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Product no AS23 4993

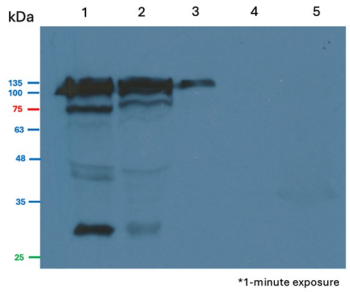
Anti-ACO1,2,3 | Aconitase

Product information

Immunogen	KLH-conjugated peptide derived from *Arabidopsis thaliana* ACO1,2,3 protein sequence. UniProt: Q42560 (ACO1), Q94A28 (ACO2), Q9SIB9 (ACO3) TAIR: AT4G35830, AT4G26970, AT2G05710
Host	Rabbit
Clonality	Polyclonal
Purity	Antigen affinity purified serum, in PBS pH 7.4
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution, add 50 µl of sterile or deionized 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.
Additional information	Antibody is recognizing S35:ACO2-GFP fusion protein.

Application information

Recommended dilution	1 : 1000 - 1: 5000 (WB)
Expected | apparent MW	98 kDa
Confirmed reactivity	*Arabidopsis thaliana, Nicotiana tabacum, Vitis vinifera*
Predicted reactivity	*Brassica napus, Capsicum annuum, Cannabis sativa, Citrus sp., Cucumis sativus, Euglena gracilis, Glycine max, Gossypium sp., Malus domestica, Manihot esculenta, Phaseolus vulgaris, Pisum sativum, Pinus pinaster, Populus sp., Solanum lycopersicum, Solanum tuberosum* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	To be added when available. Antibody released in December 2025.



Samples:
1 - 50 ug of *Arabidopsis thaliana* whole leaf extract (vegetative leaves), 4 week-old plant
2 - 50 ug of *Nicotiana benthamiana* whole leaf extract (vegetative leaves), 4 week-old plant
3 - ~20 ug (due to low yields) of mitochondria isolated from 4 week-old *Nicotiana benthamiana* vegetative leaves *While samples in lanes 1, 2, 4, and 5 were freshly extracted, the sample in lane 3 was extracted and stored for 6 months at -20°C
4 - 50 ug of *Vitis vinifera* (grapevine) whole leaf extract (vegetative leaves)
5 - 50 ug of *E. coli* protein extract
Marker: MW (kDa) BLUelf prestained protein ladder

50 µg/well of total protein extracted freshly from *A. thaliana* leaves (1), *N. benthamiana* leaves (2), *V.vinifera* grapevine leaves (4) or *E. coli* (5). Exact extraction buffer components were: 2.5 mM Tris pH 8.2, 0.05 mM KCl, 20% glycerol, 400 mM sucrose, and 5 mM $MgCl_2$. Samples were denatured with SDS loading buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, 0.01% bromophenol blue) at 100°C for 10 min. Samples were separated at RT on 12% SDS-PAGE and blotted for 1h15 minutes to PVDF (pore size of 0.45 um) using wet transfer in the cold. Blot was blocked with 3% skim milk in PBS-T for 1h at RT with agitation. Blot was incubated in the primary antibody at a dilution of 1:1000 in PBS-T containing 1% skim milk overnight at 4°C with 60 RPM agitation. The blot was washed with 25 ml of 1X PBS-T five times at 5 minutes each with 60 RPM agitation. The blot was incubated with 1:8000 secondary anti-rabbit IgG, Horseradish peroxidase-linked antibody in PBS-T

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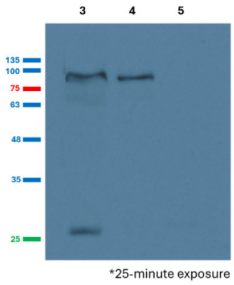
containing 1% skim milk for 2 hours with 60 RPM agitation. The blot was washed as described previously. The washed, and drained blot was incubated with 2 ml of Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific) for 5 minutes, and protein bands were exposed to X-ray films for 1 minute to capture signal.

Additional bands are a result of high protein load/well and concentrated primary antibody, which is suggested in our protocol, as it is easier to optimize it, once it is confirmed that the antibody is recognizing correct target protein.

Courtesy of Catherine Fust, PhD Candidate in the Department of Molecular and Cellular Biology, University of Guelph, Canada



3 - 20 ug of *Vitis vinifera* (grapevine) whole leaf extract (vegetative leaves) denatured in typical SDS loading buffer via boiling for 10 minutes
4 - 20 ug of *Vitis vinifera* (grapevine) whole leaf extract (vegetative leaves) denatured in typical SDS loading buffer for 15 hours at room temperature (no boiling)
5 - 20 µg of *Vitis vinifera* (grapevine) whole leaf extract (vegetative leaves) denatured in SDS loading buffer supplemented with Urea (7M) and boiled for 10 minutes

Marker: MW (kDa) BLUelf prestained protein ladder

20 µg/well of total protein extracted from *Vitis vinifera* (grapevine) leaves (3-5). Exact extraction buffer components were: 2.5 mM Tris pH 8.2, 0.05 mM KCl, 20% glycerol, 400 mM sucrose, and 5 mM $MgCl_2$. Samples were denatured with SDS loading buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, 0.01% bromophenol blue) at 100°C for 10 min (or room temp for 15 hours for sample #4). Samples were separated at RT on 12% SDS-PAGE and blotted for 1h 15 minutes to PVDF (pore size of 0.45 µm) using wet transfer in the cold. Blot was blocked with 3% skim milk in PBS-T for 1h at RT with agitation. Blot was incubated in the primary antibody at a dilution of 1:5000 in PBS-T containing 1% skim milk for 1h at room temp with 60 RPM agitation. The blot was washed with 25 ml of 1X PBS-T five times at 5 minutes each with 60 RPM agitation. The blot was incubated with 1:8000 secondary ECL™ anti-rabbit IgG, Horseradish peroxidase-linked whole antibody from donkey in PBS-T containing 1% skim milk for 2 hours with 60 RPM agitation. The blot was washed as described previously. The washed, and drained blot was incubated with 2 ml of Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific) for 5 minutes, and protein bands were exposed to X-ray films for 1 minute, 5 minutes or 25 minutes to capture signal.

Note: addition of urea did not improve antibody signal but resulted in no signal at all. Aging grapevine leaves exhibit lower ACO protein levels, therefore more sensitive detection reagent must be applied.

Courtesy of Catherine Fust, PhD Candidate in the Department of Molecular and Cellular Biology, University of Guelph, Canada