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 AS03 030S

AtpB | Positive control/quantitation standard

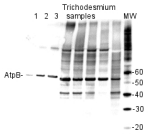
Product information

Format	Lyophilized, in glycerol.
Quantity	100 µl
Reconstitution	For reconstitution add 90 µl of milliQ 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 AtpB protein standard can be used in combination with global anti-AtpB antibodies to quantitate AtpB from a wide range of species. Global antibodies are raised against highly conserved amino acid sequences in the AtpB protein. 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 0.2µg of chlorophyll will give a AtpB signal in this range. Positive control: load per well: a 2µl load is optimal for most chemiluminescent detection systems. 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	in most gel systems AtpB migrates around 50-54 kDa
Additional information	**Concentration:** after adding 90 µl of dest. water final concentration of the standard is 0.27 pmol/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), 50mM 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.**
Selected references	Fraser et al. (2013). Photophysiological and Photosynthetic Complex Changes during Iron Starvation in Synechocystis sp. PCC 6803 and Synechococcus elongatus PCC 7942. PLOS ONE.

Application example



AtpB protein standard (AS03 030S) 0.03, 0.1, 0.26 pmol **(1-3) and total protein** from *Trichodesmium* IMS 101 extracted with Agrisera Protein Extraction Buffer (**AS08 300**) were separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Blots were blocked immediately following transfer in 2% blocking reagent 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: 50 000 for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-hen IgY horseradish peroxidase conjugated) diluted to 1:50 000 in 2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with chemiluminescence detection reagent according the manufacturer's instructions. Images of the blots were obtained using a CCD imager nd

Quantity One software Exposure time was 10 seconds.

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.