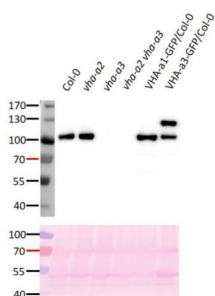


Product no **AS20 4369****Anti-V-ATPase, a3 | vacuolar H<sup>+</sup>-ATPase subunit a isoform 3****Product information**

<b>Immunogen</b>	KLH-conjugated synthetic peptide derived from <i>Arabidopsis thaliana</i> V-ATPase subunit a3, UniProt: <a href="#">Q8W4S4-1</a> , TAIR: <a href="#">At4g39080</a>
<b>Host</b>	Rabbit
<b>Clonality</b>	Polyclonal
<b>Purity</b>	Immunogen affinity purified serum in PBS pH 7.4.
<b>Format</b>	Lyophilized
<b>Quantity</b>	50 µg
<b>Reconstitution</b>	For reconstitution add 50 µl of sterile water
<b>Storage</b>	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**

<b>Recommended dilution</b>	1 : 1000 (WB)
<b>Expected   apparent MW</b>	92.8   >100 kDa ( <i>Arabidopsis thaliana</i> )
<b>Confirmed reactivity</b>	<i>Arabidopsis thaliana</i>
<b>Predicted reactivity</b>	<i>Brassica campestris</i> , <i>Brassica oleracea</i> , <i>Brassica rapa</i> , <i>Capsella rubella</i> , <i>Eutrema salsugineu</i> , <i>Noccaea caerulescens</i> Species of your interest not listed? <a href="#">Contact us</a>
<b>Not reactive in</b>	No confirmed exceptions from predicted reactivity are currently known
<b>Additional information</b>	

**Application example**

30 µg of microsomal membranes were extracted freshly from 5-day-old *Arabidopsis thaliana* seedlings grown in liquid culture with a buffer containing 350mM sucrose, 70mM Tris-HCl pH8, 10% glycerol, 3mM Na<sub>2</sub>EDTA, 0.15% BSA, 1.5% PVP-40, 4mM DTT, 1x Roche complete<sup>TM</sup> Protease Inhibitor and denatured with 1x Laemmli buffer at 50°C for 10 min. Proteins were separated on 7.5 % SDS-PAGE and blotted 1h to PVDF (pore size of 0.2 µm) using wet transfer. Blot was blocked with 3% milk for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 in 2%BSA in TBS-T (0.05%) for ON/4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly with TBS-T, then washed twice for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:25 000 in 3% milk in TBS-T for 2h/RT with agitation. The blot was washed as above and developed for 2 min with chemiluminescent detection reagent. Exposure time was 100 seconds.

**expected band sizes:** 1 - marker as indicated in the picture 2 - endogenous VHA-a3 at ~93kDa 3 - endogenous VHA-a3 at ~93kDa 4 - no band since it is the mutant (It also tells us that the AB is not recognizing VHA-a2 which is very similar to VHA-a3) 5 - no band since it is the mutant (It also tells us that the AB is not recognizing VHA-a2 which is very similar to VHA-a3) 6 - endogenous VHA-a3 at ~93kDa (It also tells us that the AB is not recognizing VHA-a1(-GFP) which is similar to VHA-a3) 7 - endogenous VHA-a3 at ~93kDa and VHA-a3-GFP at ~120kDa

Courtesy of Upendo Lupanga, Centre of Organismal Studies, University of Heidelberg, Germany