COSMO BIO CO., LTD.
Inspiration for Life Science

Agrisera
Antibodies for plant sciences

Product: Anti-RbcL (Rubisco large subunit) Global antibody

Product no: AS01 017

Product Information

Antibody clonality: Polyclonal

Raised in: Hen

Purity: Total IgY purified by PEG precipitation in PBS pH 8 with 0.02 % sodium azide

Quantity: 50 µl

Concentration: 16 µg/µl

Antibody form: Liquid . Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid or lyophilized material adhering to the cap or sides of the tubes.

Storage instructions: 4°C Do not freeze. Make aliquots.

Related products:
AS01 017S Rubisco protein standard for quantitation or to be used as a positive control.

AS03 037 Anti-RbcL (Form I and Form II) Global antibody rised in a rabbit

References:

MacKenzie et al. (2005) Inorganic carbon acclimation in *Synechococcus elongatus* alters the dynamics of macromolecular pools and photosynthetic fluxes in response to increased light. Photosynthesis Research 85: 341 - 357

Mackenzie et al. (2004) Environmental change provokes rapid macromolecular reallocations in a static population of photobionts in the lichen Lobaria pulmonaria. Lichenologist 36:425-433

Schofield et al. (2003) Changes in macromolecular allocation in nondividing algal symbionts allow for photosynthetic acclimation in the lichen Lobaria pulmonaria. New Phytologist, Vol. 159, Issue 3

Background

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the rate-limiting step of CO2 fixation in photosynthesis.
It is one of the most abundant proteins on Earth and its homology has been demonstrated from purple bacteria to flowering plants.

Immunogen: The antibody was raised against a peptide target conserved in the large subunit of Rubisco.

Peptide target absolutely conserved in Taxa:
Green algae, Liverworts, Mosses, Welwitschia, Conifers, Eudicots, Monocots, Cyanobacteria incl. Prochlorophytes

Target present but variant in some species:
Ferns, Euglenoids

Target conserved in some members, range of conservation not fully known:
gamma-proteobacteria, beta-proteobacteria, alpha-proteobacteria

Target present but imperfectly conserved in:
Red algae, Diatoms, Cryptomonad, Haptophytes, Brown Algae

Target not conserved in Type II RUBISCO found in Dinoflagellates and some photosynthetic bacteria.

Antibodies are intended for the research use only not for diagnostic or therapeutic use.

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Distributor

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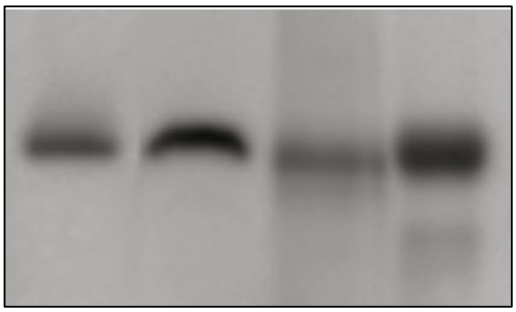
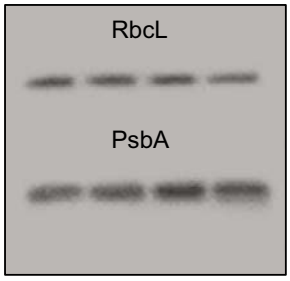
Application information

Working dilution: 1:50 000 with ECL Advance or 1: 20 000 with regular ECL



Detection of Rubisco large subunits in following samples: 1 – *Ulmus sp. ;* 2 - *Synechococcus* sp. PCC 7942; 3 – Marsh grass; 4 – Mixed phytoplankton.

Anti- RbcL antibody has been used experimentally to detect Rubisco protein using whole protein extracts from following species: Thiobacillus sp. ,cyanobacteria (*Synechococcus* sp. PCC 7942), red algae, mixed phytoplankton (dominated by diatoms) lichen *Lobaria pulmonaria, edicago sativa* L(Alfa alfa*),* Spinach, *Solanum tuberosum* (potato), *Pisum sativum* (pea),*Ulmus* (american elm), grass *Spartina alterniflora*.



