J Plant Physiol. 2018 Dec;231:310-317. doi: 10.1016/j.jplph.2018.10.007.
Umekawa and Ito (2018). Thioredoxin o-mediated reduction of mitochondrial alternative oxidase in the thermogenic skunk cabbage Symplocarpus renifolius. J Biochem. 2018 Oct 5. doi: 10.1093/jb/mvy082.
Hu et al. (2018). OsNDUFA9 encoding a mitochondrial complex I subunit is essential for embryo development and starch synthesis in rice.Plant Cell Rep. 2018 Aug 27. doi: 10.1007/s00299-018-2338-x.
Zhu et al. (2018). Mitochondrial alternative oxidase-dependent autophagy involved in ethylene-mediated drought tolerance in Solanum lycopersicum. Plant Biotechnol J. 2018 May 4. doi: 10.1111/pbi.12939.
Garmash et al. (2017). Expression profiles of genes for mitochondrial respiratory energy-dissipating systems and antioxidant enzymes in wheat leaves during de-etiolation. J Plant Physiol. 2017 Aug;215:110-121. doi: 10.1016/j.jplph.2017.05.023.
Vishwakarma et al. (2016). A discrete role for alternative oxidase under hypoxia to increase nitric oxide and drive energy production. Free Radic Biol Med. 2018 Mar 28. pii: S0891-5849(18)30148-5. doi: 10.1016/j.freeradbiomed.2018.03.045.
Zhao et al. (2016). Nitrogen deprivation induces cross-tolerance of Poa annua callus to salt stress. Biologia Plantarum 60 (3): 543–554.
Solti et al. (2016). Does a voltage-sensitive outer envelope transport mechanism contributes to the chloroplast iron uptake? Planta. 2016 Dec;244(6):1303-1313. Epub 2016 Aug 19.
Zhang et al. (2016). A High Temperature-Dependent Mitochondrial Lipase EXTRA GLUME1 Promotes Floral Phenotypic Robustness against Temperature Fluctuation in Rice (Oryza sativa L.). PLoS Genet. 2016 Jul 1;12(7):e1006152. doi: 10.1371/journal.pgen.1006152. eCollection 2016.
Meng et al. (2016). Physiological and proteomic responses to salt stress in chloroplasts of diploid and tetraploid black locust (Robinia pseudoacacia L.). Sci Rep. 2016 Mar 15;6:23098. doi: 10.1038/srep23098
Pavlovič et al. (2016). Light-induced gradual activation of photosystem II in dark-grown Norway spruce seedlings. Biochim Biophys Acta. 2016 Feb 18. pii: S0005-2728(16)30028-7. doi: 10.1016/j.bbabio.2016.02.009.
Konert et al.(2015). Protein phosphatase 2A (PP2A) regulatory subunit B' interacts with cytoplasmic ACONITASE 3 and modulates the abundance of AOX1A and AOX1D in Arabidopsis thaliana. New Phytol. 2015 Feb;205(3):1250-63. doi: 10.1111/nph.13097. Epub 2014 Oct 13.

Application example



25 µg of *Arabidopsis thaliana* mitochondrial wild type fraction **(1)** mitochondrial fraction from a mutant with increased AOX level **(2)**, total wild type leaf extract **(3)**, total leaf extract from AOX overproducing mutant **(4)** were separated on 10% gel and blotted on **nitrocellulose** membrane using wet transfer (0.22% CAPS, pH 11). Filters where blocked (1.5h) in 5% milk in TBST (1X TBS, 0,1% Tween 20), incubated with 1: 1000 anti-AOX polyclonal antibodies (2h in TBST) followed by 1 h incubation with 1: 50 000 Agrisera secondary anti-rabbit HRP-coupled antibodies (AS09 602) and visualized with chemiluminescent detection reagent, on Kodak autoradiography film for 15-60 s. Mitochondria were isolated as described by Urantowka et al. (Plant Mol Biol, 2005, 59:239-52). Mitochondrial pellets were suspended in 1X Laemmli buffer (5% beta-mercaptoetanol, 3.7% glycerol, 1.1% SDS, 23 mM Tris- HCl pH 6.8, 0.01% bromophenol blue), heated (95°C, 5 min.) and centrifuged (13 000rpm, 1 min.). Leaf extracts were prepared as described by Martinez-Garcia et al. (Plant J., 1999, 20:251-7).

