Agrisera
Part of Olink® Group

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

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Product no AS15 2955S

RbcL II | Rubisco form II positive control/quantitation standard

Product information

Format	Lyophilized in glycerol.
Reconstitution	For reconstitution add 90 µl of sterile water, Please notice that this product contains 10% glycerol and might appear as liquid but is provided lyophilized
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. 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	The RbcL protein standard can be used in a combination with Agrisera global antibiodies (AS15 2955 from rabbit) to quantitate RbcL from a wide range of species. Global antibodies are raised against highly conserved amino acid sequence. This standard is also included in following kits: Educational antibody kit - photosynthesis, Photosynthesis Tool Kit - quantitation, Rubico quantitation kit, Quantitative western blot: detailed method description, video tutorial

Application information

Recommended dilution	Standard curve: 3 loads are recommended (0.5, 2 and 4µl). For most applications a sample load of 0.2 µg of chlorophyll/well will give a RbcL signal in this range. Positive control: a 2 µl load per well is optimal for most chemiluminescent detection systems. Higher standard concentration needs to be used to allow detection by Coomasie stains. Such gels with higher standard concentration can not be used for quantitation using chemiluminescence. This standard **is stabilized and ready** and does not require heating before loading on the gel. Please note that this product contains 10% glycerol and might appear as liquid but is provided lyophilized. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently Take extra care to mix thoroughly before each use, as the proteins tend to settle with the more dense layer after freezing.
Expected | apparent MW	52,7 kDa
Additional information	**Concentration:** after re-constitution with sterile milliQ water final concentration of the standard is 0.15 pmoles/µl **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), 50 mM DTT. **This standard is ready-to-load and does not require any additions or heating. It needs to be fully thawed and thoroughly mixed prior to using. Avoid vigorous vortexing, as buffers contain detergent. Following mixing, briefly pulse in a microcentrifuge to collect material from cap.** **This standard is stabilized and ready and does not require heating before loading on the gel.** **Please note that this product contains 10% glycerol and might appear as liquid but is provided lyophilized. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently Take extra care to mix thoroughly before each use, as the proteins tend to settle with the more dense layer after freezing.** Please, use the 55 kDa size of RbcL for calculations. The pmoles in the standard refer to pmoles of rbcL monomers.
Selected references	Bausch et al. (2019). Combined effects of simulated acidification and hypoxia on the harmful dinoflagellate Amphidinium carterae. Mar Biol 166: 80. https://doi.org/10.1007/s00227-019-3528-y.

Application example

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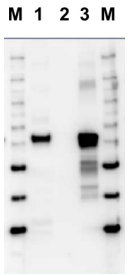

1.5 µg of total protein extract from *Rhodobacter capsulatus* **(1);** extracted with Agrisera Protein Extraction Buffer PEB (AS08 300); 0.5 pmol of recombinant RbcL I **(2)**, 0.5 pmol of **recombinant RbcL II, product** AS15 2955S **(3)** Samples were diluted with 1X sample buffer (NuPAGE LDS sample 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 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, recommended 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 of extreme femtogram sensitivity, 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.