Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

product AS07 260

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

product information

Background	The Plasma Membrane **H+ATPase** is a family of proteins of ca. 100 kDa that are believed to be exclusive to the plasma membranes of plants and fungi. The protein is anchored within biological membrane which creates an electrochemical gradient used as an energy source and is essential for uptake of most metabolites and plant responses to environment, for example movement of leaves.
Immunogen	KLH-conjugated synthetic peptide exposed to cytoplasm in H+ATPase model, 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	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 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. Do not Store this antibody in 4°C.
Tested applications	Immunofluorescence (IF), Immunolocalization (IL), Western blot (WB)
Related products	AS07 213 | Anti-V-ATPase (vacuolar marker), rabbit antibodies AS07 260PRE | H+ATPase | plasma membrane H+ATPase, pre-immune serum AS07 260P | H+ATPase plasma membrane H+ATPase peptide, control for immunolocalization studies AS13 2671 | Anti-H+ATPase plasma membrane H+ATPase, chicken antibodies Antibodies to membrane transport system Recommended secondary antibody
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	*Aesculus hippocastanum*, *Arabidopsis thaliana, Camellia sinensis* cv. Shu-chazao*, Chara australis* R.Br, *Chlamydmonas reinhardtii, Cucumis sativus, Cucurbita moschata, Glycine max* (weak)*, 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, Physcomitrella patens, Picea abies, Populus tremula, Pteris vittata* (fern)*, Ricinus communis, Spinacia oleracea, Zea mays, Vicia faba*
Predicted reactivity	Algae*, Avena sativa, Dunaliella spp., Gossypium hirsutum, Hordeum vulgare, Ostreococcus spp., Pinus thunbergii, Physocomitrella patens, Mesembruanthemum crystallinum, Mortierella elongata, Ostreococcus tauri, Saccharomyces cerevisiae, Solanum tuberosum, Ulva prolifera*
Not reactive in	*Aspergillus niger*

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Additional information

VERY IMPORTANT: please, do not heat up your samples over 70°C as this might cause H+ATPase to precipitate and there will be no signal on your Western Blot.

H+ATPase will be less abundant in mature roots and leafs.

This product can be sold containing ProClin if requested.

Selected references

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Lomin et al. (2017). Studies of cytokinin receptor–phosphotransmitter interaction provide evidences for the initiation of cytokinin signalling in the endoplasmic reticulum. Functional Plant Biology, CSIRO Publications. (*Nicotiana benthamiana*, western blot)
Kovaleva et al. (2017). Regulation of Petunia Pollen Tube Growth by Phytohormones: Identification of Their Potential Targets. DOI:10.17265/2161-6256/2016.04.004. (immunolocalization)
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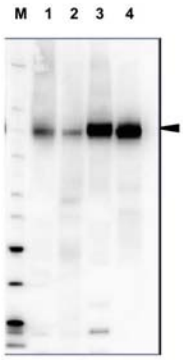
Application example



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**), were boiled 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

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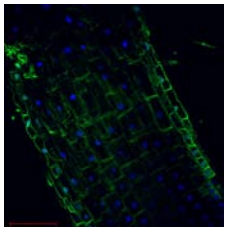
washed once for 15 min and 3 times for 5 min in TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody AS09 602) diluted to 1:20 000 in 2% blocking solution for 1h at room temperature with 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