

product **AS03 037**

RbcL | Rubisco large subunit, form I and form II (50 µl)

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 <i>rbcL</i> gene, and the small subunit (15 kDa) is coded by a family of nuclear <i>rbcS</i> genes.
immunogen	KLH-conjugated synthetic peptide conserved across all known plant, algal and (cyano)bacterial RbcL protein sequences (form I L8S8 and form II L2), including <i>Arabidopsis thaliana</i> AtCg00490, <i>Hordeum vulgare</i> P05698, <i>Oryza sativa</i> P0C510, <i>Chlamydomonas reinhardtii</i> P00877, <i>Synechococcus</i> PCC 7920 A5CKC5
antibody format	rabbit polyclonal, serum, lyophilized
quantity	50 µl 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	western blot (WB), tissue printing (TP), immunofluorescence/confocal microscopy (IF), immunogold (IG)
related products	AS03 037-100 anti-RbcL, Rubisco large subunit, form I and form II (100 µl) AS03 037-200 anti-RbcL, Rubisco large subunit, form I and form II, larger pack size (200 µl) of AS03 037, rabbit antibody AS03 037-HRP anti-RbcL, Rubisco large subunit, form I and form II. HRP conjugated AS03 037PRE Rubisco large subunit, pre-immune serum AS09 409 Rubisco quantitation kit AS01 017 anti-RbcL Rubisco large subunit, form I, chicken antibody AS07 218 anti-Rubisco 557 kDa hexadecamer, rabbit antibody to a whole protein AS01 017S anti-RbcL Rubisco protein standard for quantitative western blot or positive control AS07 259 anti-RbcS Rubisco small subunit (SSU), rabbit antibody AS07 222 RbcS Rubisco small subunit (SSU) from pea, rabbit antibody matching Agrisera secondary antibody
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 simultaneous western blot detection with RbcL, PsbA and PsaC antibodies.

