

product **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	<u>KLH</u> -conjugated synthetic peptide derived from fully conserved C-terminal consensus motif from plant AOX isoforms 1 and 2 including <i>Arabidopsis thaliana</i> AOX1A (<u>At3g22370</u>) and AOX2 (<u>At5g64210</u>), <i>Solanum lycopersicum</i> <u>Q7XBG9</u> , <i>Oryza sativa</i> <u>Q7XT33</u> .
antibody format	rabbit polyclonal, serum, lyophilized
quantity	200 µl - for reconstitution add 200 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	western blot (WB)
additional information	Protocol for plant mitochondria isolation can be found here.

application information

recommended dilution	1 : 1000 on 10-20 µg of mitochondrial protein/lane with standard ECL detection (WB)
expected apparent MW	36-40 36-40 for <i>Arabidopsis thaliana</i>
confirmed reactivity	<i>Arabidopsis thaliana</i> , <i>Lupinus luteus</i> , <i>Pisum sativum</i> , <i>Solanum tuberosum</i> , <i>Physcomitrella patens</i>
predicted reactivity	dicots including: <i>Nicotiana tabacum</i> and <i>Glycine max</i> , monocots including <i>Oryza sativa</i> , <i>S.officinarum</i> and <i>Triticum aestivum</i> , conifers, diatoms
not reactive in	<i>Chlamydomonas reinhardtii</i>
additional information	to be added when available
selected references	<u>Lang</u> , E.G.E., S.J. Mueller, S.N.W. Hoernstein, J. Porankiewicz-Asplund, M. Vervliet-Scheebaum, R. Reski (2010). Simultaneous isolation of pure and intact chloroplasts and mitochondria from moss as basis for sub-cellular proteomics. Plant Cell Reports, DOI: 10.1007/s00299-010-0935-4. (open source)

Yamasaki et al. (2007) Regulation of Copper Homeostasis by Micro-RNA in *Arabidopsis*. *J. Biol Chem* 282: 16369-16378

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 were 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 standard ECL 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

