

product **AS09 521**
ACO1 | aconitase

product information

background	Aconitase is a single subunit enzyme of the tricarboxylic acid cycle (or Krebs cycle) in the mitochondria. A cytosolic isoform is also part of the glyoxylate cycle. Aconitase catalyzes the dehydration / hydration of citrate to iso-citrate, via cis-aconitate as an intermediate. The reaction is facilitated by an iron-sulphur cluster in the active site of the enzyme. The iron-sulphur cluster is somewhat unstable, especially under oxidative stress, and loss of the cofactor leads to degradation of the protein. Alternative names: ACO, citrate hydro-lyase 1,2,3
immunogen	<i>Arabidopsis</i> ACO1 (AT4G35830 , Q42560), codon 120 – 898 (C-terminus), was cloned in fusion with a N-terminal 6xHis tag, and over-expressed in <i>E. coli</i> . 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.
antibody format	rabbit polyclonal serum lyophilized
quantity	100 µl 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 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	<i>Arabidopsis</i> 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 <i>Arabidopsis</i> isoforms are more similar in amino acid sequence to mammalian iron-regulatory proteins (IRP-1) than to the mammalian and yeast mitochondrial aconitases.

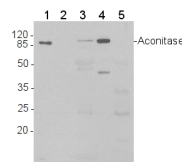
application information

recommended dilution	1:5 000 – 1:10 000 with standard ECL (WB). At higher concentrations the antibody binds aspecifically resulting in non-specific signals ≤ 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	<i>Arabidopsis thaliana</i> ACO1, ACO2 and ACO3 isoforms, <i>Solanum lycopersicum</i>

predicted reactivity	dicots including: <i>Cucurbita maxima</i> , <i>Nicotiana tabacum</i> , <i>Ricinus communis</i> , <i>Solanum tuberosum</i> , <i>Vitis vinifera</i> , monocots: <i>Oryza sativa</i> , <i>Zea mays</i> , trees: <i>Picea sitchensis</i> , <i>Populus trichocarpa</i>
not reactive in	<i>Chlamydomonas reinhardtii</i>
additional information	The antibody recognises all three <i>Arabidopsis</i> 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	<u>Arnaud</u> et al. (2007) The iron-responsive element (IRE)/iron-regulatory protein 1 (IRP1)-cytosolic aconitase iron-regulatory switch does not operate in plants. <i>Biochem. J.</i> 405: 523-531 <u>Bernard</u> et al. (2009). An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron-sulfur proteins in <i>Arabidopsis</i> . <i>Plant Physiol.</i> 151:590-602.

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 (Abcam), 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.