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

Product information

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

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

Application information

Recommended dilution	1 : 500 (IL), 1 : 5000, 2-30 μg protein/lane (WB)
Expected apparent MW	29 kDa (for Arabidopsis thaliana)
Confirmed reactivity	Arabidopsis thaliana, Amaranthus palmeri, Beta vulgaris, Brassica oleracea var. botrytis, Brassica rapa subsp. rapa, Citrus sinensis, Fortunella margarita Swingle, Oryza sativa, Papaver sp. pollen tubes (IL), Spinacia oleracea, Physcomitrium patens, Zea mays
Predicted reactivity	Arabidopsis alpina, Aundo donax, Brachypodium distachyon, Brassica campestris, Brassica napus, Brassica rapa subsp. pekinensis, Capsella rubella, Citrus clementina, Eutrema salsugineum, Glycine max, Glycine soja, Gossypium arboreum, Hoedum vulgare var. distichum, Jatropha curcas, Medicago truncatula, Mesembryanthemum crystallinum, Morus notabilis, Nicotiana tabacum, Phaseolus coccineus, Phaseolus vulgaris, Pisum sativum, Plantago major, Prunus persica, Ricinus communis, Solanum lycopersicum, Solanum tuberosum, Sorghum bicolor, Theobroma cacao, Triticum aestivum, Vitis vinifera
Not reactive in	Chlamydomonas rainhardtii. Glucina may diatoms. Saccharomyces cerevisiae
Additional information	Amount of mitochondrial fraction detected by anti-VDAC1 antibody was from 2-10 up
	Immunolocalization method description and images are available <u>here</u> Blue-native (2D BN/SDS-PAGE) methodology is described in Piechota et al. 2010
Selected references	 Boussardon et al. (2025). The atypical proteome of mitochondria from mature pollen grains. Curr Biol . 2025 Jan 21:S0960-9822(24)01705-6. doi: 10.1016/j.cub.2024.12.037. Soria et al. (2024). Functional resilience: An active oxidative phosphorylation system prevails amid foreign proteins in holoparasitic plants. Current Plant Biology Volume 37, March 2024, 100322. Wittmann at al. (2024). Dual plastid targeting of Protoporphyrinogen Oxidase 2 in Amaranthaceae promotes herbicide tolerance. Plant Physiol. 2024 Feb 8:kiae062.doi: 10.1093/plphys/kiae062. Bao et al. (2023). Aberrant accumulation of ceramides in mitochondria trigger cell death requiring autophagy in Arabidopsis. J Exp Bot . 2023 Dec 9:erad456.doi: 10.1093/plphys/kiae065. Xue et al.(2023). The PtdIns3P phosphatase MtMP promotes symbiotic nitrogen fixation via mitophagy in Medicago truncatula. iScience. 2023 Sep 15;26(10):107752.doi: 10.1016/j.isci.2023.107752. Belykh et al. (2022). Responses of genes of DNA repair, alternative oxidase, and pro-/antioxidant state in Arabidopsis thaliana with altered expression of AOX1a to gamma irradiation. Int J Radiat Biol. 2022;98(1):60-68. doi: 10.1080/09553002.2022.1998712. Epub 2021 Nov 11. PMID: 34714725. Li et al. (2021) Isolation and comparative proteomic analysis of mitochondria from the pulp of ripening citrus fruit. Hortic Res. 2021 Feb 1;8(1):31. doi: 10.1038/s41438-021-00470-w. PMID: 33518707; PMCID: PMC7848011. Tarasenko et al. (2020). Plant mitochondrial subfractions have different ability to import DNA. Theor. Exp. Plant Physiol. doi.org/10.1007/s40626-020-00167-w Garmash et al. (2020). Altered levels of AOX1a expression result in changes in metabolic pathways in Arabidopsis



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Bai et al. (2019). Overexpression of soybean GmPLD? enhances seed oil content and modulates fatty acid composition in transgenic Arabidopsis. Plant Science 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 4oC. 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 -80oC 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 Ostersetzer, The Hebrew University of Jerusalem, Israel



Fixation and Immunolocalization

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

Pollen tubes were fixed in 400 μM 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 μL 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.