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Product no AS06 203A

IDH | Isocitrate dehydrogenase (Cellular [compartment marker] of mitochondrial matrix)

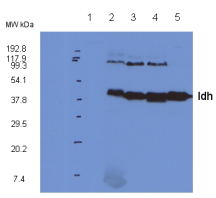
Product information

Immunogen	KLH-conjugated peptide 1 and peptide 2 conserved in all higher plants mitochondrial, NAD dependent isocitrate dehydrogenase subunits including *Arabidopsis thaliana* IDH-I Q8LFC0, At4g35260 and IDH-II P93032, At2g17130
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	Peptide used to elicit this antibody is not conserved in NADPH dependent anzymes, partially conserved across eukaryotic Idh subunits, Some conservation across bacterial which contain the NAD-dependent form of Idh (as opposed to the NADP-dependent form)

Application information

Recommended dilution	1:400 (IF), 1 : 5 000 (WB)
Expected | apparent MW	39 | 45 kDa (*Arabidopsis thaliana*)
Confirmed reactivity	*Arabidopsis thaliana, Brassica oleracea, Capsicum annuum, Lycopersicum chilense, Nicotiana benthamiana, Oryza sativa, Solanum lycopersicum, Pisum sativum, Solanum sogarandium, Solanum tuberosum, Zea mays*
Predicted reactivity	*Brachypodium distachyon, Brassica napus, Capsella rubella, Citrus sinensis, Glycine max, Hordeum vulgare, Malus x domestica, Medicago truncatula, Nicotiana tabacum, Phaseolus vulgaris, Theobroma cacao, Triticum aestivum, Vitis vinifera, Zea mays* Species of your interest not listed? Contact us
Not reactive in	*Chlamydomonas reinhardtii*
Additional information	Cellular [compartment marker] of mitochondrial matrix
Selected references	Kolodziejczak et al. (2018). m-AAA Complexes Are Not Crucial for the Survival of Arabidopsis Under Optimal Growth Conditions Despite Their Importance for Mitochondrial Translation. Plant Cell Physiol. 2018 May 1;59(5):1006-1016. doi: 10.1093/pcp/pcy041. Rurek et al. (2018). Mitochondrial Biogenesis in Diverse Cauliflower Cultivars under Mild and Severe Drought Involves Impaired Coordination of Transcriptomic and Proteomic Response and Regulation of Various Multifunctional Proteins. Preprints 2018, 2018010276 (doi: 10.20944/preprints201801.0276.v1). Fujii et al. (2016). The Restorer-of-fertility-like 2 pentatricopeptide repeat protein and RNase P are required for the processing of mitochondrial orf291 RNA in Arabidopsis. Plant J. 2016 Jun;86(6):504-13. doi: 10.1111/tpj.13185. Yin et al. (2016). Comprehensive Mitochondrial Metabolic Shift during the Critical Node of Seed Ageing in Rice. PLoS One. 2016 Apr 28;11(4):e0148013. doi: 10.1371/journal.pone.0148013. eCollection 2016. Rurek et al. (2015). Biogenesis of mitochondria in cauliflower (Brassica oleracea var. botrytis) curds subjected to temperature stress and recovery involves regulation of the complexome, respiratory chain activity, organellar translation and ultrastructure. Biochim Biophys Acta. 2015 Jan 21. pii: S0005-2728(15)00016-X. doi: 10.1016/j.bbabio.2015.01.005.

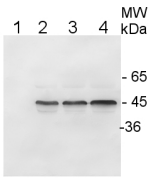
Application example



20 µg of total protein from *Arabidopsis thaliana* leaf extract **(1)**, *Arabidopsis thaliana* fraction enriched with mitochondria **(2)**, *Arabidopsis thaliana* pure mitochondria **(3)**, *Pisum sativum* pure mitochondria **(4)**, *Solanum tuberosum* pure mitochondria **(5)**, were separated on **4-12% SDS-PAGE** and blotted to **nitrocellulose**. Blots were blocked immediately following transfer in 5% milk powder in TBS. Blots were incubated in the primary antibody at a dilution of 1: 5 000 for 1h at room temperature with agitation, followed by an incubation with a secondary antibody and a series of washes. Blots were developed using chemiluminescent detection reagent.

