COSMO BIO CO., LTD.
Inspiration for Life Science

Agrisera
Antibodies for plant sciences

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

Product no: AS03 037

Product Information

Antibody clonality: Polyclonal

Raised in: Rabbit

Purity: Affinity purified IgG in PBS pH 7.4

Quantity: 100 µg

Antibody form: Lyophilized. For reconstitution please add 100 µl of sterile water.
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: -20°C or -80°C long Term storage (years). Please, avoid freezing and thawing of antibodies.
Make aliquots instead.

Related products:
AS01 017 Anti-RbcL (Form I) Global antibody rised in a hen
AS01 017S Rubisco protein standard for quantitation or to be used as a positive control.

References:

Six et al (2007) Contrasting photoacclimation strategies in oceanic and lagoon ecotypes of the eukayotic picoplankter Ostreococcus. Accepted pending revision, Limnology and Oceanography, 18 May 2007
Brown et al (2007) Resource dynamics during infection of Micromonas pusilla by virus MpV-Sp1. accepted by Environmental Microbiology, 28 May 2007.
Morash et al. (2007) Macromolecular dynamics of the photosynthetic system over a seasonal developmental progression in Spartina alterniflora. Canadian Journal of Botany, in press.
Cahoon et al. (2004) Analysis of developing maize plastids reveals two mRNA stability classes correlating with RNA polymerase type EMBO reports 5, 8, 801–806 (2004)(published on line)

Background

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the rate-limiting step of CO2 fixation in photosynthesis.
It is an enzyme that is demonstrably homologous from purple bacteria to flowering plants.

Immunogen: The antibody was raised against a peptide target conserved in all known Form I and Form II RbcL proteins (Rubisco large subunit).

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	

Peptide target is conserved in Type I and II RUBISCO and therefore will also recognize Rubisco protein from Dinoflagellates and some photosynthetic bacteria.

Antibody is therefore especially suitable for work with samples from Dinoflagellates, Haptophytes and Ochrophytes (diatoms, Raphidophytes, brown algae) as well as higher plants.

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

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Distributor

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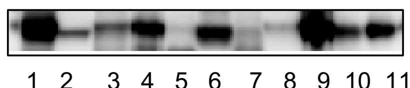
Application information

Working dilution: 1:50 000 with ECL Advance or 1: 5000 with regular ECL

Detection of Rubisco protein in different extracts.



1 2 3 4 5 6 7 8 9 10 11

Following lanes contained whole cell extracts from:

1. **Higher plants:** Spinach;
2. **Cyanobacteria:** Synechococcus PCC 7942;
3. **Glaucocystophyte:** *Cyanophora paradoxa*;
4. **Ochrophyte - Raphidophyte:** *Heterosigma akashiwo*;
5. **Ochrophyte - Diatom:** *Thalassiosira pseudonana*;
6. **Euglenoid:** *Euglena gracilis*;
7. **Prasinophyte:** *Micromonas pusilla*;
8. **Green Algae -Chlorophyte:** *Chlamydomonas reinhardtii*;
9. **Red Algae:** *Porphyra* sp.;
10. **Dinoflagellate:** *Gonyaulax polyedra*;
11. **Haptophyte:** *Emiliania huxleyi*;

Loading based on equal Chlorophyll a - 0.25 µg/line
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

Immunofluorescence/confocal microscopy: Anti-RbcL antibody has been applied for immunocytochemical straining of diatoms according to: Schmid AMM (2003) Endobacteria in the diatom Pinnularia (Bacillariophyceae). II.Host cell cycle-dependent translocation and transient chloroplant scars. J.Phycol. 39: 139-153
Wordemann, L., McDonald, K. L. & Cande, Z. W. (1986) The distribution of cytoplasmic microtubules throughout the cell cycle of the centric diatom Stephanopyxis turris: their role in nuclear migration and positioning the mitotic spindle during cytokinesis. J. Cell Biol. 102:1688-98.

Glutaraldehyde fixation has been applied and 0.1% Triton X100 for cell permeabilization.
Detergent treatment was done for 30 min to overnight and 2-3 hours of treatment were preferable.

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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 (goat anti-rabbit 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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