

This product is for research use only (not for diagnostic or therapeutic use)

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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 ug

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 ldh subunits, Some conservation across bacterial which contain the NAD-dependent form of ldh (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

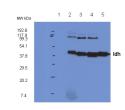
Kolodziejczak et al. (2018). m-AAA Complexes Are Not Crucial for the Survival of Arabidopsis Under Optimal Growth Conditions Despite Their Importance for Mitochondrial Translation. Plant Cell Physiol. 2018 May 1;59(5):1006-1016. doi: 10.1093/pcp/pcy041.

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).

<u>Fujii</u> 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. <u>Yin</u> 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





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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.

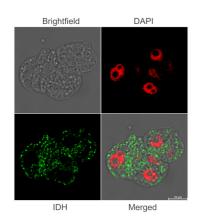
* Band detected at ca. 90 kDa is suspected to be a dimmer of ldh, since this band is depleted upon peptide competition experiment.

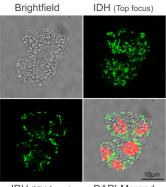
Courtesy of Dr. Olivier Keech, Umeå Plant Science Centre, Sweden



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





IDH (Mid-focus) DAPI-Merged

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.



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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 $0.2 \mu m$ filtered) in Phosphate Buffered Saline (PBS), pH 7.4 (2x stock, $0.2 \mu m$ 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.