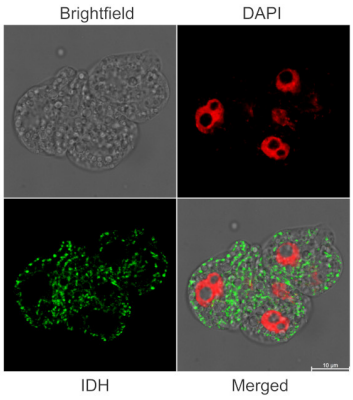
* Band detected at ca. 90 kDa is suspected to be a dimmer of Idh, since this band is depleted upon peptide competition experiment.

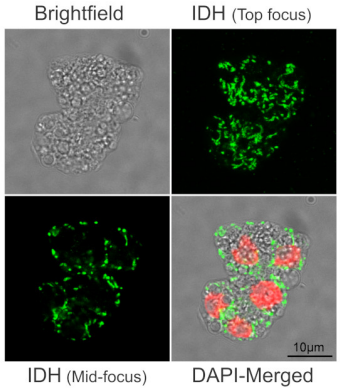
Courtesy of Dr. Olivier Keech, Umeå Plant Science Centre, Sweden



15 µg of total protein stem extract from *Lycopersicum esculentum* **(1)**, pure mitochondrial fraction isolated from stems of *Lycopersicum esculentum* **(2)**, pure mitochondrial fraction isolated from stems of *Capsicum annuum* **(3)**, pure mitochondrial fraction isolated from tubers of *Solanum tuberosum* **(4)** were separated on **10% SDS-PAGE** and blotted onto **nitrocellulose .** After blocking with 5% milk in TBST , blots were incubated with the primary antibody at a dilution of **1:1000** in TBST for 1.5h at room temperature. Following incubation and wash steps, blots were incubated with secondary Anti-Rabbit IgG , Alkaline Phosphatase Conjugate for 1 hour at a dilution of 1:40 000. Blots were developed with the alkaline phosphatase detection system using **NBT**/**BCIP**.

Courtesy of Bartosz Szabala, Institute of Plant Genetics, Polish Academy of Science, Poland





Immunofluorescent localization of IDH on suspension culture of *Arabidopsis thaliana* (upper image) *or Oryza sativa* (bottom image), using anti-IDH antibodies (AS06 203A) and anti-rabbit IgG DyLight®488 conjugated secondary antibodies (AS10 1165). DAPI staining of nuclei is pseudocolored red.

Material: Suspension cultures of *Arabidopsis thaliana,* ecotype Landsberg erecta cv.MM1 or *Oryza sativa* ssp.japonica cv. 'Unggi 9'
Fixation: Packed cell volume to fixer ratio: 250 µl : 5ml
Fixer composition and buffer: 4% (w/v) paraformaldehyde (freshly prepared as 8% stock and 0.2 µm filtered) in Phosphate Buffered Saline (PBS), pH 7.4 (2x stock, 0.2 µm filtered)
Container and method: in 6 cm Petri dish, gentle shaking at room temperature (RT)
Duration: 30 minutes (*Arabidopsis thaliana*) or 60 minutes (*Oryza sativa*). Cells were not shaken during the first 5 mins of fixation to allowed to partially recover from osmotic shock induced by formaldehyde.
Hydrophilization: no
Cell wall digestion: Yes
Packed cell volume to enzyme ratio: 100 µl : 2ml Enzyme composition: 1% (A) 1.2% (R) Cellulase (chromatically purified, powder, Worthington) 1% Pectinase (protease free, liquid, Sigma) Buffer: 0.5% (w/v) MES buffer, pH 5.6
Container and method: in 2 ml microfuge tube by rolling at room temperature (RT)
Duration: 30 minutes (*Arabidopsis thaliana*), 90 minutes (*Oryza sativa*)
Membrane permeabilization: Triton-X100 (0.2%), 10 min/RT
Antigen retrieval: no
Blocking buffer: Fish gelatin (5% v/v)
Washing buffer: PBS
Primary antibody dilution and incubation time: 1:400, ON/4ºC
Secondary antibody dilution and incubation time and supplier: anti-rabbit IgG DyLight®488 conjugated secondary antibodies (AS10 1165), 1:600, 1hn/RT
Co-staining of the nucleus (DAPI): Yes
Nucleus staining: 100 ng/ml DAPI

Courtesy of Dr. Ferhan Ayaydin, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary.