

product **AS07 260**

H⁺ATPase | plasma membrane H⁺ATPase

product information

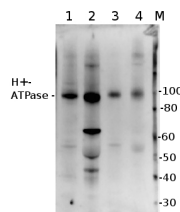
background	The Plasma Membrane H⁺ATPase is a family of proteins of ca. 100 kDa that are believed to be exclusive to the plasma membranes of plants and fungi. The protein is anchored within biological membrane which creates an electrochemical gradient used as an energy source and is essential for uptake of most metabolites and plant responses to environment, for example movement of leaves.
immunogen	<u>KLH</u> -conjugated synthetic peptide derived from available di and monocot, fern, mosses and algal plasma membrane ATPase sequences including <i>Arabidopsis thaliana</i> ATPase 1 (<u>At2g18960</u>) and ATPase 2,3,4,6,7,8,9 of <i>Arabidopsis thaliana</i> and hydrogen ATPase of <i>Chlamydomonas reinhardtii</i> (<u>Q9FNS3</u>)
antibody format	rabbit polyclonal serum lyophilized
quantity	200 µl for reconstitution add 200 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), immunofluorescence (IF)
additional information	cellular [compartment marker] for plasma membrane

application information

recommended dilution	1: 1000 - 1: 5000 with standard ECL OR 1: 10 000 with ECL-Advance, enhanced chemiluminescence (WB), 1: 600 - 1: 1000 (IF)
expected apparent MW	95 kDa (<i>Arabidopsis thaliana</i>)
confirmed reactivity	dicots including: <i>Arabidopsis thaliana</i> , <i>Cucurbita moschata</i> , <i>Glycine max</i> (weak), <i>Lycopersicon esculentum</i> , <i>Nicotiana tabacum</i> , <i>Ricinus communis</i> , <i>Spinacia oleracea</i> , monocots including: <i>Hordeum vulgare</i> , <i>Zea mays</i> , trees: <i>Populus tremula</i> , <i>Pteris vittata</i> (fern), algae: <i>Chlamydomonas reinhardtii</i> ,
predicted reactivity	dicots (including <i>Nicotiana tabacum</i> , <i>Solanum tuberosum</i> , <i>Mesembrianthemum crystallinum</i>); monocots (including

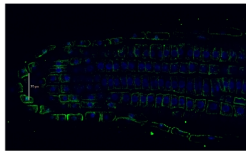
not reactive in	<p><i>Avena sativa</i>, <i>Hordeum vulgare</i>, <i>Oryza sativa</i>, conifers (<i>Pinus thunbergii</i>), mosses (<i>Physcomitrella patens</i>), algae (<i>Dunaliella</i> spp., <i>Ostreococcus</i> spp.), <i>Saccharomyces cerevisiae</i></p>
additional information	<p>no confirmed exceptions from predicted reactivity known in the moment</p> <p>for additional Western blot detection image please refer to the article below</p>
selected references	<p>Wulfetange et al. (2011). The Cytokinin Receptors of <i>Arabidopsis thaliana</i> are Locating Mainly to the Endoplasmic Reticulum. <i>Plant Physiol.</i> (in press).</p> <p>Sánchez-Nieto et al. (2011). Kinetics of the H⁺-ATPase from Dry and 5-Hours-Imbibed Maize Embryos in Its Native, Solubilized, and Reconstituted Forms. <i>Mol. Plant.</i></p> <p>Mitani et al. (2011). Isolation and functional characterization of an influx silicon transporter in two pumpkin cultivars contrasting in silicon accumulation. <i>Plant J.</i> 66(2):231-240.</p>

application example



5 µg of total protein from (1) *Zea mays* whole cell, extracted with **Protein Extraction Buffer**, PEB ([AS08 300](#)), (2) *Hordeum vulgare* leaf extracted with PEB, (3) *Spinacia oleracea* total cell extracted with PEB, (4) *Arabidopsis thaliana* were separated on **4-12% NuPage** (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Blots were blocked immediately following transfer in 2% ECL Advance blocking reagent (GE Healthcare) 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: 10 000 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 1:50 000 in 2% ECL Advance blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with ECL Advance detection reagent according to the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

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. Co-staining with DAPI visualized nucleus (blue color). Scale bar – 50 μ m.

Courtesy Dr. Taras Pasternak, Freiburg University