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Product no AS20 4377

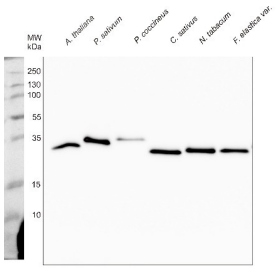
Cyt f | Cytochrome f protein (PetA) of thylakoid Cyt b6/f-complex (higher plants)

Product information

Immunogen	KLH-conjugated peptide derived from *Arabidopsis thaliana* PetA sequence: UniProt: P56771, TAIR: AtCg00540
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution add 50 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.

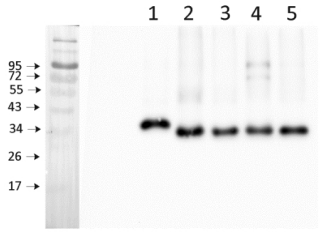
Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	31-32 kDa
Confirmed reactivity	*Arabidopsis thaliana, Cucumis sativus, Echinola crus-galli, Ficus elastica, Lupinus angustifolius, Nicotiana tabacum, Phaseolus coccineus , Picea abies, Pinus sylvestris, Pisum sativum, Solanum tuberosum, Synechococcus elongatus* PCC7942*, Zea mays*
Predicted reactivity	*Nicotiana benthamiana* Species of your interest not listed? Contact us
Not reactive in	cyanobacteria
Selected references	Lempiainen et al. (2022) Plants acclimate to Photosystem I photoinhibition by readjusting the photosynthetic machinery. Plant Cell Environ. 2022 Oct;45(10):2954-2971. doi: 10.1111/pce.14400. Epub 2022 Aug 16. PMID: 35916195.



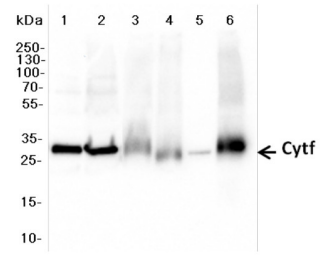
0.25 µg - 1 µg of isolated thylakoids (stored at -80°C): 1 µg of *Arabidopsis thaliana* thylakoid membranes, 1 µg of *Pisum sativum* thylakoid membranes, 0.25 µg of *Phaseolus coccineus* thylakoid membranes, 1 µg of *Cucumis sativus* thylakoid membranes, 1 µg of *Nicotiana tabacum* thylakoid membranes, 1 µg of *Ficus elastica* variegata thylakoid membranes were denatured with 150 µL double diluted Roti®-Load 1 (ROTH, Art.-Nr. K929.1) at 95 °C for 2 min. Samples containing 1 (or 0.25) µg of chlorophyll/well were separated on 14% SDS-PAGE and blotted 45 min at 100V to PVDF (pore size of 0.2 µm) using wet transfer. Blot was blocked with 5% milk 4°C/ON with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 3h/RT with agitation in TBS (+0.5% Amersham™ ECL Prime Blocking Agent; cat no: RPN418). The antibody solution was decanted and the blot was washed 2 times for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated AS09 602) diluted to 1:20 000 in TBS-T (+1% milk) for 1h/RT with agitation. The blot was washed as above and developed for 5 min with Agrisera ECLBright. The Image was recorded using ChemiDoc MP Imaging System (Bio-Rad) with automatic selection of the exposure time.
TBS (25 mM Tris, 500 mM NaCl; pH 7.5) TBS-T (TBS + 0.1% Tween20)

Courtesy of Dr. Radosław Mazur, University of Warsaw, Poland



1.0 µg of chlorophyll from chloroplasts of: *Pisum sativum* **(1)**, *Echinochloa crus-galli*, M chloroplasts **(2)**, *Echinochloa crus-galli*, BS (bundle sheath) chloroplasts **(3)**, *Zea mays* M chloroplasts **(4)**, *Zea mays BS* (bundle sheath) chloroplasts **(5)**, extracted with 0.4 M sorbitol, 50 mM Hepes NaOH, pH 7.8, 10 mM NaCl, 5 mM MgCl2 and 2 mM EDTA. Samples were denatured with Laemmli buffer at 75ºC for 5 min and were separated on 12% SDS-PAGE and blotted 30 min to PVDF using wet transfer. Blot was blocked with 5% milk for 2h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 overnight at 40ºC with agitation in 1% milk in TBS-T. 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 horse radish peroxidase conjugated, from Agrisera, AS09 602, Lot 2001) diluted to 1:20 000 in 1 % milk 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% H_2O_2 in 0.1 M Tris- HCl, pH 8.5. Exposure time in ChemiDoc System was 73 seconds.

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



Samples:
1 - 25 ug of *Picea abies* total proteins extract obtained from needles using 10% TCA/acetone, pellet was dissolved in 8M Urea, 40 mM Tris-HCl, pH 6.8, 0,1 mM EDTA, 1% SDS
2 - 25 ug of *Pinus sylvestris* total proteins extract obtained from needles using 10% TCA/acetone, pellet was dissolved in 8M Urea, 40 mM Tris-HCl, pH 6.8, 0,1 mM EDTA, 1% SDS
3 - 10 ug of *Lupinus angustifolius* thylakoid membranes
4 - 10 ug of *Solanum tuberosum* thylakoid membranes
5 - 10 ug of *Synechococcus elongatus* PCC7942 thylakoid membranes
6 - 10 ug of *Pisum sativum,* thylakoid membranes
MW markers: PageRuler™ Plus Prestained Protein Ladder from ThermoFisher Scientific, cat # 26619.

25 µg/well of total protein from *Picea abies* **(1)** and *Pinus sylvestris* **(2)** needles extracted with 10% TCA/acetone, and 10 µg/well of chlorophyll from *Lupinus angustifolius* **(3)**, *Solanum tuberosum* **(4)**, *Synechococcus elongatus* PCC7942 **(5)**, and Pisum sativum extracted with 0.4 M sorbitol, 50 mM Hepes NaOH, pH 7.8, 10 mM NaCl, 5 mM MgCl2 and 2 mM EDTA. Samples were denatured with Laemmli buffer at 70°C for 5 min and were separated on 8-16% SDS-PAGE and blotted 40 min to nitrocellulose (pore size of 0.2 um), using semi-dry transfer. Blot was blocked with 5 % milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 1h at RT with agitation in 1% milk in TBS-T. 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, AS09 602) diluted to 1:25 000 in for 1h/RT with agitation. The blot was washed as above and developed for 5 min with ECL detection kit with iBright FL1500 Imaging System (Thermo Fisher). Exposure time was 745 seconds.

Please note that detection of PetA using this antibody requires optimization of Western blot protocol in terms of protein load and incubation time, depending upon which species is analyzed.

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