

Product no **AS09 521****Anti-ACO1 | Aconitase****Product information**

Immunogen | *Arabidopsis* ACO1 ([AT4G35830](#), [Q42560](#)), codon 120 – 898 (C-terminus), was cloned in fusion with a N-terminal 6xHis tag, and over-expressed in *E. coli*. All recombinant protein accumulated in inclusion bodies, which were purified by centrifugation and solubilised in 6 M guanidine-HCl. The protein was refolded by dilution in 100 mM Tris-HCl 8.5, 10% (v/v) glycerol, 2 mM dithiothreitol, and concentrated prior to immunisation.

Host | Rabbit**Clonality** | Polyclonal**Purity** | Serum**Format** | Lyophilized**Quantity** | 100 µl**Reconstitution** | For reconstitution add 100 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 | *Arabidopsis* expresses three highly similar aconitase isozymes (ACO1/ AT4G35830, ACO2/AT4G26970 and ACO3/AT2G05710), of which ACO1 is the cytosolic isoform, while ACO2 and ACO3 are predominantly located in the mitochondria (Arnaud et al 2007, Bernard et al 2009). The combined abundance and activity of the mitochondrial aconitases is about 3 times higher than the cytosolic pool (Bernard et al 2009). The *Arabidopsis* isoforms are more similar in amino acid sequence to mammalian iron-regulatory proteins (IRP-1) than to the mammalian and yeast mitochondrial aconitases

Application information

Recommended dilution | 1 : 5 000 - 1 : 10 000 (WB). At higher concentrations the antibody binds aspecifically resulting in non-specific signals around 60 kDa, including Rubisco subunits

Expected | apparent MW | 98 kDa, Note that ACO1, ACO2 and ACO3 cannot be distinguished in size by standard SDS-PAGE,

Confirmed reactivity | *Arabidopsis thaliana* ACO1, ACO2 and ACO3 isoforms, *Brassica oleracea*, *Solanum lycopersicum*

Predicted reactivity | *Cucurbita maxima*, *Hordeum vulgare*, *Nicotiana tabacum*, *Ricinus communis*, *Solanum tuberosum*, *Vitis vinifera*, *Oryza sativa*, *Zea mays*, *Picea sitchensis*, *Populus trichocarpa*
Species of your interest not listed? [Contact us](#)

Not reactive in | *Chlamydomonas reinhardtii*, cyanobacteria

Additional information | The antibody recognises all three *Arabidopsis* aconitase isoforms (ACO1, ACO2 and ACO3, see Bernard et al 2009). Possible differences in affinity have not been precisely quantified. Sensitivity threshold is between 2 and 10 ng for WB / ECL (see figure). Antibodies will recognize aconitase isoforms in denaturing and native gel electrophoresis

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>
[Przybyla-Toscano et al. \(2021\)](#) Protein lipoylation in mitochondria requires Fe-S cluster assembly factors NFU4 and NFU5. *Plant Physiol.* 2021 Oct 28;kiab501. doi: 10.1093/plphys/kiab501. Epub ahead of print. PMID: 34718778.
[Rurek et al. \(2018\)](#). Mitochondrial Biogenesis in Diverse Cauliflower Cultivars under Mild and Severe Drought Involves Impaired Coordination of Transcriptomic and Proteomic Response and Regulation of Various Multifunctional Proteins. *Preprints 2018*, 2018010276 (doi: 10.20944/preprints201801.0276.v1).
[Setiati et al. \(2014\)](#). Root phosphoenolpyruvate carboxylase and NAD-malic enzymes activity increase the ammonium-assimilating capacity in tomato. *J Plant Physiol.* 171:49-63.
[Birke et al. \(2012\)](#). Cysteine biosynthesis, in concert with a novel mechanism, contributes to sulfide detoxification in mitochondria of *Arabidopsis thaliana*. *Biochem J.* May 2, ahead of print.

application example

Western blot analysis of **1)** 5 ng purified 6xHis-AtACO1 (Δ 119, 87 kDa); **2)** 2 ng 6xHis-AtACO1; **3)** Total protein (15 μ g) from *Arabidopsis thaliana* leaves were extracted with 2 volumes 50 mM Tris-HCl pH 8.0, 5% (v/v) glycerol, 1% (w/v) sodium dodecyl sulphate, 10 mM NaEDTA, 1 mM phenylmethanesulfonyl fluoride; **4)** 15 μ g of purified mitochondria from *Arabidopsis thaliana* cell culture, **5)** 15 μ g of protein from *Arabidopsis thaliana* chloroplasts

Proteins were separated by SDS-PAGE and blotted onto nitrocellulose (Whatman Protran BA 83, 0.2 μ m). Blots were blocked in Tris-buffered saline (TBS) with 0.1% (v/v) Tween 20 and 5% (w/v) dried skimmed milk for 1 h at room temperature, and incubated with anti-AtACO1 antibodies diluted 1:10,000 in fresh block solution (10 mL per 8 x 6 cm blot) for 2 h at room temperature. The blot was washed 3 times with block solution, then incubated with horse-radish peroxidase conjugated anti-rabbit IgG antibodies, diluted 1: 5,000 in block solution, for 45 minutes. The blot was washed 2 times with block solution and 2 times with TBS-Tween. The signal was developed with standard ECL reagents and Kodak X-Omat LS film.

Note: as visible in lane 2, detection of recombinant AtACO1 falls below 2 ng of recombinant protein.

