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Product no AS01 017

Anti-RbcL | Rubisco large subunit, form I (chicken)

Product information

Immunogen	KLH-conjugated synthetic peptide derived from all known plant,algal and cyanobacterial RbcL (Rubisco large subunit of Rubisco Form I) sequences, including *Arabidopsis thaliana* UniProt: O03042, TAIR: AtCg00490, *Synechococcus* sp. Q3ALL1
Host	Chicken
Clonality	Polyclonal
Purity	Purified, total IgY (chicken egg yolk immunoglobulin) in PBS pH 8. Contains 0.02 % sodium azide.
Format	Liquid
Quantity	50 µl
Storage	Store at 4°C; make aliquots to avoid working with a stock. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	Peptide target used to elicit this antibody is not conserved in type II Rubisco found in dinoflagellates and some photosynthetic bacteria

Application information

Recommended dilution	(IL) tested on a grass species, formaldehyde-fixed and paraffin-embedded tissue following the protocol from Gonzalez et al, (1998) Plant Physiol, V, 116, 1 : 10 000-1 : 20 000, 2 µg of total cellular protein, (WB)
Expected | apparent MW	52.7 kDa (*Arabidopsis thaliana*), 52.5 kDa (cyanobacteria), 52.3 kDa (*Chlamydomonas reinhardtii*)
Confirmed reactivity	*Arabidopsis thaliana, Fragilariopsis cylindrus, Lobaria pulmonaria, Medicago sativa,* mixed phytoplankton, *Microcystis aeruginosa* PCC7806, *Pinus strobus*, *Pisum sativum, Solanum tuberosum, Spartina alterniflora, Spinacia oleracea, Synechococcus* sp. PCC7842, *Thiobacillus* sp. *Ulmus* sp.
Predicted reactivity	Algae, Dicots, Conifers, Liverworts, Mosses, Prochlorophytes, Welwitschia
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	This antibody detects RbcL protein from 102.6 fmoles and has been used as a control to ensure adequate permeabilization and fixation of toxic cyanobacterial cells in immunolabeling experiments (method based on: Orellana & Perry (1995) J Phycol 31: 785-794). Antibody has been used in immunolabelling of intact cyanobacterial cells fixed with ethanol using a secondary anti-IgY antibody conjugated with a fluorochrome. For Rubisco quantification using quantitative western blot technique, anti-RbcL antibody, (AS03 037) combined with Rubisco ready to use standard (AS01 017) is recommended.
Selected references	Guljamow et al. (2021) Diel Variations of Extracellular Microcystin Influence the Subcellular Dynamics of RubisCO in Microcystis aeruginosa PCC 7806. Microorganisms. 2021 Jun 10;9(6):1265. doi: 10.3390/microorganisms9061265. PMID: 34200971; PMCID: PMC8230624. (IF) Morin et al. (2019). Morin et al. (2019). Response of the sea-ice diatom Fragilariopsis cylindrus to simulated polar night darkness and return to light. Limnology and Oceanography. 9999, 2019, 1–20. (sea-ice diatom) Lv et al. (2019). Uncoupled Expression of Nuclear and Plastid Photosynthesis-Associated Genes Contributes to Cell Death in a Lesion Mimic Mutant. Plant Cell. 2019 Jan;31(1):210-230. doi: 10.1105/tpc.18.00813. Gellért et al. (2018). A single point mutation on the cucumber mosaic virus surface induces an unexpected and strong interaction with the F1 complex of the ATP synthase in Nicotiana clevelandii plants. Virus Res. 2018 Jun 2;251:47-55. doi: 10.1016/j.virusres.2018.05.005. Robert et al. (2015). Leaf proteome rebalancing in Nicotiana benthamiana for upstream enrichment of a transiently expressed recombinant protein. Plant Biotechnol J. 2015 Aug 19. doi: 10.1111/pbi.12452.

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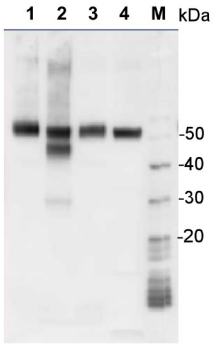
Application example



1 µg of total protein from samples such as *Arabidopsis thaliana* leaf **(1)** , *Hordeum vulgare* leaf **(2)**, *Zea mays* leaf **(3)**, *Chlamydomonas reinhardtii* total cell **(4)**, were extracted with Protein Extraction Buffer PEB (AS08 300). Samples were diluted with 1X sample buffer (NuPAGE LDS sample buffer (Invitrogen) supplemented with 50 mM DTT and heat at 70°C for 5 min and keept on ice before loading. Protein samples were separated on 4-12% Bolt Plus gels, LDS-PAGE and blotted for 70 minutes to PVDF using tank transfer. Blots were blocked immediately following transfer in 2% blocking reagent (GE RPN 2125; Healthcare) or 5% non-fat milk dissolved 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. Blots were incubated in the primary antibody at a dilution of 1: 10 000 (in blocking reagent) for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, and then washed 1x15 min and 3x5 min with TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, secondary antibody AS10 1489, Agrisera) diluted to 1:25 000 in blocking reagent for 1h at room temperature with agitation. The blots were washed as above. The blot was developed for 5 min with chemiluminescent detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 30 seconds.