**UCP | Uncoupling protein**

### Product Information

**Background**
UCP (uncoupling protein) is an inner membrane mitochondrial protein that can dissipate the proton gradient before it can be used to provide the energy for oxidative phosphorylation. Synonyms: AtUCP, Uncoupling protein 2.

**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**
Affinity purified serum in PBS, pH 7.4

**Format**
Lyophilized in PBS pH 7.4

**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 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**
Western blot (WB)

**Related products**
- Antibodies to other plant mitochondrial proteins
- Plant and algal protein extraction buffer

### Application Information

**Recommended dilution**
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 triliboides, Nicotiana tabacum, Oryza sativa, Populus trichocarpa, Ricinus communis, Saccharum officinarium (sugarcane), Triticum aestivum*

**Not reactive in**
No confirmed exceptions from predicted reactivity are currently known.

### Additional Information

**Selected references**
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) H₂O₂, 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

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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 were 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, Wroclaw University, Poland