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**
KLH-conjugated synthetic peptide derived from fully conserved C-terminal consensus motif from plant AOX isoforms including *Arabidopsis thaliana* AOX1A.


**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 antibodies
- 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, Kandelia candel, Eriphorum vaginatum, Hordeum vulgare, Lupinus luteus, Nicotiana tabacum, Oryza sativa, Picea abies, Pismum sativum, Poa annua, Robinia pseudoacacia, Solanum lycopersicum, Solanum tuberosum, Symphoricarpos renifolius, Physcomitrella patens, Tigliopus californicus, *Tritium 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), Nicotiana benthamiana,
Oryza brachyantha, Populus tremula, Picea sitchensis, Saccharum officinarum, Sauromatum venosum, Sorghum bicolor, Selaginella moellendorffii, Tetrahymena thermophila, Zea mays, Vigna unguiculata, Vitis vinifera

Not reactive in Candida 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.

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 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).

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).

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 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 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 OPHOS complexes (complex I, II, III, IV and ATP synthase= complex V) was additionally indicated.

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