Agrisera
Part of Thermo Fisher Scientific

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

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Product no AS01 017S

RbcL | Rubisco positive control/quantitation standard

Product information

Format Lyophilized in glycerol.

Quantity 100 µl

Reconstitution For reconstitution, check the label on the tube. 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 (AS01 017 from chicken or AS03 037 from a 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: three protein standard loads are recommended.
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 (standard concentration 7.9 µg/mL). Such gels with higher standard concentration can not be used for quantitation using chemiluminescence.

This standard **is stabilized** does not require heating before loading on the gel or addition of any buffer.

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.

Why can I not see the standard band using Coomasie stain? The reason that you do not see Rubisco standard on a gel is, that you have probably used it in concentration which is recommended for western blot detection, and it is too low to allow to see this protein using Coomasie stain. In such a case, you should load more Rubisco standard on a gel and stain it with more sensitive Coomasie stain or with silver. You can not use such a gel for western blot, as using higher concentration of this standard will not work for quantitation using western blot technique.

Selected references

Capo-Bauca et al. (2023). Carbon assimilation in upper subtidal macroalgae is determined by an inverse correlation between Rubisco carboxylation efficiency and CO2 concentrating mechanism effectiveness. New Phytol. 2023;237(6):2027-2038. doi:10.1111/nph.18624
Capo-Bauca et al. (2022) Correlative adaptation between Rubisco and CO2-concentrating mechanisms in seagrasses. Nat Plants. 2022 Jun;8(6):706-716. doi: 10.1038/s41477-022-01171-5. Epub 2022 Jun 20. Erratum in: Nat Plants. 2022 Jun 29;: PMID: 35729266.
Perera-Castro et al (2022). Limitations to photosynthesis in bryophytes: certainties and uncertainties regarding methodology. J Exp Bot. 2022;73(13):4592-4604. doi:10.1093/jxb/erac189

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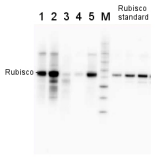
Poor et al. (2018). Comparison of changes in water status and photosynthetic parameters in wild type and abscisic acid-deficient sitiens mutant of tomato (Solanum lycopersicum cv. Rheinlands Ruhm) exposed to sublethal and lethal salt stress. J Plant Physiol. 2018 Dec 8;232:130-140. doi: 10.1016/j.jplph.2018.11.015.
Dai et al. (2018). Visualizing Individual RuBisCO and its Assembly into Carboxysomes in Marine Cyanobacteria by Cryo-Electron Tomography. J Mol Biol. 2018 Aug 20. pii: S0022-2836(18)30411-X. doi: 10.1016/j.jmb.2018.08.013.

Application example



2 µg 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 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 ug 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 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.