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### Product no AS15 2955

# RbcL II | Rubisco large subunit, form II

### **Product information**

Immunogen KLH-conjugated synthetic peptide conserved in known RbcL form II protein sequences

Host Rabbit

Clonality Polyclonal

Purity Serum

Format Lyophilized

Quantity 50 μl

**Reconstitution** For reconstitution add 50 μl of sterile water

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 (IF), (WB)

Expected | apparent

Confirmed reactivity

Alexandrium catenella, Amphidinium carterae, Chaetoceros neogracilis, Rhodobacter capsulatus, Rhodospirillum

51.7 kDa

Predicted reactivity

Acidithiobacillus ferrooxidans, Dechloromonas aromatica, Gonyaulax polyedra, Leptothrix cholodnii, Magnetovibrio blakemorei, Rhodobacter sphaeroides, Thiobacillus denitrificans, Rhodopseudomonas palustris

And with a mismatch in one single amino acid:

Gallionella capsiferriformans, Mariprofundus ferrooxydans, Thioalkalicoccus limnaeus, Methanomethylovorans hollandica, Methanococcoides burtonii, Methanosaeta concili, Methanolobus tindarius, Methanohalophilus mahii, and the dinoflagellate Symbiodinium sp. (ex Stylophora pistillata)

Species of your interest not listed? Contact us

**Not reactive in** Burkholderi, *Cyanobium* sp.

**Additional information** Protein extraction from diatoms protocol can be found here.

Selected references

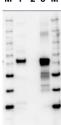
Cho et al. (2021). SxtA localizes to chloroplasts and changes to its 3'UTR may reduce toxin biosynthesis in non-toxic Alexandrium catenella (Group I). Harmful Algae, 2021,101972,ISSN 1568-9883,

https://doi.org/10.1016/j.hal.2020.101972. Immunolocalization

Bausch et al. (2019). Combined effects of simulated acidification and hypoxia on the harmful dinoflagellate Amphidinium carterae. Marine Biology, June 2019, 166:80.

Long et al. (2018). Carboxysome encapsulation of the CO2-fixing enzyme Rubisco in tobacco chloroplasts. Nat Commun. 2018 Sep 3;9(1):3570. doi: 10.1038/s41467-018-06044-0.

# **Application example**



1.5 µg of total protein extract from Rhodobacter capsulatus (1); extracted with Agrisera Protein Extraction Buffer PEB (AS08 300); 0.5 pmol of recombinant RbcL I (2), 0.5 pmol of recombinant RbcL II (3) Samples were diluted with 1X sample buffer (NuPAGE LDS sample buffer

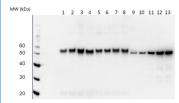


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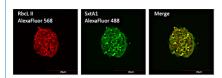
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(Invitrogen) supplemented with 50 mM DTT and heat at 70°C for 5 min and keept on ice before loading. Protein samples were separated on 4-12% Bolt Plus gels, LDS-PAGE and blotted for 70 minutes to PVDF using tank transfer. Blots were blocked immediately following transfer in 2% blocking reagent or 5% non-fat milk dissolved in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 10 000 (in blocking reagent) for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, and then washed 1x15 min and 3x5 min with TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody AS10 1489, Agrisera) diluted to 1:25 000 in blocking reagent for 1h at room temperature with agitation. The blots were washed as above. The blot was developed for 5 min with chemiluminescent detection reagent of extreme femtogram sensitivity, according the manufacturers instructions. Images of the blots were obtained using a CCD imager (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 30 seconds.



Quantitative Immunoblot of Form II RbcL from *Amphidinium carterae* (Dinoflagellata). Algae were extracted from PC filters in Agrisera Protein Extraction Buffer (AS08 300) with 3 breakage cycles (60 seconds, 6.5 m/s) in a FastPrep-24 with D-matrix ceramic beads (MP Biomedicals). Total algal protein extract (2 µg, lanes 1-8) and a range of loads of recombinant form II RbcL standard (AS15 2955S, lanes 9-13, 37.5, 75, 150, 300, 450 fmol) was separated on a Bolt polyacrylamide gel (ThermoFisher) and bloSed onto PVDF. Following antibody incubations (primary antibody AS15 2955, 1:20000; secondary antibody AS09 602, 1:20 000), protein signal was developed using chemiluminescence detection reagent and visualised using a VersaDoc Imager (BioRad).

Courtesy of Environmental Proteomics N.B, Canada



Multiplex immunofluorescence detection of SxtA1 and RbcL, form II by confocal laser scanning microscopy in diatom *Alexandrium catenella* (Group I) as described in <u>Cho</u> et al. (2021).

Anti-RbcL form II antibodies were used in diltution of 1: 10 000.