Agrisera

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product AS03 037

RbcL | Rubisco large subunit, form I and form II

product information

Background

This antibody is especially suitable for quantifying of Rubisco in plant and algal samples.

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the rate-limiting step of CO2 fixation in photosynthetic organisms. It is demonstrably homologous from purple bacteria to flowering plants and consists of two protein subunits, each present in 8 copies. In plants and green algae, the large subunit (~55 kDa) is coded by the chloroplast rbcL gene, and the small subunit (15 kDa) is coded by a family of nuclear rbcS genes.

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 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.

Tested applications

Immunofluorescence/confocal Immunolocalization (IL) (IF), Immunogold (IG), Tissue Printing (TP), Western blot (WB)

Related products

AS03 037A | Anti-RbcL | Rubisco large subunit, form I and form II (50 µg affinity purified), rabbit antibodies
AS03 037-HRP| Anti-RbcL | Rubisco large subunit, form I and form II (40 µg, HRP-conjugated), rabbit antibodies
AS15 2955 | Anti-RbcL II | Rubisco large subunit, form II (50 µl), rabbit antibodies
AS15 2955S | RbcL II | Rubisco form II positive control/quantitation standard
AS01 017 | Anti-RbcL | Rubisco large subunit, form I, chicken antibodies
AS01 017S | Rubisco protein standard for quantitative western blot or positive control
AS03 037PRE | Rubisco large subunit, pre-immune serum
AS09 409 | Rubisco quantitation kit
AS15 2994 | Rubisco ELISA quantitation kit
AS07 218 | Anti-Rubisco | 557 kDa hexadecamer, rabbit antibodies to a whole protein
AS07 259 | Anti-RbcS | Rubisco small subunit (SSU), rabbit antibodies

Plant and algal protein extraction buffer

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 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 (WB)

Expected | apparent MW

52.7 kDa (*Arabidopsis thaliana*), 52.5 kDa (cyanobacteria), 52.3 (*Chlamydomonas reinhardtii*)

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Confirmed reactivity

Agostis stolonifera cv. 'Penncross', Arabidopsis thaliana, Apium graveolens, Artemisia annua, Attheya longicornis, Baculogypsina sphaerulata (benthic foraminifer), Beta vulgaris, Begonia sp., *Bienertia sinuspersici, Brassica napus, Kandelia candel, Chaetoceros furcellatus, Colobanthus quitensis, Cicer arietinum, Chlamydomonas raudensis, Chlamydomonas reinhardtii, Colobanthus quitensis* Kunt Bartl, *Coscinodiscus concinnus, Cyanophora paradoxa, Cylindrospermopsis raciborskii* CS-505, *Cynara cardunculus, Emiliana huxleyi, Euglena gracilis, Fortunella margarita* Swingle, *Fraxinus mandshurica, Fucus vesiculosus, Glycine max, Gonyaulax polyedra, Guzmania* hybrid, *Heterosigma akashiwo, Hordeum vulgare, Jatropha curcas, Karenia brevis* (C.C.Davis) s) G.Hansen & Ø.Moestrup (Wilson isolate), *Liquidambar formosana, Malus domestica, Medicago truncatula, Micromonas pusila, Nicotiana benthamiana, Petunia hybrida* cv. Mitchell, *Phaeodactylum tricornutum, Physcomitrella patens, Porosira glacialis,, Porphyra sp., Ricinus communis, Robinia pseudoacacia, Saccharum* sp., *Schima superba, Skeletonema marinoi, Stanleya pinnata, Spinacia oleracea,* lichens, *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), Dicots, *Benincasa hispida*, Beta-proteobacteria, Conifers, Cryptomonads, Cyanobacteria (prochlorophytes), Gamma-proeobacteria, Liverworts, Monocots, Mosses, *Suaeda glauca, Welwitschia, Zosteria marina*

For detection in Rhodospirillaceae use product AS15 2955

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).

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

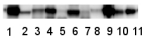
Selected references

Saha et al. (2019). Dynamics of protein accumulation from the 3'end of viral RNA is different from the rest of the genome in potato virus A infection. J Virol. 2019 Jul 24. pii: JVI.00721-19. doi: 10.1128/JVI.00721-19. (loading control)
Schober et al. (2019). Organelle Studies and Proteome Analyses on Mitochondria and Plastids Fractions from the Diatom Thalassiosira pseudonana. Plant Cell Physiol. 2019 Jun 10. pii: pcz097. doi: 10.1093/pcp/pcz097.
Lacour et al. (2019). Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the Arctic diatom Chaetoceros neogracilis. Polar Biol (2019). https://doi.org/10.1007/s00300-019-02507-2.
Contreras et al. (2019). UV-B shock induces photoprotective flavonoids but not antioxidant activity in Antarctic Colobanthus quitensis (Kunth) Bartl. Environmental and Experimental Botany, Volume 159, March 2019, Pages 179-190
Deng et al. (2019). Integrated proteome analyses of wheat glume and awn reveal central drought response proteins under water deficit conditions. J Plant Physiol. 2019 Jan;232:270-283. doi: 10.1016/j.jplph.2018.11.011.
Kong et al. (2018) Interorganelle Communication: Peroxisomal MALATE DEHYDROGENASE2 Connects Lipid Catabolism to Photosynthesis through Redox Coupling in Chlamydomonas. Plant Cell. 2018 Aug;30(8):1824-1847. doi: 10.1105/tpc.18.00361
Pao et al. (2018). Lamelloplasts and minichloroplasts in Begoniaceae: iridescence and photosynthetic functioning. J Plant Res. 2018 Mar 2. doi: 10.1007/s10265-018-1020-2. (ImmunoGold)
Deng et al. (2018). Comparative Proteome Analysis of Wheat Flag Leaves and Developing Grains Under Water Deficit. Front Plant Sci. 2018 Apr 10;9:425. doi: 10.3389/fpls.2018.00425. eCollection 2018.
Ravi et al. (2018). Separation Options for Phosphorylated Osteopontin from Transgenic Microalgae Chlamydomonas reinhardtii. Int J Mol Sci. 2018 Feb 16;19(2). pii: E585. doi: 10.3390/ijms19020585.
Wu et al. (2018). Control of Retrograde Signaling by Rapid Turnover of GENOMES UNCOUPLED 1. Plant Physiol. 2018 Jan 24. pii: pp.00009.2018. doi: 10.1104/pp.18.00009.
_im et al. (2017). Effect of cell cycle arrest on intermediate metabolism in the marine diatom Phaeodactylum tricornutum. Proc Natl Acad Sci U S A. 2017 Sep 19;114(38):E8007-E8016. doi: 10.1073/pnas.1711642114.
Arena et al. (2017). Eco-physiological and Antioxidant Responses of Holm Oak (Quercus ilex L.) Leaves to Cd and Pb. Water, Air, & Soil Pollution December 2017, 228:459.
Jespersen et al. (2017). Metabolic Effects of Acibenzolar-S-Methyl for Improving Heat or Drought Stress in Creeping Bentgrass. Front Plant Sci. 2017 Jul 11;8:1224. doi: 10.3389/fpls.2017.01224. eCollection 2017. (western blot, Agostis stolonifera cv. 'Penncross')
Neto et al. (2017). Cyclic electron flow, NPQ and photorespiration are crucial for the establishment of young plants of

