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Product no AS09 409
Rubisco quantitation kit (Western blot)

Product information

Background	**The Rubisco quantitation kit is suitable for quantitation of Rubisco in plant and algal samples** using quantitative immunoblotting. It contains **anti-RbcL antibody, Rubisco protein standard** and the **Protein Extraction Buffer** for quantitative protein extraction. This kit is a good alternative to quantification of Rubisco by ELISA as it allows separation of Rubisco from a total complex cell protein mixture, which can be troublesome in case of ELISA method.
Immunogen	KLH-conjugated synthetic peptide was used to elicit anti-RbcL antibody. No baculovirus was used in production of this product.
Host	Primary antibody: Rabbit. Secondary antibody: Goat.
Clonality	Polyclonal
Quantity	1 x 50 µl of AS03 037, RbcL	Rubisco large subunit, form I and form II (amount enough for 50-100 Western blots)
	1 x 100 µl of AS01 017S, Rubisco protein standard (0.15 pmoles / µl, amount enough for generation of standard curve in 34 assays (standard curve: 0.0625 pmoles, 0.125 pmoles, 0.25 pmoles)
	2 x 2 ml of AS08 300, Protein extraction buffer (amount enough for 48 isolations of plant material, using 500 µl 1x PEB for 100 mg fresh weight) or 120 isolations of algal material (using 200 µl 1x PEB for cell amounts corresponding to 4-10 µg total chlorophyll)
	5 x 10 µl of AS09 602, Goat anti-rabbit IgG (H&L), HRP conjugated(amount enough for 50-100 Western blots)
	1 X 10 ml of AS16 ECL-N-10, AgriseraECL Bright 10 ml (5 ml of component A + 5 ml of component B, trial pack)
Storage	RbcL antibody and protein standard: Please store at -20°C (6 month) or -80°C for long term storage(years). Please, avoid freezing and thawing of reconstituted antibodies, make aliquots instead.PEB extraction buffer:Stable at RT for at least 1 month. For short-term storage please stoore (1 month) at 4°C and for long term storage (1 year) at -20°C.
Tested applications	Western blot (WB)
Related products	Rubisco/Carbon metabolism antibody collection
	AS15 2994	Rubisco ELISA quantitation kit
	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
	AS07 218	Anti-Rubisco	557 kDa hexadecamer, rabbit antibodies, to a whole protein
	AS07 259	Anti-RbcS	Rubisco small subunit (SSU), rabbit antibodies
	matching secondary antibody

Application information

Recommended dilution	1 : 5000-1 : 10000 (WB)
Expected | apparent MW	**Rubisco protein standard**: 52.7 kDa (*Arabidopsis thaliana*), 52.5 kDa (cyanobacteria), 52.3 (*Chlamydomonas reinhardtii*)
Confirmed reactivity	*Arabidopsis thaliana, Chlamydomonas reinhardtii, Cyanophora paradoxa, Cynara cardunculus var altilis, Emiliana huxleyi, Euglena gracilis, Gonyaulax polyedra, Heterosigma akashiwo, Micromonas pusila, Porphyra sp. Spinacia oleracea, Synechococcus PCC 7942, Thalassiosira pseudonana*

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Predicted reactivity	Alpha proteobacteria, Algae (brown and red), Beta-proteobacteria, Dicots, Conifers, Cryptomonad, Cyanobacteria, Gamma-proeobacteria, Liverworts, Mosses, Prochlorophytes, Pelwitschia Species of your interest not listed? Contact us
Additional information	**Quantitative western blot:** detailed method description, video tutorial Discussion over some critical aspects of quantitative western blot: Heidebrecht et al. (2009). Improved semiquantitative Western blot technique with increased quantification range. J. Immunological Methods. 35:40-48. For high resolution images, please visit the specific product page at www.agrisera.com
Selected references	Defez et al. (2019). Bacterial IAA-Delivery into Medicago Root Nodules Triggers a Balanced Stimulation of C and N Metabolism Leading to a Biomass Increase. Microorganisms. 2019 Sep 29;7(10). pii: E403. doi: 10.3390/microorganisms7100403. Sorrentino et al. (2018). Performance of three cardoon cultivars in an industrial heavy metal-contaminated soil: Effects on morphology, cytology and photosynthesis. J Hazard Mater. 2018 Jun 5;351:131-137. doi: 10.1016/j.jhazmat.2018.02.044. Mota et al. (2015). Effects of heavy metals on Cyanothece sp. CCY 0110 growth, extracellular polymeric substances (EPS) production, ultrastructure and protein profiles. J Proteomics. 2015 Apr 29;120:75-94. doi: 10.1016/j.jprot.2015.03.004. Thamatrakoln et al. (2013). Death-specific protein in a marine diatom regulates photosynthetic responses to iron and light availability. Proc Natl Acad Sci U S A. 2013 Dec 10;110(50):20123-8. doi: 10.1073/pnas.1304727110. Supplemental material describes western blot quantification method.

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

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Application example

2 µg of total protein from various plant extracts (**1-5**) extracted with Agriera Protein Extraction Buffer 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.

Agrisera Western Blot protocol and video tutorials