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Product no AS09 577A

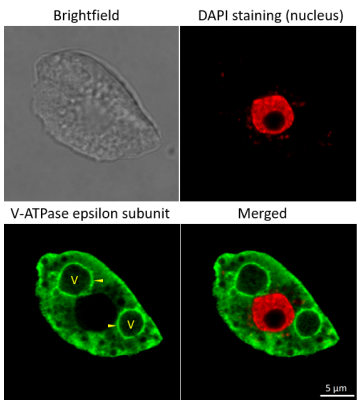
Anti-V-ATPase | Epsilon subunit of tonoplast H+ATPase (affinty purified, goat antibody)

Product information

Immunogen	KLH-conjugated synthetic peptide chosen from subunit E of plant V-ATPase including *Arabidopsis thaliana* At4g11150. Peptide is conserved in vacuolar H+-ATPase subunit E, isoform 1 to 3 (VHA-E1).
Host	Goat
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	200 µg
Reconstitution	For reconstitution add 100 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: 600 (IF), 1 : 1000-1 : 3000 (WB)
Expected | apparent MW	26 | 31 kDa (*Arabidopsis thaliana*)
Confirmed reactivity	*Arabidopsis thaliana, Avena strigosa, Medicago truncatula, Nicotiana tabacum, Oryza sativa, Solanum lycopersicum*
Predicted reactivity	Algae, *Chlamydomonas reinhardtii, Hordeum vulgare, Malus domestica, Mesembryanthemum sp., Petunia sp.,Phaseolus sp. , Physcomitrium patens, Pteris vittata (fern), Ricinus communis, Thellungiella sp., Zea mays, Vitis vinifera* Bull frog, Chicken, Bovine, D*rosophila melanogaster*, Human, Mouse, Rat Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
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. 2 hours incubation with primary antibody is recommended over over night incubation which can contribute to increased background.



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 (AS09 577A) and donkey anti-Goat IgG (H&L), DyLight® 488 conjugated secondary antibodies (AS10 1116, 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: 100 µl : 2 ml 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 min/RT
Antigen retrieval: No
Blocking buffer: Fish gelatin (5% v/v)
Washing buffer: PBS
Primary antibody dilution and incubation time: 1:600, 4ºC/ON
Secondary antibody: donkey anti-Goat IgG (H&L), DyLight® 488 conjugated secondary antibodies (AS10 1116, Agrisera), 1:600, 1h/RT
Co-staining of the nucleus (DAPI): Yes
Cell wall and nucleus staining: 100 ng/ml DAPI

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