

Product no **AS07 212****VDAC1-5 | Voltage-dependent anion-selective channel protein 1-5****Product information**

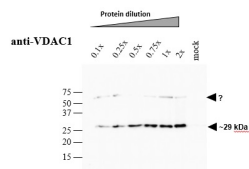
<b>Immunogen</b>	KLH-conjugated peptide conserved in all known higher plant VDAC proteins including <i>Arabidopsis thaliana</i> VDAC1 UniProt: <a href="#">Q9SRH5</a> , TAIR: <a href="#">AT3G01280</a> , VDAC2 UniProt: <a href="#">F4K3R8-1</a> , TAIR: <a href="#">AT5G67500</a> , VDAC3 UniProt: <a href="#">Q9SMX3-1</a> , TAIR: <a href="#">AT5G15090</a> , VDAC4 UniProt: <a href="#">Q9FKM2-1</a> , TAIR: <a href="#">AT5G57490</a> , VDAC5 UniProt: <a href="#">Q9M2W6-1</a> , TAIR: <a href="#">AT3G49920</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.

**Additional information** Cellular [compartment marker] of mitochondrial outer membrane for western blot,**Application information**

<b>Recommended dilution</b>	1 : 500 (IL), 1 : 5000, 2-30 µg protein/lane (WB)
<b>Expected   apparent MW</b>	29 kDa (for <i>Arabidopsis thaliana</i> )
<b>Confirmed reactivity</b>	<i>Arabidopsis thaliana</i> , <i>Amaranthus palmeri</i> , <i>Beta vulgaris</i> , <i>Brassica oleracea</i> var. botrytis, <i>Brassica rapa</i> subsp. <i>rapa</i> , <i>Citrus sinensis</i> , <i>Fortunella margarita</i> Swingle, <i>Oryza sativa</i> , <i>Papaver</i> sp. pollen tubes (IL), <i>Spinacia oleracea</i> , <i>Physcomitrium patens</i> , <i>Zea mays</i>
<b>Predicted reactivity</b>	<i>Arabidopsis alpina</i> , <i>Aundo donax</i> , <i>Brachypodium distachyon</i> , <i>Brassica campestris</i> , <i>Brassica napus</i> , <i>Brassica rapa</i> subsp. <i>pekinensis</i> , <i>Capsella rubella</i> , <i>Citrus clementina</i> , <i>Eutrema salsugineum</i> , <i>Glycine max</i> , <i>Glycine soja</i> , <i>Gossypium arboreum</i> , <i>Hoedum vulgare</i> var. <i>distichum</i> , <i>Jatropha curcas</i> , <i>Medicago truncatula</i> , <i>Mesembryanthemum crystallinum</i> , <i>Morus notabilis</i> , <i>Nicotiana tabacum</i> , <i>Phaseolus coccineus</i> , <i>Phaseolus vulgaris</i> , <i>Pisum sativum</i> , <i>Plantago major</i> , <i>Prunus persica</i> , <i>Ricinus communis</i> , <i>Solanum lycopersicum</i> , <i>Solanum tuberosum</i> , <i>Sorghum bicolor</i> , <i>Theobroma cacao</i> , <i>Triticum aestivum</i> , <i>Vitis vinifera</i>
	Species of your interest not listed? <a href="#">Contact us</a>
<b>Not reactive in</b>	<i>Chlamydomonas reinhardtii</i> , <i>Glycine max</i> , diatoms, <i>Saccharomyces cerevisiae</i>
<b>Additional information</b>	Amount of mitochondrial fraction detected by anti-VDAC1 antibody was from 2-10 µg.  Immunolocalization method description and images are available <a href="#">here</a> Blue-native (2D BN/SDS-PAGE) methodology is described in Piechota et al. 2010
<b>Selected references</b>	<a href="#">Wittmann</a> et al. (2024). Dual plastid targeting of Protoporphyrinogen Oxidase 2 in Amaranthaceae promotes herbicide tolerance. <i>Plant Physiol.</i> 2024 Feb 8; kiae062. doi: 10.1093/plphys/kiae062. <a href="#">Bao</a> et al. (2023). Aberrant accumulation of ceramides in mitochondria trigger cell death requiring autophagy in <i>Arabidopsis</i> . <i>J Exp Bot.</i> 2023 Dec 9; erad456. doi: 10.1093/jxb/erad456. <a href="#">Xue</a> et al. (2023). The PtdIns3P phosphatase MtMP promotes symbiotic nitrogen fixation via mitophagy in <i>Medicago truncatula</i> . <i>iScience.</i> 2023 Sep 15; 26(10):107752. doi: 10.1016/j.isci.2023.107752. <a href="#">Belykh</a> et al. (2022) Responses of genes of DNA repair, alternative oxidase, and pro-/antioxidant state in <i>Arabidopsis thaliana</i> with altered expression of AOX1a to gamma irradiation. <i>Int J Radiat Biol.</i> 2022;98(1):60-68. doi: 10.1080/09553002.2022.1998712. Epub 2021 Nov 11. PMID: 34714725. <a href="#">Li</a> et al. (2021) Isolation and comparative proteomic analysis of mitochondria from the pulp of ripening citrus fruit. <i>Hortic Res.</i> 2021 Feb 1; 8(1):31. doi: 10.1038/s41438-021-00470-w. PMID: 33518707; PMCID: PMC7848011. <a href="#">Tarasenko</a> et al. (2020). Plant mitochondrial subfractions have different ability to import DNA. <i>Theor. Exp. Plant Physiol.</i> doi.org/10.1007/s40626-020-00167-w <a href="#">Garmash</a> et al. (2020). Altered levels of AOX1a expression result in changes in metabolic pathways in <i>Arabidopsis thaliana</i> plants acclimated to low dose rates of ultraviolet B radiation. <i>Plant Sci.</i> 2020 Feb; 291:110332. doi: 10.1016/j.plantsci.2019.110332. <a href="#">Bai</a> et al. (2019). Overexpression of soybean GmPLD? enhances seed oil content and modulates fatty acid composition in transgenic <i>Arabidopsis</i> . <i>Plant Science</i> Volume 290, January 2020, 110298.