Simultaneous detection of PsbA and RbcL using Agrisera Global antibodies in *Synechococcus* sp. 7942.
(2 ug of total cellular protein was loaded per lane)

Detailed experimental conditions are described on page 3

Expected molecular weight of RbcL mature chain (slightly longer precursors synthesized) on SDS PAGE:
Cyanobacteria: 52.5 kDa
Arabidopsis thaliana: 52.7 kDa
Chlamydomonas reinhardtii: 52.3 kDa

Immunolocalization – tested on a grass species, formaldehyde-fixed and paraffin-embedded tissue. Following the protocol from Gonzalez et al.Plant Physiol. V. 116, April 1998.

Antibody has been used as a control to ensure adequate permeabilization and fixation of toxic cyanobacterial cells in immunolabeling experiements. The method the protocol is based on: Orellana MV and MJ Perry. 1995. Optimization of an immunofluorescent assay of the internal enzyme ribulose-1,5-bisphosphate carboxylase (RubisCO) in single phytoplankton cells. J. Phycol. 31: 785-794.

Immunolabeling of intact cyanobacterial cells fixed with ethanol using a secondary anti-IgY antibody conjugated with a fluorochrome.

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Experimental conditions for a Western blot detection:

Sample preparation: Leaf tissue was weighed and snap frozen in liquid nitrogen and stored at -80°C until processing. Frozen leaves were placed in a pre-chilled mortar and ground in liquid nitrogen with a pestle until a fine powder was obtained. Algal cultures were centrifuged to form a pellet and frozen at -80°C.

A single extraction buffer was used for disruption and solubilization of all species. Samples were suspended in 140 mM Tris base, 105mM Tris-HCl, 0.5mM ethylenediaminetetraacetic acid (EDTA), 2% Lithium dodecyl sulfate (LDS), 10% glycerol, 0.1mg/mL PefaBloc SC (AEBSF) protease inhibitor (Roche). Leaf tissue was solubilized at 0.1 to 1.0 mg tissue per μL extraction buffer.

Samples suspended in extraction buffer were immediatly refrozen in liquid nitrogen and then sonicated with a microtip attachment at a setting of 30%, until just thawed. To avoid heating, samples were then refrozen immediately in liquid nitrogen.

Following disruption, samples were centrifuged for 3 min at 10 000 x g to remove insoluble material and unbroken cells. Check for color in the pellet, as this is the best indicator of incomplete breakage. The protein content was assayed using the Bio-Rad DC Protein Assay using bovine gamma-globulin in extraction buffer as a standard. Samples in lithium dodecyl sulphate extraction buffer were brought to 50 mM dithiothreitol (DTT) final concentration and the volume was adjusted with 1X sample buffer. Cellular extracts were then heated at 70°C for 5 min. Following heating, samples were pulsed briefly in a microfuge to collect all of the material at the bottom of the tube.

Gel electrophoresis: Proteins were separated by electrophoresis on 4-12% acrylamide gradient mini-gels (NuPAGE Bis-Tris gels, Invitrogen) in MES SDS running buffer (Invitrogen) in an XCell Sure-Lock Tank (Invitrogen). Gels were electrophoresed at 200V for 35 minutes. Following electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes pre-wetted in methanol and equilibrated in 1X transfer buffer (Invitrogen) using the XCell blot module (Invitrogen) for 80 minutes at 30V.

Western Blot development: Blots were blocked immediately following transfer in 2% ECL Advance blocking reagent (GE Healthcare) 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 or overnight at 4C. Primary and secondary antibodies were used at a dilution of 1:10 000 to 1:100 000 in 2% ECL Advance Blocking solution. Blots were incubated in the primary antibody solution for 1h at room temperature 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 room temperature with agitation. Blots were incubated in secondary antibody (anti-hen IgY horse radish peroxidase conjugated, from Abcam) diluted to 1:50 000 in 2% ECL Advance blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with ECL Advance detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

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