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Product no AS19 4257

TROL | Thylakoid rhodanese-like protein

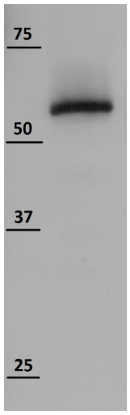
Product information

Immunogen	KLH-conjugated peptide derived from *Arabidopsis thaliana* TROL (thylakoid rhodanase-like protein) protein sequence, UniProt: Q9M158, TAIR: AT4G01050
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl, of sterile water
Storage	Lyophilized antibody can be stored 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 - 1 : 3000 (WB)
Expected | apparent MW	55-60 kDa
Confirmed reactivity	*Arabidopsis thaliana, Nicotiana tabacum, Pisum sativum*
Predicted reactivity	*Brassica rapa, Eutrema salsugineum* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	**TROL protein can be used as a chloroplast dual-localization marker:** for the thylakoids (the major portion of the protein) and the chloroplastic inner envelope. Since the envelopes make a very small portion of the total membranes, the envelope form of TROL is detectable only when isolated envelopes are applied. For AgriseraECLBright and Agrisera matching secondary antibodies goat anti-rabbit HRP conjugated, dilution 1: 25 000 1h/RT incubation (AS09 602), anti-TROL antibodies can be used in dilution of 1:3000. Recommendation: 2 µg chlorophyll/well. With increased protein or chlorophyll load/well background bands may be detected. Check here.
Selected references	Vojta and Fulgosi (2019). Topology of TROL protein in thylakoid membranes of Arabidopsis thaliana. Physiologia Plantarum, Special Issue on: “Photosynthesis”. DOI: 10.1111/ppl.12927

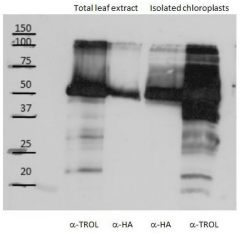
Application example



Western blot on *Arabidopsis thaliana* chloroplasts (2 µg of chlorophyll) using anti-TROL antibodies: Chloroplasts were isolated from 5 week old *Arabidopsis thaliana* by mixing and filtering through 1-layer Miracloth filter and 4-layers of gauze. Chloroplasts were isolated in 330 mM Sorbitol, 20 mM Tris/HCl pH 8.4, 5 mM EDTA, 10 mM Na_2CO_3, 0.1% BSA and washed twice in the same buffer and pelleted at 1000g for 5 min at 4°C.

Chlorophyll concentration was determined and sample corresponding to 2 µg chlorophyll was separated by 12 % SDS-PAGE. After wet Western transfer for 1h15min on nitrocellulose membrane (0.45µm WC), blot was blocked with 5% milk for 3 times for 10 min at RT with agitation. Blot was incubated in the anti-TROL primary antibody at a dilution of 1:2000 at 4°C/ON with agitation, in PBS buffer containing 1%Tween 20 and 5% milk. The antibody solution was decanted and the blot was rinsed briefly, then washed 3 times for 10 min in the same buffer at RT with agitation. Blot was incubated in anti-rabbit IgG horse radish peroxidase conjugate diluted to 1:50 000 in PBS buffer containing 1%Tween 20 and 5% milk for 1h/RT with agitation. The blot was washed 2 times for 10 min in PBS buffer containing 1% Tween 20 and developed using 1 ml 1.13 mM luminol (Alfa Aesar) solution in 0.1M Tris(HCl) pH 8.6, 100 µl 6.7 mM p-hidroxy coumaric acid (Sigma) in DMSO and 0.3 µl 35% H_2O_2 (Kemika). ECL results was analysed by 3-minute exposure on X-ray films.

Courtesy of Dr. Lea Vojta and Dr. Hrvoje Fulgosi, Laboratory for Molecular Plant Biology and Biotechnology, Division of Molecular Biology, Institute Ruđer Bošković,Croatia



Western blot on TROL-overexpressing *Arabidopsis thaliana* line using anti-TROL antibodies: TROL overexpression line was constructed by transformation of knock-out *Arabidopsis thaliana* plants by plasmid vector pH7WG2.0 containing TROL-HA-FLAG construct. Total leaf extract from *Arabidopsis thaliana* corresponding to 20 mg of wet leaf tissue and isolated chloroplasts corresponding to 25 µg of chlorophyll, were loaded on 15 % SDS-PAGE. Western transfer was performed for 1 hour at 200 mA (wet blot). Membrane was immunodecorated with anti-TROL (1:2000) and anti-HA (1:1000) primary antibodies overnight at 4°C. As secondary antibody, anti-rabbit IgG-HRP conjugate (1:50000) was used for anti-TROL and anti-rat IgG-HRP conjugate (1:30000) for anti-HA. Detection was performed using ECL and exposure on X-ray film.

Courtesy of Dr. Lea Vojta, Laboratory for Molecular Plant Biology and Biotechnology, Division of Molecular Biology, Institute Ruđer Bošković, Croatia