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Product no AS07 260

Anti-H+ATPase | Plasma membrane H+ATPase (rabbit antibody)

Product information

Immunogen	KLH-conjugated synthetic peptide, derived from available di and monocot, fern, mosses and algal plasma membrane ATPase sequences including *Arabidopsis thaliana* ATPase 1 (UniProt: P20649, TAIR: At2g18960) and ATPase 2 (UniProt: P19456 , TAIR: At4g30190), 3 (UniProt: P20431, TAIR: At5g57350), 4 (UniProt: Q9SU58, TAIR: At3g47950), 6 (UniProt: Q9SH76, TAIR: At2g07560), 7 (UniProt: Q9LY32, TAIR: At3g60330), 8 (UniProt: Q9M2A0, TAIR: At3g42640), 9 (UniProt: Q42556, TAIR: At1g80660), 11 (UniProt: Q9LV11, TAIR: At5g62670) of *Arabidopsis thaliana* and hydrogen ATPase of *Chlamydomonas reinhardtii* (Q9FNS3)
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. Do not Store this antibody in 4°C.
Additional information	Cellular [compartment marker] for plasma membrane

Application information

Recommended dilution	1 : 600-1 : 1000 (IF), 1 : 100 (IL), 1 : 1000-1 : 10 000 (WB)
Expected | apparent MW	90-95 kDa (*Arabidopsis thaliana,* depending upon an isoform)
Confirmed reactivity	*Actinidia chinensis, Aesculus hippocastanum*, *Arabidopsis thaliana*, *Camellia sinensis* cv. Shu-chazao, *Chara australis* R.Br, *Chlamydmonas reinhardtii, Cucumis sativus, Cucurbita moschata, Glycine max, Kandelia obovata, Hordeum vulgare, Lolium perenne, Lycopersicon esculentum, Malus x domestica Borkh.* c.v. Fuji, *Marchantia polymorpha, Medicago truncatula, Nicotiana benthamiana, Nicotiana tabacum, Noccaea caerulescens, Oryza sativa, Petunia hybrida, Phalenopsis Sogo Yukidian cultivar V3, Physcomitrium patens, Picea abies, Pisum sativum, Populus tremula, Pteris vittata* (fern), *Ricinus communis, Salicornia bigelovii, Spinacia oleracea, Solanum lycopersicum, Tagetes erecta, Tetraselmis chuii, Vicia faba, Zea mays*
Predicted reactivity	*Amaranthus hypochondriacus,Avena sativa,Beta vulgaris, Cyanidioschyzon merolae, Dunaliella spp., Galdieria sulphuraria, Gossypium hirsutum, Hordeum vulgare, Ostreococcus spp., Pinus thunbergii, Physocomitrella patens, Mesembruanthemum crystallinum, Mortierella elongata, Nannochloropsis gaditana* CCMP526, *Ostreococcus tauri, Prosopis alba, Rosa chinensis Jacq., Saccharomyces cerevisiae, Solanum tuberosum, Sorghum bicolor, Spinacia oleracea, Triticum aestivum, Ulva prolifera, Ustilago maydis* Species of your interest not listed? Contact us
Not reactive in	*Allium sp., Aspergillus niger, Citrus limon, Colobanthus apetala, Cuminium cyminum, Curcuma amada, Deschampsia antractica, Lupinus luteus, Morinda citrifolia, Trigonella foenum, Vicia faba*
Additional information	**VERY IMPORTANT: Please, do not heat up your samples above 70°C as this may cause H+ATPase to precipitate, and there will be no signal on your Western Blot.** Before SDS-PAGE, centrifuge your samples at room temperature at 10 000 rpm/1 min to remove any aggregates. H+ATPase will be less abundant in mature roots and leafs and therefore detection may require use of very sensitive reagents. This product can be sold containing ProClin if requested.
Selected references	Salazar et al. (2024). SOS1 tonoplast neo-localization and the RGG protein SALTY are important in the extreme salinity tolerance of Salicornia bigelovii. Nat Commun. 2024 May 20;15(1):4279.doi: 10.1038/s41467-024-48595-5. Mosesso et al. (2024). Arabidopsis CaLB1 undergoes phase separation with the ESCRT protein ALIX and modulates autophagosome maturation. Nat Commun. 2024 Jun 19;15(1):5188. doi: 10.1038/s41467-024-49485-6. Li et al. (2024). An NLR paralog Pit2 generated from tandem duplication of Pit1 fine-tunes Pit1 localization and function. Nat Commun. 2024 May 30;15(1):4610. doi: 10.1038/s41467-024-48943-5.

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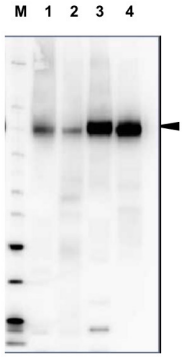
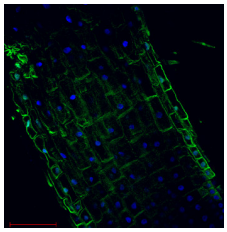


20 µg of total protein from *Arabidopsis thaliana* **(1)**, *Hordeum vulgare* (2), *Zea mays* (3), *Nicotiana tabaccum* plasma membrane fraction, 2.5 µg **(4)**, extracted with **P**rotein **E**xtration **B**uffer, PEB (**AS08 300**, homogenate the tissue with 3 to 5 volumes of the homogenizing buffer), were denaturated for 10 min. in 70°C and separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Blots were blocked immediately following transfer in blocking reagent 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 for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in 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) diluted to 1:20 000 in 2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with chemiluminescence detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad). Exposure time was 2 min.

Immunolocalization



Plasma membrane H+ATPase localization in*Arabidopsis thaliana* roots**.**
Arabidopsis thaliana, elongation zone, H+ATPase (green). *Arabidopsis thaliana* roots were fixed in para-formaldehyde for 30 minutes. Tissue cleaning has been performed before immunolocalization. Anti-rabbit H+ATPase | plasma membrane primary antibody diluted in 1: 300 and anti-rabbit IgG secondary antibody conjugated with Alexa 555. Co-staining with DAPI visualized nucleus (blue color). Scale bar – 100 µm.

Courtesy Dr. Taras Pasternak, Freiburg University, Germany