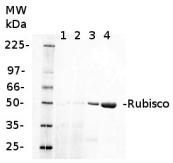
Ricinus communis and Jatropha curcas exposed to drought. Plant Biol (Stuttg). 2017 Apr 12. doi: 10.1111/plb.12573. (Jatropha curcas and Ricinus communis, western blot)
Ribeiro et al. (2017). Increased sink strength offsets the inhibitory effect of sucrose on sugarcane photosynthesis. J Plant Physiol. 2017 Jan;208:61-69. doi: 10.1016/j.jplph.2016.11.005.
Baumgart et al. (2017). Heterologous expression of the Halothiobacillus neapolitanus carboxysomal gene cluster in Corynebacterium glutamicum. J Biotechnol. 2017 Mar 27. pii: S0168-1656(17)30124-4. doi: 10.1016/j.jbiotec.2017.03.019.
Kolesinski et al. (2017). Is RAF1 protein from Synechocystis sp. PCC 6803 really needed in the cyanobacterial Rubisco assembly process? Photosynth Res. 2017 Jan 20. doi: 10.1007/s11120-017-0336-4.
Castiglia et al. (2016). High-level expression of thermostable cellulolytic enzymes in tobacco transplastomic plants and their use in hydrolysis of an industrially pretreated Arundo donax L. biomass.Biotechnol Biofuels. 2016 Jul 22;9:154. doi: 10.1186/s13068-016-0569-z. eCollection 2016.
Meng et al. (2016). Physiological and proteomic responses to salt stress in chloroplasts of diploid and tetraploid black locust (Robinia pseudoacacia L.). Sci Rep. 2016 Mar 15;6:23098. doi: 10.1038/srep23098
Heinnickel et al. (2016). Tetratricopeptide repeat protein protects photosystem I from oxidative disruption during assembly. Proc Natl Acad Sci U S A. 2016 Mar 8;113(10):2774-9. doi: 10.1073/pnas.1524040113
Young et al. (2015). Antarctic phytoplankton down-regulate their carbon-concentrating mechanisms under high CO2 with no change in growth rates. Marine Ecology Progress Series 532:13-28.
Li at al. (2015). Salt stress response of membrane proteome of sugar beet monosomic addition line M14. J Proteomics. 2015 Apr 3. pii: S1874-3919(15)00109-8. doi: 10.1016/j.jprot.2015.03.025.
Krasuska et al. (2015). Switch from heterotrophy to autotrophy of apple cotyledons depends on NO signal. Planta. 2015 Jul 18.
Janeczko et al. (2015). Disturbances in production of progesterone and their implications in plant studies. Steroids. 2015 Feb 9. pii: S0039-128X(15)00054-9. doi: 10.1016/j.steroids.2015.01.025.
Kolesinski et al. (2014). Rubisco Accumulation Factor 1 from Thermosynechococcus elongatus participates in the final stages of ribulose-1,5-bisphosphate carboxylase/oxygenase assembly in Escherichia coli cells and in vitro. FEBS J. 2014 Jul 12. doi: 10.1111/febs.12928
Pandey and Pandey-Rai (2014). Modulations of physiological responses and possible involvement of defense-related secondary metabolites in acclimation of Artemisia annua L. against short-term UV-B radiation. Planta. 2014 Jul 15.
Liang et al. (2014). Cyanophycin mediates the accumulation and storage of fixed carbon in non-heterocystous filamentous cyanobacteria from coniform mats. PLoS One. 2014 Feb 7;9(2):e88142. doi: 10.1371/journal.pone.0088142. eCollection 2014. (immunogold)
Mayfield et al. (2014). Rubisco Expression in the Dinoflagellate Symbiodinium sp. Is Influenced by Both Photoperiod and Endosymbiotic Lifestyle. Mar Biotechnol, Jan 22.

Application example

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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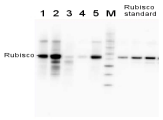
1 µg of chlorophyll from Cryptophyte samples **(1,2)** and 1 µg of chlorophyll **(3)** or 10 µg of total protein **(4)** from *Arabidopsis thaliana* leaves extracted either with 2ml of 100 mM TrisHCl, 50 mM EDTA, 250 mM NaCl, 0.05% SDS (Sample 1) or 10 mL of 50 mM Hepes-KOH (pH 7.8), 330 mM sorbitol, 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 70C 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 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.
