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Product no AS04 054

Anti-AOX1/2 | Plant alternative oxidase 1 and 2

Product information

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 the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
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, Glycine max, Hordeum vulgare, Lupinus luteus, Nicotiana tabacum, Oryza sativa, Picea abies, Pisum sativum, Poa annua, Robinia pseudoacacia, Rosa hybrida, Solanum lycopersicum, Solanum tuberosum, Symplocarpus renifolius, Physcomitrium patens, Tigriopus californicus, Triticum aestivum, Zea mays*
Predicted reactivity	*Aegilops tauschii, Brachypodium distachyon, Capsella rubella, Citrus sinensis, Citrus clementina, Corylus heterophylla, Crocus sativus, Cucumis sativus, Daucus carota, Hypericum perforatum, Lotus japonicus, Malus x domestica, Medicago truncatula, Medicago sativa, Mangifera indica, Naegleria gruberi* (amoeba), *Nelumbo nucifera, Nicotiana benthamiana, Oryza brachyantha, Populus tremula, Picea sitchensis,Pyrus communis,Saccharum officinarum, Sauromatum venosum, Sorghum bicolor, Selaginella moellendorffii, Tetrahymena thermophila, Vigna radiata, Vigna unguiculata, Vitis vinifera* Species of your interest not listed? Contact us
Not reactive in	*Candidia albicans, Chlamydomonas reinhardtii* (use an antibody to algal AOX1, AS06 152), *Stomolophus* sp2
Additional information	According to Konert et al. (2015) AOX antibody is recognizing AOX1A and AOX1D. This product can be sold containing ProClin if requested.
Selected references	Hoa et al. (2024). Proteomic analysis on symbiotic differentiation of mitochondria in soybean nodules. Comparative Study Plant Cell Physiol. 2004 Mar;45(3):300-8. doi: 10.1093/pcp/pch035. Soria et al. (2024).Functional resilience: An active oxidative phosphorylation system prevails amid foreign proteins in holoparasitic plants. Current Plant Biology Volume 37, March 2024, 100322. Rodrigues et al. (2023). Germination of Pisum sativum L. Seeds Is Associated with the Alternative Respiratory Pathway. Biology (Basel). 2023 Oct 9;12(10):1318.doi: 10.3390/biology12101318. Brito et al. (2022) The role of the electron-transfer flavoprotein: ubiquinone oxidoreductase following carbohydrate starvation in Arabidopsis cell cultures. Plant Cell Rep. 2022 Jan 15. doi: 10.1007/s00299-021-02822-1. Epub ahead of print. PMID: 35031834. Pascual et al (2021). ACONITASE 3 is part of the ANAC017 transcription factor-dependent mitochondrial dysfunction response, Plant Physiology, 2021;, kiab225, https://doi.org/10.1093/plphys/kiab225 Challabathula et al. (2021) Differential modulation of photosynthesis, ROS and antioxidant enzyme activities in stress-sensitive and -tolerant rice cultivars during salinity and drought upon restriction of COX and AOX pathways of mitochondrial oxidative electron transport, Journal of Plant Physiology,Volume 268,2022,153583, ISSN 0176-1617,https://doi.org/10.1016/j.jplph.2021.153583.

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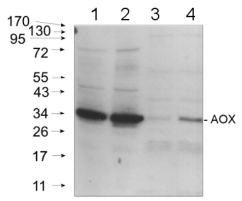
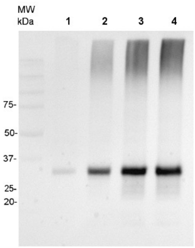
Oh et al. (2021) Alternative oxidase (AOX) 1a and 1d limit proline-induced oxidative stress and aid salinity recovery in Arabidopsis. Plant Physiol. 2021 Dec 17:kiab578. doi: 10.1093/plphys/kiab578. Epub ahead of print. PMID: 34919733.
Pavlovic & Kocab. (2021) Alternative oxidase (AOX) in the carnivorous pitcher plants of the genus Nepenthes: what is it good for? Ann Bot. 2021 Dec 18:mcab151. doi: 10.1093/aob/mcab151. Epub ahead of print. PMID: 34922341.
Lang et al. (2011).Simultaneous isolation of pure and intact chloroplasts and mitochondria from moss as the basis for sub-cellular proteomics. Plant Cell Rep. 2011 Feb;30(2):205-15.doi: 10.1007/s00299-010-0935-4.



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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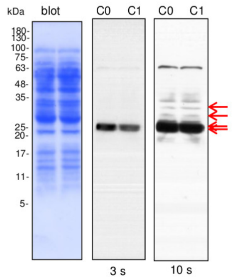
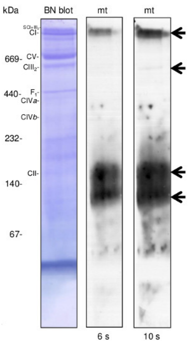


Lines C0, C1- 10 µg of cauliflower mitochondrial proteins (C0- controls; C1- plants grown in mild drought conditions) isolated as described by Rurek et al., 2015 (doi:10.1016/j.bbabio.2015.01.005) were separated by 12% SDS- PAGE and electroblotted in semi-dry conditions (Towbin buffer) to Immobilon-P 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