

product **AS01 017S**
RbcL | Rubisco protein standard

product information

background	<p>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.</p> <p>Source of a standard: RbcL protein purified from spinach.</p>
immunogen	does not apply
antibody format	does not apply
quantity	250 µl lyophilized, for reconstitution add 225 µl of milliQ water per each tube
storage	store lyophilized/reconstituted at -20 °C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.
tested applications	western blot (WB)
additional information	<p>Global antibodies are raised against highly conserved amino acid sequences in the RbcL protein. The RbcL protein standard can therefore be used in combination with global anti-RbcL antibodies to quantitate RbcL from a wide range of species.</p> <p>Quantitative western blot: detailed method description.</p>

application information

recommended dilution	<p>Standard curve: 3 loads are recommended (0.5, 2 and 4µl). For most applications a sample load of 0.2µg of chlorophyll will give a RbcL signal in this range.</p> <p>positive control: a 2µl load per well is optimal for most chemiluminescent detection systems. Higher standard concentration needs to be used to allow detection by Coomassie stains. Such gels with higher standard concentration can not be used for quantitation using chemiluminescence.</p> <p>This standard is stabilized and does not require heating before loading on the gel.</p>
expected apparent MW	52.7 kDa
confirmed reactivity	does not apply
predicted reactivity	does not apply

not reactive in | no confirmed exceptions from predicted reactivity currently known

additional information | **Concentration:** after adding 225 μ l of milliQ water final concentration of the standard is 0.15 pmoles/ μ l

Protein standard buffer composition: Glycerol 10%, Tris Base 141 mM, Tris HCl 106 mM, LDS 2%, EDTA 0.51 mM, SERVA® Blue G250 0.22 mM, Phenol Red 0.175 mM, pH 8.5, 0.1mg/ml PefaBloc protease inhibitor (Roche), 50mM DTT.

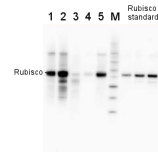
Rubisco protein was purified directly from plant tissue.

Please, use the 55 kDa size of RbcL for calculations. The pmoles in the standard refer to pmoles of rbcL monomers.

selected references | [MacKenzie](#) et al (2005). Large reallocations of carbon, nitrogen and photosynthetic reductant among phycobilisomes, photosystems and Rubisco during light acclimation in *Synechococcus elongatus* are constrained in cells under low environmental inorganic carbon. *Arch of Microbiol.* 183: 190 - 202. [Bouchard](#) et al. (2006) UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. *Photochem and Photobiol* 82: 936-951. [Morash](#) et al. (2007) Macromolecular dynamics of the photosynthetic system over a seasonal developmental progression in *Spartina alterniflora*. *Can J. of Bot.* 85: 476-483(8)

application example

2 μ g of total protein from various plant extracts (1-5) extracted with 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 select the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample.

Note: Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5 μ g total protein, depending on the abundance of the target protein.

Quantitation: When quantitated standards are included on the blot, the samples can be quantitated using the available software. Excellent quantitation can be obtained with images captured on the Bio-Rad Fluor-S-Max or equivalent instrument using Bio-Rad QuantityOne software. The contour tool is used to select the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample. Using above protocol linear standard curves are generated over 1-1.5 orders of magnitude range in target load. It is important to note that immunodetections usually show a strongly sigmoidal signal to load response curve, with a region of trace detection of low loads, a pseudolinear range and a region of saturated response with high loads. For immunoquantitation it is critical that the target proteins in the samples and the standard curve fall within the pseudolinear range. Our total detection range using this protocol spans over 2 orders of magnitude, but the quantifiable range is narrower.

Quantitative western blot: [detailed method description](#).