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Product no AS13 2704 Anti-Lhcb1-P | LHCII type I chlorophyll a/b-binding protein, phopshorylated Product information

Immunogen	KLH-conjugated synthetic peptide RKT*VAKPKGP, where T* indicates phospho-Thr
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	25 μg
Reconstitution	For reconstitution add 25 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 : 10 000 (WB)
Expected apparent MW	25 25 kDa for Arabidopsis thaliana
Confirmed reactivity	Arabidopsis thaliana, Echinochloa crus-galli,, Oryza sativa, Panicum miliaceum, Pisum sativum, Zea mays
Predicted reactivity	Arachis hypogaea, Colobanthus quitensis Kunt Bartl, Hordeum vulgare, Mesembryanthemum crystallinum, Nicotiana tabacum, Phaseolus vulgaris, Silene vulgaris, Solanum lycopersicum, Spinacia oleracea, Species of your interest not listed? <u>Contact us</u>
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	<u>Collombat</u> et al. (2025). Arabidopsis conditional photosynthesis mutants abc1k1 and var2 accumulate partially processed thylakoid preproteins and are defective in chloroplast biogenesis. Commun Biol . 2025 Jan 22;8(1):111. doi: 10.1038/s42003-025-07497-y. <u>Nilsson</u> et al. (2020). PSB33 protein sustains Photosystem II in plant chloroplasts under UVA light. J Exp Bot. 2020 Sep 15;eraa427.doi: 10.1093/jxb/eraa427. <u>Rudenko</u> et al. (2019). The role of carbonic anhydrase a-CA4 in the adaptive reactions of photosynthetic apparatus: the study with a-CA4 knockout plants. Protoplasma (2019). https://doi.org/10.1007/s00709-019-01456-1 <u>Rantala</u> and Tikkanen et al. (2018). Phosphorylation-induced lateral rearrangements of thylakoid protein complexes upon light acclimation. Plant Direct Vol. 2, Issue 2. <u>Rantala</u> et al. (2017). Proteomic characterization of hierarchical megacomplex formation in Arabidopsis thylakoid membrane. Plant J. 2017 Dec;92(5):951-962. doi: 10.1111/tpj.13732. <u>Fristedt</u> et al. (2017). PSB33 sustains photosystem II D1 protein under fluctuating light conditions. Journal of Experimental Botany doi:10.1093/jxb/erx218.



1,5 µg of chlorophyll from *Zea mays*, mesophyll (1) and bundle sheath (2), *Panicum miliaceum*, mesophyll (3) and bundle sheath (4), *Echinochloa crus-galli*, mesophyll (5) and bundle sheath (6), *Arabidopsis thaliana* (7), *Pisum sativum* (8) chloroplasts extracted with 0.4 M sorbitol, 50 mM HEPES NaOH, pH 7.8, 10 mM NaCl, 5 mM MgCl2 and 2 mM EDTA were loaded to lanes. Samples were denatured with Laemmli buffer at 75 0C for 5 min and were separated on 12% SDS-PAGE, and blotted 30 min to PVDF using wet transfer. Blot was blocked with 5% BSA for 2h at room temperature (RT) with agitation. Blot was incubated in the primary antibody Anti-Lhcb1-P (LOT 2201) at a dilution of 1: 10 000 in 1% BSA in TBS-T overnight at 40C with agitation. The antibody solution was decanted, and the blot was washed 4 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG HRP conjugated, from Agrisera, <u>AS09 602</u>, LOT 2106) diluted to 1: 20 000 in



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1 % BSA in TBS-T for 1h at RT with agitation. The blot was washed 5 times for 5 min in TBS-T and 2 times for 5 min in TBS, and developed for 1 min with 1.25 mM luminol, 0.198 mM coumaric acid and 0.009% H2O2 in 0.1 M Tris- HCI, pH 8.5. Exposure time in ChemiDoc System was 153 seconds.



Courtesy Dr. Wioleta Wasilewska-Dębowska, Warsaw University, Poland

1 μg of thylakoid membranes isolated from *Arabidopsis thaliana* wilde-type and mutants were solubilized with 3X LB (6 M urea, 12% SDS, 30% glycerol, 100 mM DTT, 150 mM Tris pH7.0, 0.8% Comassie G-250). 1 μg of total chlorophyll was loaded and separated on 16% SDS-PAGE, and then blotted for 2 h onto nitrocellulose membrane. Blots were blocked with milk powder for 2 h and then incubated in the primary antibody solution, at a dilution of 1: 5 000/2.5 h, RT incubation, which was then decanted and the blot was washed 3 times for 5 min in TBST. Membrane was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:10 000 in for 1h, followed by washing steps as above. All the steps fallowing transfer were performed in room temperature (RT) with agitation. Membrane was developed for 5 min with ECL according to the manufacturer's instructions and recorded using FujiFilm CCD camera with 30 s increment time for around 5 min.

Courtesy of a PhD candidate Małgorzata Pietrzykowska, Umeå Plant Science Centre, Sweden