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Product no AS07 212

VDAC1-5 | Voltage-dependent anion-selective channel protein 1-5

Product information

Background	**VDAC** proteins are porin-type, beta-barrel diffusion pores. Prominently localized in the outer mitochondrial membrane and involved in metabolite exchange.
Immunogen	KLH-conjugated peptide conserved in all known higher plant VDAC proteins including *Arabidopsis thaliana* VDAC1 UniProt: Q9SRH5 , TAIR: AT3G01280, VDAC2 UniProt F4K3R8-1 , TAIR: AT5G67500, VDAC3 UniProt: Q9SMX3-1, TAIR: AT5G15090, VDAC4 UniProt: Q9FKM2-1, TAIR:AT5G57490, VDAC5 UniProt: Q9M2W6-1, TAIR: AT3G49920
Host	Rabbit
Clonality	Polyclonal
Purity	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 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	Blue-native PAGE (2D BN/SDS-PAGE), Western blot (WB)
Related products	AS07 212-ALP | Anti-VDAC1-5 | Voltage-dependent anion-selective channel protein 1, ALP-conjugated (40 µg) AS07 212-HRP | Anti-VDAC1-5 | Voltage-dependent anion-selective channel protein 1, HRP-conjugated (40 µg) AS04 054 | Anti-AOX1/2 rabbit antibody, marker of mitochondrial inner membrane AS06 203A | Anti-Idh rabbit antibody, marker of mitochondrial matrix collection of antibodies to other mitochondrial proteins
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, Beta vulgaris, Brassica oleracea* var. botrytis, *Brassica rapa* subsp. *rapa, Citrus sinensis, Fortunella margarita* Swingle, *Oryza sativa, Papaver* sp. pollen tubes (IL), *Spinacia oleracea*, *Physcomitrella patens*
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, Zea mays* Species of your interest not listed? Contact us
Not reactive in	*Chlamydomonas reinhardtii, Glycine max, Zea mays*, diatoms, *Saccharomyces cerevisiae*

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Additional information

Amount of mitochondrial fraction detected by anti-VDAC1 antibody was from 2-10 µg.

Immunolocalization method description and images are available here
Blue-native (2D BN/SDS-PAGE) methodology is described in Piechota et al. 2010
For high resolution images, please visit the specific product page at www.agrisera.com

Selected references

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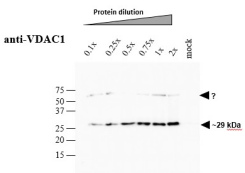
For high resolution images, please visit the specific product page at www.agrisera.com

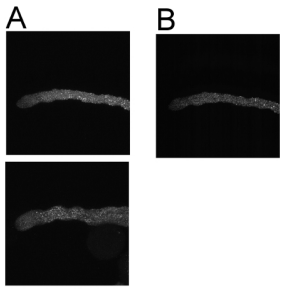
Application example



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 20ºC, 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.

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