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Product no AS07 204

FtsH3 + FtsH10 | ATP-dependent zinc metalloprotease FtsH3 + FtsH10 (mitochondrial)

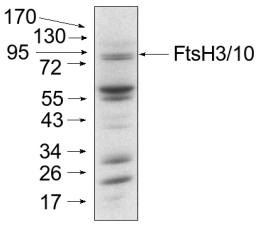
Product information

Immunogen	KLH-conjugated peptide dereived from sequences of *Arabidopsis thaliana* FtsH3 and FtsH10 with localization to mitochondria Q84WU8, At2g29080 and Q8VZI8, At1g07510
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	200 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	Blue-native (2D BN/SDS-PAGE) methodology is described in Piechota et al. 2010

Application information

Recommended dilution	1 : 500-1 : 1000 (WB)
Expected | apparent MW	80 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Arabidopsis thaliana*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
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. Piechota et al. (2010). Identification and characetization of high-molecular-weight complexes fromed by m-AAA proteases and prohibitins in mitochondria of Arabidopsis thaliana. J Biol Chem. 2010 Apr 23;285(17):12512-21. doi: 10.1074/jbc.M109.063644.

Application example



Total protein from *Arabidopsis thaliana* mitochondria (20 µg) were separated on 10% acrilamide gel and electrophoresis prepared according to Schägger and von Jagov (Anl. Biochem., 1987, 166:368-379). After running the gel, proteins were transferred to nitrocellulose membrane using wet transfer (0.22% CAPS, pH 11). Transfer was checked by Ponceau S staining. Blot was destained by several quick washings in distilled water and 1 washing in 1X TBS (10 mM T pH 7.5, 150 mM NaCl) (10-15 min.).Blot was blocked by 1.5 hour in 5% milk in TBST (1X TBS, 0,1 20) After blocking blot was washed quickly twice in TBST and incubated 2 hours with primary antibody (dilution 1: 1000 TBST (dilution 1:1000). Washing: two quick washings in TBST and 3 x 10 min. washings in TBST. Then blot was incubated 45-60 min. with a secondary anti-rabbit antibodies conjugated to peroxidase (dilution 1:10000) in TBST. Washing: as above. After washing blot was incubated 1-2 min. in ECL solution and exposed to Kodak autoradiography film. Exposure time was 15-60 seconds.

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 (95ºC, 5 min.) and centrifuged (13 000rpm, 1 min.).

Courtesy Dr. J. Piechota, Wrocław University, Poland