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This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

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Product no AS09 409set

Rubisco quantitation kit (Western blot)

Product information

This set contains the following products:
AS03 037, RbcL | Rubisco large subunit, form I and form II (50 µl)
AS01 017S, Rubisco protein standard (100 µl)
AS08 300, Protein extraction buffer (2 x 2 ml)
AS09 602, Goat anti-rabbit IgG (H&L), HRP conjugated(2 x 10 µl)

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 1 x 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) 2 x 10 µl of AS09 602, Goat anti-rabbit IgG (H&L), HRP conjugated (amount enough for 50-100 Western blots)
Storage	RbcL antibody and protein standard: Please store at -20°C (6 months) 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.

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*
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.
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.

Application example

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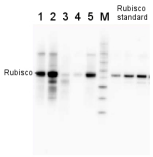

2 µg of total protein from various plant extracts (**1-5**) extracted with Agriera Protein Extraction Buffer PEB (**AS08 300**) separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Markers MagicMarks (Invitrogen) (**M**) and Rubisco protein standard (AS01 017S) at **0.0625 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