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Product no AS12 1850

Anti-UCP | Uncoupling protein

Product information

Immunogen	KLH-conjugated synthetic peptide derived from known UCP protein sequences, including UCP1 (AT3G54110) and UCP2 (AT5G58970) of *Arabidopsis thaliana*
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	100 µg
Reconstitution	For reconstitution add 100 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 conserved in both isoforms, UCP1 and UCP2 of *Arabidopsis thaliana*.

Application information

Recommended dilution	1: 200 (IL), 1 : 2000 (WB)
Expected | apparent MW	32 kDa
Confirmed reactivity	*Arabidopsis thaliana, Splanum lycopersicum, Triticum aestivum, Vicia faba (protoplasts)*
Predicted reactivity	*Citrus sinensis, Dracunulus vulgaris, Glycine max, Litchi chinensis, Medicago tribuloides, Nannochloropsis gaditana, Nicotiana tabacum, Oryza sativa, Populus trichocarpa, Ricinus communis, Saccharum officinarium (sugarcane), Triticum aestivum* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	Czobor et al. (2019). Comparison of the response of alternative oxidase and uncoupling proteins to bacterial elicitor induced oxidative burst. PLoS One. 2019 Jan 10;14(1):e0210592. doi: 10.1371/journal.pone.0210592. Takáč et al. (2018). Shot-Gun Proteomic Analysis on Roots of Arabidopsis pld 1 Mutants Suggesting the Involvement of PLD 1 in Mitochondrial Protein Import, Vesicular Trafficking and Glucosinolate Biosynthesis. Int J Mol Sci. 2018 Dec 26;20(1). pii: E82. doi: 10.3390/ijms20010082. (immunolocalization) Garmash et al. (2017). Expression profiles of genes for mitochondrial respiratory energy-dissipating systems and antioxidant enzymes in wheat leaves during de-etiolation. J Plant Physiol. 2017 Aug;215:110-121. doi: 10.1016/j.jplph.2017.05.023. Florez-Sarasa et al. (2016). Impaired cyclic electron flow around Photosystem I disturbs high-light respiratory metabolism. Plant Physiol. 2016 Oct 19. pii: pp.01025.2016. Liu et al. (2015). Silencing of mitochondrial uncoupling protein gene aggravates chilling stress by altering mitochondrial respiration and electron transport in tomato. Acta Physiologiae Plantarum November 2015, 37:223. Long et al. (2015). Contributions of photosynthetic and non-photosynthetic cell types to leaf respiration in Vicia faba L. and their responses to growth temperature. Plant Cell Environ. 2015 Apr 1. doi: 10.1111/pce.12544. Grabelnych et al. (2014). Mitochondrial Energy Dissipating Systems (Alternative Oxidase, Uncoupling Proteins, and External NADH Dehydrogenase) Are Involved in Development of Frost Resistance of Winter Wheat Seedlings. ISSN 0006 2979, Biochemistry (Moscow), 2014, Vol. 79, No. 6, pp. 506 519. © Pleiades Publishing, Ltd., 2014. Barreto et al. (2014). Overexpression of UCP1 in tobacco induces mitochondrial biogenesis and amplifies a broad stress response. BMC Plant Biol. 2014 May 28;14(1):144.

Application example

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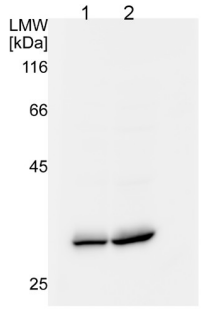
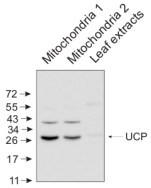


25 µg **(1)** or 50 µg **(2)** of mitochondria, isolated from 14-day old Col-0 plants (*Arabidopsis thaliana*) grown in hydroponic cultures, were separated on a 12.5% acrylamide-SDS-PAGE. The unstained peqGOLD low molecular weight (LMW) protein marker was used as a molecular weight standard. The gel was subsequently incubated in transfer buffer (40 mM glycine, 100 mM Tris, 0.375 % (w/v) SDS, pH 8.9-9) for 30 minutes at room temperature. Semidry western blotting was performed with 3 layers of whatman paper soaked in transfer buffer and blotted at 0.8 mA/ cm2 gel for one hour at room temperature. Blocking was performed for one hour in TBS-T (150 mM NaCl, 10 mM Tris pH 7.4, 0.1 % (v/v) Tween-20) with the addition of 3 % (w/v) non-fat milk at room temperature. Primary antibody (1:2000 dilution) incubation was performed at 4°C/ON in the presence of 1 % (w/v) non-fat milk in TBS-T. Secondary antibody goat anti-rabbit HRP conjugated (AS09 602 Agrisera), at 1:10 000 dilution) was incubated 1h/RT in TBS-T. Chemiluminescence was detected using a 1:1 ratio mixture of ECL 1 (100 mM Tris, 1 % (w/v) luminol, 0.44 % (w/v) coumaric acid, pH 8.5) and ECL 2 (100 mM Tris, 0.18 % (v/v) H_2O_2, pH 8.5) solution and visualized using an Image QuantLAS4000 image visualizer (GE healthcare). The membrane was exposed for 60 seconds.
UCP was detected at a size of approximately 29 kDa.

Courtesy Dr. Tamara Hechtl, Munich University, Germany



10 µg of mitochondrial fraction from *Arabidopsis thaliana* and 25 µg of *Arabidopsis thaliana* leaf extract were separated on 10% gel and blotted on nitrocellulose membrane using wet transfer (0.22% CAPS, pH 11). Filters where blocked (1.5h) in 5% milk in TBST (1X TBS, 0,1% Tween 20), incubated with 1: 5000 anti-UCP antibodies (2h in TBST) followed by incubation with 1: 10 000 secondary anti-rabbit (1h) HRP-coupled antibodies from Agrisera, AS09 602 and visualized with standard ECL on Kodak autoradiography film for 15-60 s. Mitochondria were isolated as described by Urantowka et al. (Plant Mol Biol, 2005, 59:239-52). Mitochondrial pellets were suspended in 1X Laemmli buffer (5% beta-mercaptoetanol, 3.7% glycerol, 1.1% SDS, 23 mM Tris- HCl pH 6.8, 0.01% bromophenol blue), heated (95°C, 5 min.) and centrifuged (13 000rpm, 1 min.). Leaf extracts were prepared as described by Martinez-Garcia et al. (Plant J., 1999, 20:251-7).
Courtesy Dr. Janusz Piechota, Wrocław University, Poland