**Lang** et al. (2011). Simultaneous isolation of pure and intact chloroplasts and mitochondria from moss as the basis for sub-cellular proteomics. *Plant Cell Rep.* 2011 Feb;30(2):205-15. doi: 10.1007/s00299-010-0935-4.

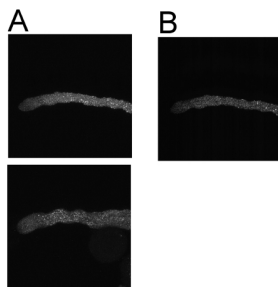
## Application example



Crude membrane proteins were separated on 12% SDS-PAGE and blotted 1h to PVDF. Blots were blocked immediately following transfer in 5% blocking reagent (BioRad, 170-6404) in 50 mM Tris, 150 mM sodium chloride pH 7.5 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody in 1: 5000 dilution for over-night at 4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 5 min in TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (Goat anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:5000 in 0.2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 1~2 min with chemiluminescent detection reagent, according the manufacturers instructions. Images of the blots were obtained using a CCD imager (LAS4000 GE) and by ImageQuant software (GE).

*Arabidopsis thaliana* membrane extraction and SDS-PAGE analysis About 200 mg (gFW) *Arabidopsis* seedlings (3-week-old), grown on 1% MS-agar plates, was ground with mortar and pestle in the presence of 2 ml extraction buffer [75 mM MOPS-KOH, 0.6 M Sucrose, 4 mM EDTA, 0.2% PVP-40, 0.2% BSA, 8 mM L-cystein, pH 7.6] and the protease inhibitor cocktail 'complete Mini' from Roche Diagnostics GmbH (Mannheim, Germany). Crude membrane extracts were prepared essentially as described in Colas des Francs-Small et al. (2012). The membranous fraction was obtained by centrifugation at 22,000 g for 10 min at 4°C. The pellet containing the crude membranous fraction was washed twice with wash buffer [37.5 mM MOPS-KOH, 0.3 M Sucrose, 2 mM EDTA pH 7.6]. The samples were kept frozen at -80°C until used. For SDS-PAGE, an aliquot equivalent to 10 mg (i.e. 1x dilution) of crude *Arabidopsis* membrane extracts was solubilized in 3x Laemmli sample buffer (Bio-Rad) and the proteins were analyzed by SDS-gel electrophoresis

Courtesy of Dr. Oren Ostersetzter, The Hebrew University of Jerusalem, Israel



### Fixation and Immunolocalization

**(A)** full confocal stacks; **(B)** Single confocal section

Pollen tubes were fixed in 400  $\mu$ M 3-maleimodobenzoic acid N-hydroxysuccinimide ester (MBS, Pierce) for 6 min at 20°C, followed by 2% formaldehyde (1 h, 4°C). Cells were washed three times in 1x TBS then once in MES buffer (15 mM MES, pH 5.0), then incubated in 0.05% cellulose/0.05% macerozyme with 0.1% Triton X-100 in MES buffer containing 0.1 mM PMSF and 1 % BSA for 15 min. Cells were washed once in MES, then twice in TBS and then incubated in blocking solution (1% BSA in TBS) for 30 min at room temperature. Pollen was incubated with anti-VDAC1 antibodyies diluted in blocking solution (at 1:500) overnight at 4°C. Following TBS washes pollen was then incubated with the secondary antibody for 1.5 h at room temperature followed by further TBS washes. Pollen tubes were mounted on slides with 5  $\mu$ L of Vectashield + DAPI (Vector Laboratories, USA) and coverslips sealed with nail varnish.

Method taken from Poulter et al (submitted) Actin-binding proteins implicated in formation of the punctate actin foci stimulated by the self-incompatibility response in *Papaver*. Submitted to *Plant Physiology*.

Courtesy Professor Noni Franklin-Tong, University of Birmingham, UK