application information

recommended dilution	1: 5000 - 10 000 with standard ECL (WB), 1: 800 (TP), immunofluorescence/confocal microscopy (IF), 1: 1000 (IG), 1: 250 for images see Prins et al. (2008), detailed protocol available on request
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expected apparent MW	52.7 kDa (<i>Arabidopsis thaliana</i>), 52.5 kDa (cyanobacteria), 52.3 (<i>Chlamydomonas reinhardtii</i>)
confirmed reactivity	<i>Arabidopsis thaliana</i> , <i>Apium graveolens</i> , <i>Artemisia annua</i> , <i>Baculogypsina sphaerulata</i> (benthic foraminifer), <i>Bienertia sinuspersici</i> , <i>Kandelia candel</i> , <i>Cicer arietinum</i> , <i>Chlamydomonas raudensis</i> , <i>Chlamydomonas reinhardtii</i> , <i>Colobanthus quitensis</i> Kunt Bartl, <i>Cyanophora paradoxa</i> , <i>Cylindrospermopsis raciborskii</i> CS-505, <i>Emiliana huxleyi</i> , <i>Euglena gracilis</i> , <i>Fraxinus mandshurica</i> , <i>Fucus vesiculosus</i> , <i>Glycine max</i> , <i>Gonyaulax polyedra</i> , <i>Guzmania hybrid</i> , <i>Heterosigma akashiwo</i> , <i>Hordeum vulgare</i> , <i>Karenia brevis</i> (C.C.Davis) s) G.Hansen & Ø.Moestrup (Wilson isolate), <i>Liquidambar formosana</i> , <i>Malus domestica</i> , <i>Medicago truncatula</i> , <i>Micromonas pusila</i> , <i>Nicotiana benthamiana</i> , <i>Physcomitrella patens</i> , <i>Porphyra sp.</i> , <i>Schima superba</i> , <i>Stanleya pinnata</i> , <i>Spinacia oleracea</i> , lichens, <i>Symbiodinium sp.</i> , <i>Synechococcus PCC 7942</i> , <i>Thalassiosira pseudonana</i> , <i>Thermosynechococcus elongatus</i> , <i>Prochlorococcus sp.</i> (surface and deep water ecotype), <i>Triticum aestivum</i> , dinoflagellate endosymbionts (genus <i>Symbiodinium</i>), extreme acidophilic verrucomicrobial methanotroph <i>Methylococcoides burtonii</i> strain SolV, <i>Thalassiosira punctigera</i> , <i>Tisochrysis lutea</i> , <i>Vitis vinifera</i>
predicted reactivity	di and monocots, conifers, mosses, liverworts, welwitschia, green algae, red algae, brown algae, cryptomonad, cyanobacteria including prochlorophytes, gamma-proteobacteria, beta-proteobacteria, alpha proteobacteria, <i>Suaeda glauca</i>
not reactive in	no confirmed exceptions from predicted reactivity known in the moment
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).
selected references	Krasuska et al. (2015). Switch from heterotrophy to autotrophy of apple cotyledons depends on NO signal. <i>Planta</i>. 2015 Jul 18. Janeczko et al. (2015). Disturbances in production of progesterone and their implications in plant studies. <i>Steroids</i>. 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 <i>Thermosynechococcus elongatus</i> participates in the final stages of ribulose-1,5-bisphosphate carboxylase/oxygenase assembly in <i>Escherichia coli</i> cells and in vitro. <i>FEBS J</i>. 2014 Jul 12. doi: 10.1111/febs.12928 Pandey and Pandey-Raj (2014). Modulations of physiological responses and possible involvement of defense-related secondary metabolites in acclimation of <i>Artemisia annua</i> L. against short-term UV-B radiation. <i>Planta</i>. 2014 Jul 15. Liang et al. (2014). Cyanophycin mediates the accumulation and storage of fixed carbon in non-heterocystous filamentous cyanobacteria from coniform mats. <i>PLoS One</i>. 2014 Feb 7;9(2):e88142. doi: 10.1371/journal.pone.0088142. eCollection 2014. (immunogold) Mayfield et al. (2014). Rubisco Expression in the Dinoflagellate <i>Symbiodinium sp.</i> Is Influenced by Both Photoperiod and Endosymbiotic Lifestyle. <i>Mar Biotechnol</i>, Jan 22. Seveso et al. (2013). Exploring the effect of salinity changes on the levels of Hsp60 in the tropical coral <i>Seriatopora caliendrum</i>. June 29. (<i>Symbiodinium sp.</i> antibody reactivity) Losh et al. (2013). Rubisco is a small fraction of total protein in marine phytoplankton. <i>New Phytol</i>. April 198 (1):52-8. Chen et al. (2013). Photosynthetic and antioxidant responses of <i>Liquidambar formosana</i> and <i>Schima superba</i> seedlings to sulfuric-rich and nitric-rich simulated acid rain. <i>Plant Physiol & Biochem</i>. Li et al. (2012). MAP Kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in <i>Arabidopsis</i>. <i>New Phytol</i>. ahead of print - RbcL antibody used as loading control. Zhao et al. (2011). Expansins are involved in cell growth mediated by abscisic acid and indole-3-acetic acid under drought stress in wheat. <i>Plant Cell Rep</i>. Nov (RbcL antibody used as a loading control)

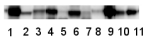
Johnson (2011). Manipulating RuBisCO accumulation in the green alga, *Chlamydomonas reinhardtii*. Plant Mol Biol. May 24.

Kubien et al. (2011). Quantification of the amount and activity of Rubisco in leaves. Methods Mol Biol. 2011;684:349-62.

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Zilliges et al (2011) The Cyanobacterial Hepatotoxin Microcystin Binds to Proteins and Increases the Fitness of *Microcystis* under Oxidative Stress Conditions. PLoS ONE.

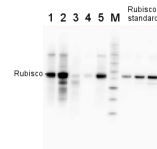
application example



0.25 µg of chlorophyll a/lane from *Spinacia oleracea* (1), *Synechococcus* PCC 7942 (2), *Cyanophora 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).

application example

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.