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Product no AS07 213

V-ATPase | Epsilon subunit of tonoplast H+ATPase

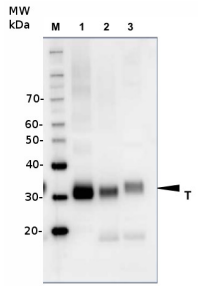
Product information

Immunogen	KLH-conjugated synthetic peptide chosen from subunit E of plant V-ATPase including *Arabidopsis thaliana* UniProt: Q39258-1, TAIR: At4g11150. Peptide is conserved in vacuolar H+-ATPase subunit E, isoform 1 to 3 (VHA-E1).
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
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.
Additional information	Cellular [compartment marker] of tonoplast membrane. This product can be sold containing ProClin if required.

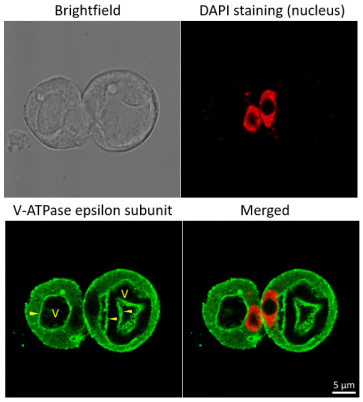
Application information

Recommended dilution	1: 100 (IF), 1 : 50 (IHC), 1 : 2000-1 : 5000 (WB)
Expected | apparent MW	26 | 31 kDa (*Arabidopsis thaliana*)
Confirmed reactivity	*Ananas comosus, Arabidopsis thaliana, Cucumis sativus, Chara australis , Chlamydomonas reinhardtii, Fortunella margarita* Swingle*, Hordeum vulgare, Lycopersicum esculentum, Lilium longiflorum, Malus x domestica Borkh.* c.v. Fuji*, Medicago truncatula, Mesembryanthemum crystallinum, Nicotiana tabacum, Noccaea caerulescens, Oryza sativa, Petunia hybrida* cv. Mitchell*, Populus* sp.*, Pteris vittata (fern), Thellungiella sp., Triticum aestivum, Zea mays, Vitis vinifera*
Predicted reactivity	*Brachypodium dystachyon, Capsella rubella, Chenopodium quinoa, Citrus clementina, Citrus unshiu, Citrus limon, Eucalypsus grandis, Glyxine max, Glycine soja, Lotus japonicus, Phaseolus sp., Physcomitrium patens, Populus trichocarpa, Prunus persica, Ricinus communis, Riticum aestivum, Solanum lycopersicum, Solanum tuberosum, Sorghum bicolor Theobroma cacao, Vitis vinifera,* Bull frog, Chicken, Bovine, *Drosophila melanogaster*, Human, Mouse, Rat Species of your interest not listed? Contact us
Not reactive in	*Avicennia* sp., mangrove plants, *Schizosaccharomyces pombe*
Additional information	V-ATPase is very sensitive for the redox of the SDS buffer. We recommend using at least 50-100 mM DTT freshly prepared before handling the sample. Immunostaining protocol using V-ATPase antibodies can be found here.
Selected references	Collins et al. (2020). EPSIN1 Modulates the Plasma Membrane Abundance of FLAGELLIN SENSING2 for Effective Immune Responses . Plant Physiol. 2020 Feb 24. pii: pp.01172.2019. doi: 10.1104/pp.19.01172 Lang et al. (2011).Simultaneous isolation of pure and intact chloroplasts and mitochondria from moss as the basis for sub-cellular proteomics. Plant Cell Rep. 2011 Feb;30(2):205-15.doi: 10.1007/s00299-010-0935-4.

Application example



10 µg of total protein from samples such as *Arabidopsis thaliana* leaf **(1)** , *Hordeum vulgare* leaf **(2)**, *Zea mays* leaf **(3)** were extracted with Protein Extraction Buffer PEB (AS08 300). Samples were diluted with 1X sample buffer (NuPAGE LDS sample buffer (Invitrogen) supplemented with 50 mM DTT and heat at 70°C for 5 min and keept on ice before loading. Protein samples were separated on 4-12% Bolt Plus gels, LDS-PAGE and blotted for 70 minutes to PVDF using tank transfer. Blots were blocked immediately following transfer in 2% blocking reagent or 5% non-fat milk dissolved in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 5 000 (in blocking reagent) for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, and then washed 1x15 min and 3x5 min with TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody AS09 602, Agrisera) diluted to 1:25 000 in blocking reagent for 1h at room temperature with agitation. The blots were washed as above. The blot was developed for 5 min with chemiluminescent detection reagent, according the manufacturers instructions. Images of the blots were obtained using a CCD imager (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 30 seconds.



Immunofluorescent localization of V-ATPase epsilon subunit of tonoplast H+ATPase in suspension culture of *Oryza sativa* ssp. japonica cv. 'Unggi 9', using goat anti-V-ATPase, epsilon subunit of tonoplast antibodies (AS07 213) and donkey anti-rabbit IgG, DyLight® 488 conjugated (AS10 1165, Agrisera). Vacuolar membrane, tonoplast, is highlighted by yellow arrowheads. DAPI staining of nuclei is pseudocolored red.

Method

Material: Suspension cultures of *Oryza sativa* ssp. japonica cv. 'Unggi 9
Fixation: Packed cell volume to fixer ratio: 250 µl : 5ml
Fixer composition and buffer: 4% (w/v) paraformaldehyde (freshly prepared as 8% stock and 0.2 µm filtered) 0.01% (v/v) Triton-X100 in Phosphate Buffered Saline (PBS), pH 7.4 (2x stock, 0.2 µm filtered)
Container and method: in 6 cm Petri dish, gentle shaking at room temperature (RT) Duration: 40 min
Hydrophilization: No
Cell wall digestion: Yes Packed cell volume to enzyme ratio: 100ul : 2ml Enzyme composition: 1% (A) 1.2% (R) Cellulase (chromatically purified, powder, Worthington) 1% (A) 1.2% (R) Pectinase (protease free, liquid, Sigma) Buffer: 0.5% (w/v) MES buffer, pH 5.6 Container and method: in 2 ml microfuge tube by rolling at room temperature (RT)
Duration: 60 min
Membrane permeabilization: Triton-X100 (0.35%) 7 mins/RT
Antigen retrieval: No
Blocking buffer: Fish gelatin (5% v/v) Washing buffer: PBS
Primary antibody dilution and incubation time: 1:300, ON/4°C
Secondary antibody: donkey anti-rabbit IgG, DyLight® 488 conjugated (AS10 1165, Agrisera), 1:600, 1h/RT
Co-staining of the nucleus (DAPI):
Cell wall and nucleus staining: 100 ng/ml DAPI

Courtesy of Dr. Ferhan Ayaydin, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary.