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: Q39213, TAIR: AT3g22360, AOX1C UniProt: Q62048, TAIR: AT3g27620, and AOX2, UniProt: Q62049, TAIR: AT5G64210, Solanum lycopersicum UniProt: Q7XBG9, Oryza sativa UniProt: Q7XT35, 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 AOX1 | alternative oxidase from Chlamydomonas reinhardtii, rabbit antibody
AS10 699 AOX | alternative oxidase monoclonal antibody
AS04 054PRE | AOX1/2 | plant alternative oxidase 1 and 2, pre-immune serum
AS04 054S | AOX | AOX positive control/quantitation standard

Recommended secondary antibody (for western blot with ECL detection)

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.

This product can be sold containing ProClin if requested.

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, Kandelia candel, Eriphorum vaginatum, Hordeum vulgare, Lupinus luteus, Nicotiana tabacum, Picea abies, Pisum sativum, Poa annua, Robinia pseudoacacia, Solanum lycopersicum, Solanum tuberosum, Physcomitrella patens

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), Nicotiana benthamiana, Oryza brachyantha, Oryza sativa, Populus tremula, Picea sitchensis, Saccharum officinarum, Sauromatum venosum, Sorghum bicolor, Selaginella moellendorffii, Tetrahymena thermophila, Zea mays, Vigna unguiculata, Vitis vinifera

Not reactive in Chlamydomonas reinhardtii

Additional information

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

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Selected references


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, Wroclaw University, Poland
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 IgG, 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 standard ECL 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 OPHOS 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.