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Product no AS15 2981

HSP26,5 | Heat shock protein 26,5 (mitochondrial)

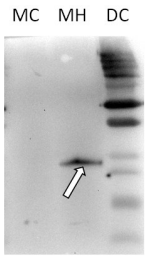
Product information

Immunogen	KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* HSP26.5, mitochondrial, UniProt: Q9SSQ8, TAIR: At1g52560
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution add 25 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	Detection on total extracts needs to be optimized. Antibody is recognizing HSP23.6 synthesized *in vitro* using the PURExpress in Vitro Protein Synthesis Kt (NEB).

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	26,5 | 23 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Glycine soja* Species of your interest not listed? Contact us
Additional information	Please, note that there might be no HSPs accumulation below temperature of 32-34°C. HSPs are induced when the plant experience temperatures higher than the growing temperature with around 10°C. So, the HSPs induction temperatures for plants grown at 18C differ from these for plants grown at 24C. Another very effective parameter is the humidity. When using low humidity the plant has a chance to cool down through transpiration. In this case the HSPs induction requires higher temperatures.

Application example



Mitochondrial enriched fractions were prepared from 4-week old *Arabidopsis thaliana* Col-0 rosette leaves (control, or heat shocked 3h 38°C) using the method of Huang et al (2013, Nature Comm. 4:2558). Crude mitochondria obtained from 3-4 g leaves were suspended in 50 µL washing buffer (0.4 M sucrose, 50 mM Tris pH 7.5, 3mM EDTA, 0.1% BSA). For western blot analysis, 40 µL of suspension were mixed with 10 µL of 5X SDS-PAGE sample buffer (40 mM Tris pH 6.8, 0.8 % SDS, 0.4 % bromophenol blue, DTT 0.2 M, 20 % glycerol), heat denatured at 95 °C for 3 min, separated on 13.5 % SDS-PAGE, and blotted 1h to PVDF (0.2 µm Immobilon PSQ transfer membrane, Millipore) using tank transfer (10 mM CAPS pH 11, 10 % ethanol). After staining with Ponceau red, blot was blocked with TBS containing 1.5 % Tween 20 by incubation at room temperature (RT). Blot was briefly rinsed three times with TBS-T (TBS with 0.05 % Tween 20) and incubated overnight at 4°C with the primary

antibody at a dilution of 1:1 000 in TBS-T. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 15 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (goat anti-rabbit IgG horse radish peroxidase conjugated, from Agrisera) diluted to 1:75 000 in TBS-T for 1h at RT with agitation. The blot was washed as above and developed for 2 min with Clarity Western ECL (Biorad). DC: dual color markers (Biorad), 5 µL MC: 10 µL of mitochondria enriched fractions from control leaves MH: 10 µL of mitochondria enriched fractions from heat-shocked leaves.

Remark: Protein were not measured in the extracts because of the presence of BSA. Coomassie blue staining confirmed similar protein content of crude mitochondria extract (estimated to around 2µg/µL). Similar protein content of MC and MH extracts is also supported by the background with the AB HSP23.5 antibody.

Courtesy Dr. David Macherel, Mitostress team, IRHS, France.