Courtesy Dr. Janusz Piechota, Wrocław University, Poland



20 µg of mitochondrial protein isolated from 2-week-old *Arabidopsis thaliana* seedlings (Smakowska et al., 2016) extracted with a buffer containing urea, thiourea, CHAPS and Triton X-100 (Heidorn-Czarna et al., 2018) were denaturated with Laemmli buffer at 95°C for 5 min and separated on 12% SDS-PAGE. Wild-type grown at 22°C (1), mutant grown at 22°C (2), wild-type grown at 30°C (3), mutant grown at 30°C.

Afterwards the gel was blotted for 1.5h to nitrocellulose membrane using wet-transfer. Blot was blocked with 5% milk in TBS-T at 4°C/ON with agitation. Blot was incubated in the primary antibody (anti-AOX1/2, AS04 054) at a dilution 1:1000 in 5% milk in TBS-T for 1.5h /RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 2 times for min in TBS-T at RT with agitation.

Blot was incubated in Agrisera matching secondary antibody (goat anti-rabbit IgG, HRP-conjugated, AS09 602) diluted to 1:20 000 in 5% milk in TBS-T for 1h/RT with agitation. The blot was washed as above and developed with chemiluminescence using GBox imager (Syngene).

Courtesy Dr. Małgorzata Heidorn-Czarna, University of Wrocław, Poland

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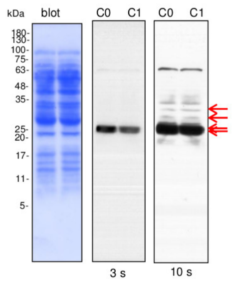

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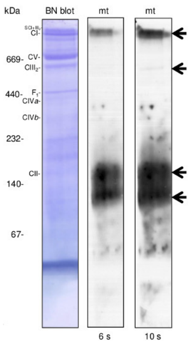
Lines C0, C1- 10 µg of cauliflower mitochondrial proteins (C0- controls; C1- plants grown in mild drought conditions) isolated as described by Rurek et al., 2015 (doi:10.1016/j.bbabio.2015.01.005) were separated by 12% SDS- PAGE and electroblotted in semi-dry conditions (Towbin buffer) to Immobilon-P membrane (Millipore). Blots were CBB R 250 briefly stained, destained, wet-scanned and after completed destaining, they were blocked in 5% skimmed milk (dissolved in PBS-T containing 0.1% Tween 20) in 1h, RT. Primary antisera (at 1: 1000, diluted in 2% skimmed milk in PBS-T) were bound by overnight incubation of blots at +4 O C. After blot washing (2 times quick, 2 times of 5 min, and 10 min at the end), secondary goat anti-rabbit IgGs, HRP- conjugated (Agrisera, AS09 602; at 1: 50 000, diluted in 2% milk/ PBS-T) were bound in 1 h, RT. Blots were washed (as above) with copious amounts of PBS-T and chemiluminescence signals acquired by using chemiluminescent detection reagents on RTG film between 3 s and 2 min (periods of the given image acquisition were indicated).



100 µg of cauliflower mitochondria were pelleted and proteins were digitonin solubilised (30 min at 4°C) at the detergent: protein ratio 4:1 (g:g) using ACA 750 buffer. Unsolubilised material was further pelleted and supernatant after complementation with Serva Blue was loaded onto 4.5-16% gradient BN gel. After separation, protein complexes in the gel were denatured and reduced (in the presence of SDS and 2-mercaptoethanol) and then they were electroblotted and immunodetected essentially in the same manner as it was indicated for SDS-PAGE blots. Four complexes containing alternative oxidase were detected (the most abundant ca.150 and 120 kDa). This data is very similar to the one obtained for green tissue mitochondria of Arabidopsis and Medicago (see Gelmap project; https://gelmap.de/). Mobility of known OHPHOS complexes (complex I, II, III, IV and ATP synthase= complex V) was additionally indicated.

Courtesy Dr. Michał Rurek, Department of Molecular and Cellular Biology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland