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

Product no AS03 037

Anti-RbcL | Rubisco large subunit, form I (rabbit)

Product information

Immunogen	KLH-conjugated synthetic peptide conserved across all known plant, algal and cyanobacterial RbcL protein sequences (form I L8S8 and form II L2), including, *Arabidopsis thaliana* O03042, *Hordeum vulgare* P05698, *Oryza sativa* P0C510, *Chlamydomonas reinhardtii* P00877, *Synechococcus* PCC 7920 A5CKC5
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
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.
Additional information	Anti-RbcL can be used as a cellular [compartment marker] of plastid stroma (cytoplasm in cyanobacteria) and detects RbcL protein from 31.25 fmoles. As both forms (I and II) are detected it is suitable for work with samples from Dinoflagellates, Haptophytes and Ochrophytes (diatoms, Raphidophytes, brown algae) as well as higher plants. This antibody together with Agrisera Rubisco protein standard is very suitable to quantify Rubisco in plant and algal samples.Example of a simulataneous western blot detection with RbcL, PsbA and PsaC antibodies. This antibody is not suitable for use in immunoprecipiation. This product can be sold containing ProClin if requested.

Application information

Recommended dilution	Immunofluorescence/confocal microscopy (IF), 1: 1000 (IG), 1: 250 for images see Prins et al. (2008), detailed protocol available on request, 1: 800 (TP), 1: 5000 - 10 000 and more depending upon protein load and detection method (WB)
Expected | apparent MW	52.7 kDa (*Arabidopsis thaliana*), 52.5 kDa (cyanobacteria), 52.3 (*Chlamydomonas reinhardtii*)
Confirmed reactivity	*Agostis stolonifera cv. Penncross, Arabidopsis thaliana, Apium graveolens, Artemisia annua, Atrichum undulatum, Attheya longicornis, Baculogypsina sphaerulata (benthic foraminifer), Beta vulgaris, Begonia* sp., *Bienertia sinuspersici, Brassica napus, Kandelia candel, Cannabis sativa* L., *Chaetoceros furcellatus, Chlorococcum dorsiventrale, Colobanthus quitensis, Cicer arietinum, Chenopodium quinoa, Chlamydomonas raudensis, Chlamydomonas reinhardtii, Colobanthus quitensis* Kunt Bartl, *Chlorella sorokiniana, Chlorella vulgaris, Coscinodiscus concinnus, Cyanophora paradoxa, Cylindrospermopsis raciborskii* CS-505, *Cynara cardunculus, Emiliana huxleyi, Euglena gracilis, Ficus carica, Fortunella margarita* Swingle, *Fraxinus mandshurica, Fucus vesiculosus, Gladieria sulphuraria, Glycine max, Gonyaulax polyedra, Gongolaria barbata, Guzmania* hybrid, *Heterosigma akashiwo, Hevea, Hordeum vulgare, Hypnum cupressiforme, Jatropha curcas, Karenia brevis* (C.C.Davis) s) G.Hansen & Ø.Moestrup (Wilson isolate), *Kochia prostrata, Lathyrus sativus, Liquidambar formosana, Malus domestica, Medicago truncatula, Micromonas pusila, Nicotiana benthamiana, Nicotiana tabacum, Panicum virgatum, Petunia hybrida* cv. Mitchell, *Phaeodactylum tricornutum, Physcomitrium patens, Pisum sativum, olytrichum formosum, Porosira glacialis,, Porphyra sp., Ricinus communis, Robinia pseudoacacia, Rhytidiadelphus squarrosus, Saccharum* sp., *Schima superba, Skeletonema costatum* (diatom), *Skeletonema marinoi (diatom), Solanum lycopersicum*, *Spinacia oleracea,* lichens, *Stanleya pinnata, Symbiodinium sp., Synechococcus PCC 7942, Synechococcus elongatus* UTEX 2973, *Rhoeo discolor, Thalassiosira pseudonana, Thermosynechococcus elongatus, Triticum aestivum, Prochlorococcus sp.* (surface and deep water ecotype), *Triticum aestivum,* dinoflagellate endosymbionts (genus Symbiodinium), extreme acidophilic verrucomicrobial methanotroph *Methylacidiphilum fumariolicum* strain SolV, *Thalassiosira punctigera, Tisochrysis lutea, Verbascum lychnitis, Vitis vinifera, Quercus ilex*
Predicted reactivity	Alpha proteobacteria, Algae (brown and red) including *Galdieria sulphuraria*, *Arthrospira platensis*, Dicots, *Benincasa hispida*, *Kalanchoe fedtschenkoi*; Beta-proteobacteria, Conifers, Cryptomonads, Cyanobacteria (prochlorophytes), *Eragrostis tef,* Gamma-proeobacteria, Liverworts, *Manihot esculenta*, Marchantia polymorpha, *Miscanthus giganteus*, Monocots, Mosses, *Suaeda glauca, Welwitschia; Nannochloropsis* sp., *Picochlorum sp., Porphyridium purpureum, Zea mays, Zosteria marina* For detection in Rhodospirillaceae use product AS15 2955 Species of your interest not listed? Contact us

Not reactive in No confirmed exceptions from predicted reactivity are currently known

Additional information This antibody was used in:

Immunocytochemical staining of diatoms according to Schmid (2003) J Phycol 39: 139-153 and Wordemann et al. (1986) J Cell Biol 102: 1688-1698.

Immunofluorescence Dreier et al. (2012). FEMS Microbial Ecol., March 2012.

Western blot and tissue printing during a student course Ma et al. (2009).

As a loading control Sun et al. (2020).

Protocol for Rubisco quantification using this antibody can be found here.

Selected references

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Zhou et al. (2024). Antagonistic systemin receptors integrate the activation and attenuation of systemic wound signaling in tomato. Dev Cell. 2024 Nov 26:S1534-5807(24)00670-1. doi: 10.1016/j.devcel.2024.11.005.
Pica et al. (2024). Functional ecological traits in young and adult thalli of canopy-forming brown macroalga Gongolaria barbata (Phaeophyta) from a transitional water system. PeerJ. 2024 Sep 12:12:e17959. doi: 10.7717/peerj.17959.
Phukan et al. (2024). Externally supplied ascorbic acid moderates detrimental effects of UV-C exposure in cyanobacteria. Photochem Photobiol Sci. 2024 Jul 12. doi: 10.1007/s43630-024-00612-8.
Zhao et al. (2024). Psb28 protein is indispensable for stable accumulation of PSII core complexes in Arabidopsis.Plant J. 2024 May 26. doi: 10.1111/tpj.16844.
Nuamzanei et al. (2024). Impact of polyvinyl chloride (PVC) microplastic on growth, photosynthesis and nutrient uptake of Solanum lycopersicum L. (Tomato). Environ Pollut. 2024 Apr 16:123994. doi: 10.1016/j.envpol.2024.123994.
Ciesielska et al. (2024). S2P2-the chloroplast-located intramembrane protease and its impact on the stoichiometry and functioning of the photosynthetic apparatus of A. thaliana. Front Plant Sci. 2024 Mar 15:15:1372318. doi: 10.3389/fpls.2024.1372318.
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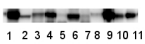
Rredhi et al. (2023). The UV-A Receptor CRY-DASH1 Up- and Downregulates Proteins Involved in Different Plastidial Pathways. J Mol Biol. 2023 Sep 10:168271.doi: 10.1016/j.jmb.2023.168271.
Hao and Malnoë (2023). A Simple Sonication Method to Isolate the Chloroplast Lumen in Arabidopsis thaliana.Bio Protoc. 2023 Aug 5; 13(15): e4756.
Chen et al. (2023) Producing fast and active Rubisco in tobacco to enhance photosynthesis. Plant Cell. 2023;35(2):795-807. doi:10.1093/plcell/koac348
Garcia et al. (2023) Effects of RuBisCO and CO2 concentration on cyanobacterial growth and carbon isotope fractionation [published online ahead of print, 2023 Jan 5]. Geobiology. 2023;10.1111/gbi.12543. doi:10.1111/gbi.12543
Minagawa, Dann. (2023) Extracellular CahB1 from Sodaline magerasimenkoae IPPAS B-353 Acts as a Functional Carboxysomal beta-Carbonic Anhydrase in Synechocystis sp. PCC6803. Plants (Basel). 2023;12(2):265. Published 2023 Jan 6. doi:10.3390/plants12020265
Vidal-Meireles, et al. (2023)The lifetime of the oxygen-evolving complex subunit PSBO depends on light intensity and carbon availability in Chlamydomonas. Plant Cell Environ. 2023;46(2):422-439. doi:10.1111/pce.14488
Capo-Bauca et al. (2023). Carbon assimilation in upper subtidal macroalgae is determined by an inverse correlation between Rubisco carboxylation efficiency and CO2 concentrating mechanism effectiveness. New Phytol. 2023;237(6):2027-2038. doi:10.1111/nph.18623

Western blot



0.25 µg of chlorophyl a/lane from *Spinacia oleracea* (1), *Synechococcus* PCC 7942 (2), C*yanophora paradoxa* (3), *Heterosigma akashiwo* (4), *Thalassiosira pseudonana* (5), *Euglena gracilis* (6), *Micromonas pusilla* (7), *Chlamydomonas reinhardtii* (8), *Porphyra* sp (9), *Gonyaulax polyedra* (10), *Emiliania huxleyi* (11) extracted with PEB (**AS08 300**), were separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **nitrocellulose**. Filters were blocked 1h with 2% low-fat **milk powder** in TBS-T (0.1% TWEEN 20) and probed with anti-RbcL antibody (AS03 037, **1:50 000**, 1h) and secondary anti-rabbit (**1:20000,** 1 h) antibody (HRP conjugated, recommended secondary antibody AS09 602) in TBS-T containing 2% low fat milk powder. Antibody incubations were followed by washings in TBS-T. All steps were performed at RT with agitation. Blots were developed for 5 min with ECL Advance detection reagent according the manufacturers instructions (GE Healthcare). Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

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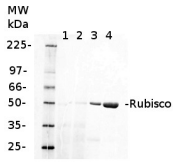
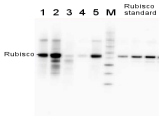


1 µg of chlorophyll from Cryptophyte samples **(1,2)** and 1 µg of chlorophyll **(3)** or 10 µg of total protein **(4)** from *Arabidopsis thaliana* leaves extracted either with 2 ml of 100 mM TrisHCl, 50 mM EDTA, 250 mM NaCl, 0.05% SDS (Sample 1) or 10 mL of 50 mM Hepes-KOH (pH 7.8), 330 mM sorbitol, 10 m EDTA, 5 mM NaCl, 5 mM MgCl2, 5 mM sodium ascorbate and 0.2% BSA (Sample 2). Samples were denatured with 1:1 Amersham WB Loading Bufferv at 70°C for 10 min and were separated on pre-casted 13.5% Amersham WB gel and blotted for 30 min to Amersham WB PVDF using wet transfer. Blots were blocked with 2% Amersham ECL Blocking Agent for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 (rabbit anti-Rubisco AS03 037) for 1.5 h 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. Membrane was cut in half and left part was incubated in anti-rabbit DyLight® 550 secondary antibody from Agrisera (AS11 1782) diluted to 1:2 000 in TBST for 1h at RT with agitation. The blot was scanned using Cy3 channel of Amersham WB System.

Courtesy of Dr. Małgorzata Wessels, Agrisera



2 µg of total protein from various plant extracts (**1-5**) extracted with PEB (**AS08 300**) separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Markers MagicMarks (Invitrogen) (**M**) and Rubisco protein standard (AS01 017S) at **0.0625 pmol, 0.125 pmol, 0.25 pmol**.

Following standard western blot procedure this image has been obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad). The contour tool of the software is used to the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample.

Note: Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5 ug total protein, depending on the abundance of the target protein.
