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Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS19 4318

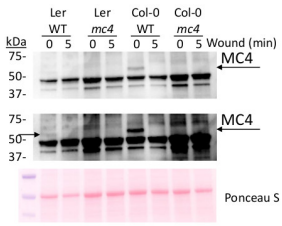
Anti-MC4 | Metacaspase-4

Product information

Immunogen	KLH-conjugated peptide derived from *Arabidopsis thaliana* MC4, UniProt: O64517, TAIR: At1g79340
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. 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	45.5 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Raphanus sativus, Noccaea caerulescens* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	Full-length MC4 (zMC4) disappears upon wounding. Accumulation of specific lower weight bands of active MC4 subunits, p20) is not detected with this antibody.
Selected references	To be added when available, antibody released in November 2020.



10 day old *Arabidopsis thaliana* seedlings, Ler wild type (WT), Col-0 (WT) and MC4 T-DNA insertion mutants Ler mc4 and Col-0 mc4. Grown on ½ MS medium (no addition of sugar). • Complete seedlings were grinded on liquid nitrogen and tissue powder was immediately mixed with denaturing Laemmli buffer preheated at 70 °C and further denatured at 70 °C for 10 min (= 0 minutes of wounding). Alternatively, tissue powder was kept at room temperature for 5 minutes and mixed with denaturing Laemmli buffer preheated at 70 °C and further denatured at 70 °C for 10 min (= 5 minutes of wounding). • An equal volume of leaf grinded on liquid nitrogen was mixed with denaturing 2x Laemmli buffer preheated at 70 °C and further denatured at 70 °C for 10 min. Samples were separated on 12 % SDS-PAGE (stainfree, BioRad) and blotted 7 minutes to PVDF (pore size of 20 um), using semi-dry (BioRad Turboblot) . Blot was blocked with 5% milk for 2h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 ON/4°C in PBS-T, 1% milk, with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed three times for 10 min in PBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (Goat anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:10 000 in PBS-T, 1% milk, for 2h/RT with agitation. The blot was washed as above and developed for 1 min with Agrisera ECL Superbright. Exposure time was 1-10 seconds.

Clear band at correct height of approximately 55 kDa in Col-0 at 0 minutes of wounding. After 5 minutes, MC4 autocatalytically cleaves itself to be activated, so the full length MC4 band at 55 kDa disappears.
This band is clearly absent in Col-0 mc4 which is a clear evidence for specific detection of MC4 in Col-0.

Courtesy Dr. Simon Stael,VIB-Ugent Center for Plant Systems Biology, Ghent University, Belgium