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Product no AS18 4240

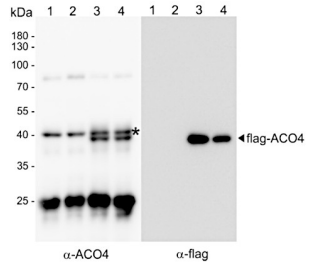
Anti-ACO4 | 1-aminocyclopropane-1-carboxylate oxidase 4

Product information

Immunogen	KLH-conjugated peptide derived from ACO4 of *Arabidopsis thaliana*, UniProt: Q06588, TAIR: AT1G05010
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
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 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.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	36.6 | 40 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Camelina sativa, Capsella rubella, Eutrema salsugineum*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	To be added when available, antibody released in June 2021.



Samples: 1 and 2. pBA002 vector as negative control (technical replicates).
3 and 4. pBA002:AtACO4 (technical replicates).

Total protein extracts were prepared from *Nicotiana benthamiana* whole leaves agroinfiltrated with the indicated vectors.

18 µg/well of total protein extracted freshly from *Nicotiana benthamiana* with following buffer components: Tris-HCl pH 7.5 100 mM, Urea 8 M, Triton x-100 (0.2%), Sarkosyl (0.2%), PMSF 1mM, Pepstatin 1 µg/mL, Leupeptin (1 µg/mL), NEM 2 mM and Iodoacetamida 10 mM and denatured with 4x Laemmli Sample Buffer at 95 °C for 5 min. Protein samples were separated on 12% SDS-PAGE and blotted for 1h to PVDF/nitrocellulose (pore size of 0.2 µm), using semi-dry transfer. Blot was blocked with 3% milk in TBS-T for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 (anti-ACO4) or 1:2000 (anti-flag, Agrisera, No. AS15 3036, Lot. 1902) in 3% milk in TBS-T ON/4°C with agitation (anti-ACO4 and anti-FLAG in corresponding membrane). The antibody solution was removed and the blot was washed three times for 10 min in TBS-T at RT with agitation. Blot was incubated with Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated AS09 602) or ECLTM Peroxidase labelled anti-mouse antibody) diluted to 1:25 000 for 1h/RT with agitation. The blot was washed as above and developed for 30 min with AgriseraECLSuperBright. Exposure time was 60 seconds.
Asterisk denotes a band recognized in all samples: both in negative control as in ACO4 samples, whereas the second band is exclusive of the ACO4 samples with the same MW as the ACO4 samples with FLAG-tag. The second ban may be an orthologus protein in *N. benthamiana*.

Courtesy of Diana Fuertes Bailón (L.Maria Lois Lab), CRAG, Barcelona, Spain