

product **AS07 213-10**

V-ATPase | epsilon subunit of tonoplast H⁺ATPase (10 µl)

product information

background	Plant vacuole V-ATPase is responsible for energization of transport of ions and metabolites, and acts as well 'house-keeping' and as a stress response enzyme. V-ATPase is a multi-subunit enzyme composed of a membrane sector and a cytosolic catalytic sector. It is related to the FoF1 ATP synthase. Alternative protein names: Vacuolar proton pump subunit E, Protein EMBRYO DEFECTIVE 2448
immunogen	<u>KLH</u> -conjugated synthetic peptide chosen from subunit E of plant V-ATPase including <i>Arabidopsis thaliana</i> <u>At4g11150</u> . Peptide is conserved in vacuolar H ⁺ -ATPase subunit E, isoform 1 to 3 (VHA-E1).
antibody format	rabbit polyclonal serum lyophilized
quantity	10 µl for reconstitution add 10 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 tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.
tested applications	western blot, (WB) immunohistochemistry (IHC)
additional information	cellular [compartment marker] of tonoplast membrane

application information

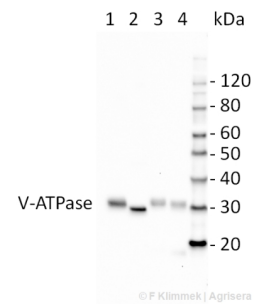
recommended dilution	1 : 2 000 - 1 : 5000 with alkaline phosphatase or ECL (WB), 1:50 (IHC)
expected apparent MW	26 31 kDa (<i>Arabidopsis thaliana</i>)
confirmed reactivity	<i>Ananas comosus</i> , <i>Arabidopsis thaliana</i> , <i>Chlamydomonas reinhardtii</i> , <i>Hordeum vulgare</i> , <i>Lycopersicon esculentum</i> , <i>Lilium longiflorum</i> , <i>Mesembryanthemum</i> sp., <i>Nicotiana tabacum</i> , <i>Petunia</i> sp., <i>Pteris vittata</i> (fern), <i>Thellungiella</i> sp., <i>Zea mays</i>
predicted reactivity	dicots including <i>Phaseolus</i> sp., <i>Ricinus communis</i> , <i>Vitis vinifera</i> and monocots including: <i>Oryza sativa</i> , <i>Zea mays</i> , algae, <i>Physcomitrella patens</i> , bull frog, chicken, bovine, <i>Drosophila melanogaster</i> , human, mouse, rat
not reactive in	no confirmed exceptions from predicted reactivity known in the moment
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

[Lang](#), E.G.E., S.J. Mueller, S.N.W. Hoernstein, J. Porankiewicz-Asplund, M. Vervliet-Scheebaum, R. Reski (2010). Simultaneous isolation of pure and intact chloroplasts and mitochondria from moss as basis for sub-cellular proteomics. *Plant Cell Reports*, DOI: 10.1007/s00299-010-0935-4. (open source). [Pertl](#) et al. (2009). The pollen organelle membrane proteome reveals highly spatial-temporal dynamics during germination and tube growth of lily pollen. *j. Proteome Res.* 11:5142.5152. [Hinton](#) et al. (2009). Function of a Subunit Isoforms of the V-ATPase in pH Homeostasis and in Vitro Invasion of MDA-MB231 Human Breast Cancer Cells. *J.Biol.Chem.* 24:16400-16408. [Reuveni](#) et al. (2001). Decrease in vacuolar pH during petunia flower opening is reflected in the activity of tonoplast H⁺-ATPase. *J. of Plant Physiol.* 158:991-998.

application example

10 µg of total leaf protein extracted with PEB (**AS08 300**) from (1) *Arabidopsis thaliana*, (3) *Zea mays*, and (4) *Hordeum vulgare* together with (2) cytosolic extract from *Arabidopsis thaliana* leaves were separated on **4-12% NuPage** (Invitrogen) **LDS-PAGE** and blotted 80 min (30V) to **nitrocellulose**. Filter was blocked 1h with **2% low-fat milk powder** in TBS-T (0.1% TWEEN 20) and probed with **anti-V-ATPase** (AS07 213, **1:2000**, 1h) and secondary anti-rabbit (**1:40000**, 1h) antibody (HRP conjugated, Abcam) in TBS-T containing 2% low fat milk powder. Antibody incubations were followed by washings in TBS-T (15, +5, +5, +5 min). All steps were performed at RT with agitation. Signal was detected with **SuperSignal West Pico** (Thermo Scientific) using a GenoPlex Chemi CCD (accumulated signal 10 x 30s exposure, bin 2x2).



application example

Standard IF protocol for plant material has been used including slight fixation with formaldehyde followed by washing and incubation with primary and secondary antibodies conjugated to fluorescent dyes. Green dye visualization of anti-V-ATPase antibody (Alexa 488 Molecular Probes), red – anti-tubulin antibody.

