

Agrisera

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Product no **AS03 037**

RbcL | Rubisco large subunit, form I (rabbit)

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 [A5CKG5](#)

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 simultaneous western blot detection with RbcL, PsbA and PsbC 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*, *Cannabis sativa* L., *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*, *Ficus carica*, *Fortunella margarita* Swingle, *Fraxinus mandshurica*, *Fucus vesiculosus*, *Glycine max*, *Gonyaulax polyedra*, *Guzmania* hybrid, *Heterosigma akashiwo*, *Hevea*, *Hordeum vulgare*, *Jatropha curcas*, *Karenia brevis* (C.C.Davis) s) G.Hansen & Ø.Moestrup (Wilson isolate), *Lathyrus sativus*, *Liquidambar formosana*, *Malus domestica*, *Medicago truncatula*, *Micromonas pusilla*, *Nicotiana benthamiana*, *Nicotiana tabacum*, *Petunia hybrida* cv. Mitchell, *Phaeodactylum tricornutum*, *Physcomitrella patens*, *Pisum sativum*, *Porosira glacialis*, *Porphyra* sp., *Ricinus communis*, *Robinia pseudoacacia*, *Saccharum* sp., *Schima superba*, *Skeletonema costatum* (diatom), *Skeletonema marinoi* (diatom), *Stanleya pinnata*, *Spinacia oleracea*, lichens, *Symbiodinium* sp., *Synechococcus* PCC 7942, *Synechococcus elongatus* UTEX 2973, *Rhodo* *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 fumarolicum* 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, *Chlorella vulgaris*, Conifers, Cryptomonads, Cyanobacteria (prochlorophytes), Gamma-proteobacteria, Liverworts, *Manihot esculenta*, Monocots, Mosses, *Suaeda glauca*, *Welwitschia*; *Nannochloropsis* sp., *Zostera 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).

Protocol for Rubisco quantification using this antibody can be found [here](#).

For high resolution images, please visit the specific product page at www.agrisera.com

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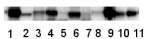
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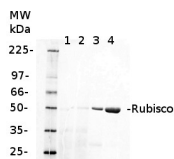
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Application example

Western blot

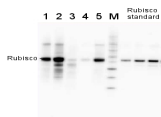


0.25 µg of chlorophyll a/lane from *Spinacia oleracea* (1), *Synechococcus* PCC 7942 (2), *Cyanophora paradoxa* (3), *Heterosigma akashiwo* (4), *Thalassiosira pseudonana* (5), *Euglena gracilis* (6), *Micromonas pusilla* (7), *Chlamydomonas reinhardtii* (8), *Porphyra* sp (9), *Gonyaulax polyedra* (10), *Emiliana 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**, 1h) 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).



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 Buffer 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. Malgorzata 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.