

RbcL | Rubisco positive control/quantitation standard

Cat PA00991

Size 100 μ l

Host

Clonality

Confirmed reactivity

Format

Lyophilized in glycerol.

Reconstitution

For reconstitution add 90 μ l 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.

Application

Western blot (WB)

Recommended dilution

Standard curve: three protein standard loads are recommended. For most applications a sample load of 0.2 μ g of chlorophyll/well will give a RbcL signal in this range. Positive control: a 2 μ l 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. 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

The RbcL protein standard can be used in a combination with Agrisera global antibodies 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, Rubisco quantitation kit, Quantitative western blot: detailed method description, video tutorial, Concentration: after re-constitution with sterile 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), 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

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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 Coomassie 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 Coomassie stain. In such a case, you should load more Rubisco standard on a gel and stain it with more sensitive Coomassie 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.

Description

Rubisco(ribulose-1, 5-diphosphate 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 shown to range from purple bacteria to flowering plants. Source of Rubisco Standard: The protein of Rubisco is purified directly from the plant tissue, spinach.

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