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contact: support@agrisera.com

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS11 1754

Anti-ATPase AAA2 domain

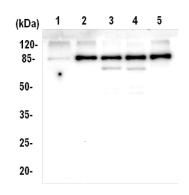
Product information

Immunogen	KLH-conjugated synthetic peptide derived from proteins containing AAA2 domain, including Arabidopsis thaliana ClpB1 P42730, At1g74310
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	200 μΙ
Reconstitution	For reconstitution add 200 μ l 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.

Application information

Recommended dilution	1 : 2000 (WB)	
Confirmed reactivity	Arabidopsis thaliana, Chlamydomonas reinhardtii, Lupinus angustifolius, Pisum sativum, Solanum tuberosum, Synechococcus sp. PCC 7942, Zea mays L.	
Predicted reactivity	AAA2 domain containing proteins including Saccharomyces cerevisiae HSP104. Nannochloropsis gaditana ClpB chaperone	
Not reactive in No confirmed exceptions from predicted reactivity are currently known		

Application example



The 20 µg of soluble proteins from 3-week old *Solanum tuberosum* (1) and *Arabidopsis thaliana* (2), one-week old *Lupinus angustifolius* (3), *Pisum sativum* (4) and *Zea mays* L. (5) leaves extracted with buffer containing 50 M Hepes-KOH, pH 7.5, 330 M sorbitol, 2 M EDTA, 1 M MgCl2, 5 M ascorbate, 0.05% BSA were mixed with sample buffer and denatured for 5 min at 70°C. Samples were separated on 10% SDS-PAGE and blotted 1h to nitrocellulose membrane (Amersham Protran) using tank wet-transfer (Bio-Rad) in standard transfer buffer in presence of 20% methanol. Transfer of proteins to the membrane was checked using 1% Ponceau S staining before the blocking step. Blots were blocked in buffer (5% low-fat milk in 1xPBS, 0.1% Tween-20) for 1h at room temperature (RT) with agitation. Blots were incubated in the primary antibody at a dilution of 1:2 000 for 1 h at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, and then washed once for 15 min and 3 times for 5 min in PBS-T at RT with agitation. Blot was incubated in secondary antibody (goat anti-rabbit IgG, <u>AS09</u> <u>602</u>, Agrisera) diluted to 1:30 000 (Agrisera) in for 1h at RT with agitation. The blot was washed as above and developed for 5 min with Clarity Western ECL Substrate and ChemiDoc detection system (Bio-Rad). Exposure time was 60 seconds.

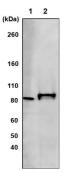
Courtesy Dr. Elena Pojidaeva, Laboratory of Plant Gene Expression, Timiryazev Institute of Plant Physiology RAS, 127276 Moscow Russia



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0.3 µg of total protein from *Synechococcus* sp. PCC7942 (1) and 20 µg of leaf soluble proteins from 3-week old *Arabidopsis thaliana* leaves (2) were separated on **3-8 % Tris-acetate NuPage gel (invitrogen)** and blotted 1,5 h to **supported introcellulose**. Blots were blocked for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 2 000 in 1xTBS-T for 1h at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, from Agrisera, <u>AS09 602</u>) diluted to 1:75 000 in for 1h at RT with agitation. The blot was washed as above and developed for 5 min with ECL according to the manufacturers instructions. Exposure time was 120 seconds.

Courtesy of Dr. A. Clarke, Göteborg University, Sweden