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**Product no AS04 054**

# AOX1/2 | Plant alternative oxidase 1 and 2

## Product information

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|---|---|
| **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. |
| **Additional information** | Mitochondrion inner membrane marker. Possibly in the inner surface of the inner mitochondrial membrane.<br><br>Protocol for a plant mitochondria preparation can be found here.<br><br>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

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| **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 truncatula, Medicago sativa, 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, Zea mays, Vigna radiata, Vigna unguiculata, Vitis vinifera*<br>*Species of your interest not listed? Contact us* |
| **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.<br><br>This product can be sold containing ProClin if requested.<br><br>For high resolution images, please visit the specific product page at www.agrisera.com |
| **Selected references** | 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<br>Makino et al. (2020). Induction of Terminal Oxidases of Electron Transport Chain in Broccoli Heads Under Controlled Atmosphere Storage. Foods, 9 (4)<br>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<br>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.<br>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. |

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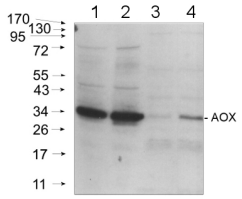
## Application example




**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**

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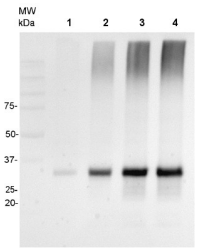


20 µg of mitochondrial protein isolated from 2-week-old *Arabidopsis thaliana* seedlings (Smakowska et al., 2016) extracted with a buffer containing urea, thiourea, CHAPS and Triton X-100 (Heidorn-Czarna et al., 2018) were denaturated with Laemmli buffer at 95°C for 5 min and separated on 12% SDS-PAGE.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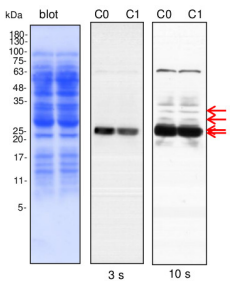
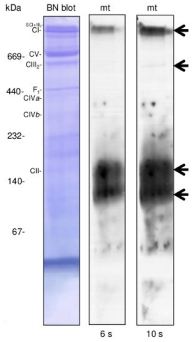


Lines C0, C1- 10 µg of cauliflower mitochondrial proteins (C0- controls; C1- plants grown in mild drought conditions) isolated as described by Rurek et al., 2015 (doi:10.1016/j.bbabio.2015.01.005) were separated by 12% SDS- PAGE and electroblotted in semi-dry conditions (Towbin buffer) to Immobilon-P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