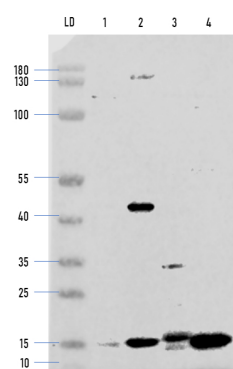


Product no **AS22 4711****Anti-PetD | Cytochrome b6-f complex subunit 4****Product information**

Immunogen	KLH-conjugated peptide derived from <i>Arabidopsis thaliana</i> PetD protein sequence, UniProt: P56774 , TAIR: AtCg00730
Host	Rabbit
Clonality	Polyclonal
Purity	Antigen affinity purified serum, in PBS pH 7.4
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution, add 50 µl, of sterile or deionized water.
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

Application information

Recommended dilution	1 : 1000 (WB)
Expected apparent MW	17.4 kDa
Confirmed reactivity	<i>Arabidopsis thaliana</i> , <i>Synechocystis</i> sp., <i>Synechococcus</i> sp., <i>Cyanobacterium aponinum</i> , <i>Chlorogloeopsis</i> sp.
Predicted reactivity	<i>Gossypium</i> sp., <i>Helianthus annuus</i> , <i>Hordeum vulgare</i> , <i>Gossypium</i> sp., <i>Zea mays</i> Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	To be added when available, antibody available in October 2023.

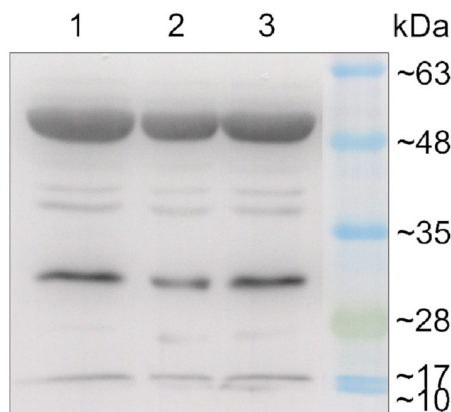
**Samples:**

LD: PAGE ruler Prestained (ThermoFisher)

- 1 - *Synechocystis* sp.
- 2 - *Synechococcus* sp.
- 3 - *Cyanobacterium* sp.
- 4 - *Chlorogloeopsis* sp.

5 µg/well of total proteins were loaded/well. Proteins were extracted freshly from 10 ml of exponentially growing cyanobacterial cultures (0.5-1 OD730). Lysis was performed via beadbeating (0.1 mm glass beads) 3 times (20s 6000 rpm and 2 min on ice) in precooled 100 mM Tris-HCl (pH 7.6) 10% glycerol and cComplete™, EDTA-free protease Inhibitor Cocktail (Merck). Then extracts were centrifuged at 17.000g for 25 min at 4°C to retrieve cytosolic fraction. Protein concentration was quantified using BCA assay and a BSA standard. Samples were denatured in 50 mM Tris-HCl (pH 6.8), 100 mM DTT, 2% SDS, 5 mM EDTA and 10% glycerol (final concentrations) + bromophenol blue stain. Denatured for 10 min at 95°C and separated in a 8-16% gradient SDS-PAGE using MOPS buffer. Samples were blotted in a Nitrocellulose membrane in a wet transfer system during 1.5h at constant current of 150 mA. Membrane was blocked overnight in 5% milk PBS-Tween buffer in agitation. Then, incubation of primary antibody (1:1000) performed during 1h at RT in 2.5% milk PBS-T. The antibody solution was decanted, and the blot was rinsed briefly twice, then washed 3 times for 15 min in PBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, [AS09 602](#)) diluted to 1:25 000 for 1h/RT with agitation in 2.5% milk PBS-T. The blot was washed as above and adding a last step with only PSB (no Tween added). And developed for 5 min with Agrisera [AgriseraECLSuperBright](#) with a LI-COR Odyssey

Fx Imager. Exposure time was 300 seconds.



Samples:

- 1 - 10 ug of 4 days old of wild-type *Arabidopsis thaliana* seedlings extract
- 2 - 5 ug of 4 days old of wild-type *Arabidopsis thaliana* seedlings extract
- 3- 10 ug of 4 days old of gun5-1 mutant seedlings extract

Exact buffer components were: (50 mM Tris-HCl pH 7.5, 10% glycerol, 150 mM NaCl, 10 mM MgCl₂, 5 mM EDTA, 5 mM DTT, 0.5% (v/v) Triton X-100, and 1 × protease inhibitors) and denatured with 4X SDS sample loading buffer (200 mM Tris-Cl (pH 6.8), 8% SDS (sodium dodecyl sulfate), 0.4% Bromophenol blue, 40% glycerol) at 95 °C 10 min. Samples were separated in the RT on 15 % SDS-PAGE and blotted for 0.5 h to PVDF (pore size of 0.2 um), using: semi-dry at room temperature. Blot was blocked with 5 % milk for: 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 2 000 at 4 °C with agitation overnight. 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 matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, [AS09 602 Agrisera](#)) diluted to 1: 50 000 for 2 h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent: AS16 ECL-N-10 [AgriseraBright](#) (mid picogram). Exposure time was 1 minute. Ther high cross reactivity above 50 kDa can be optimized by increasing blocking to 10 % nonfat milk and incubation of a primary antibody at 1: 2000 1h/RT.

Courtesy of Dr. Duorong Xu, LMU München, Germany