Agrisera
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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 AS15 2873S

SBP | Sedoheptulose-1,7-bis phosphatase positive control/quantitation standard

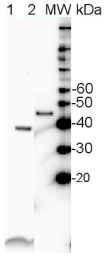
Product information

Format	Lyophilized
Reconstitution	For reconstitution add 85 µl of sterile water, Please note that this product contains 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 SBPase calibrated protein standard can be used in combination with Agrisera global anti-SBPase antibiodies (AS15 2873) to quantitate SBPase from a wide range of species. Global antibodies are raised against highly conserved amino acid sequence. Quantitative western blot: detailed method description and video tutorial.

Application information

Recommended dilution	Standard curve: 3 loads are recommended (0.5, 2 and 4 µl). 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	27 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.**

Application example



10 µg of total protein from *Arabidopsis thaliana* leaf **(1)** , SBPase protein standard AS15 2873S **(2)** were extracted with Agrisera Protein Extraction Buffer PEB (AS08 300). 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 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 AS09 602, 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.