Agrisera

contact: support@agrisera.com

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

product AS04 054S

AOX | AOX positive control/quantitation standard

product information

Background

Alternative oxidases (**AOX**) are quinol oxidases located in the inner mitochondrial membrane of plants. They function as terminal oxidases in the alternate electron transport pathway, oxidizing ubiquinone to reduce oxygen to water.

Source of AOX standard: AOX standard source is *Sphingomonas wittichii* strain RW1, overexpressed in E.coli bearing an N-terminal his6 tag.

Format

Lyophilized

Quantity

120 µl

Reconstitution

For reconstitution add 100 µl of milliQ water. Please note 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 tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

Tested applications

Western blot (WB)

Related products

Primary antibodies matching AOX standard are:

AS04 054 | Anti-AOX1/2 | plant alternative oxidase 1 and 2, rabbit antibodies

Plant and algal protein extraction buffer

Additional information

The AOX calibrated protein standard can be used in combination with Agrisera global anti-AOX antibodies (AS04 054) to quantitate AOX from a wide range of species. Global antibodies are raised against highly conserved amino acid sequence.

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

27 kDa

Additional information

Concentration: 0.1 pmol/µl. After re-constitution with sterile milliQ water, the final concentration of the AOX monomer is.0.1 pmol/µl. While a dimer is present in the lane, only the 27 kDa monomer contributes to the calibration.

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.

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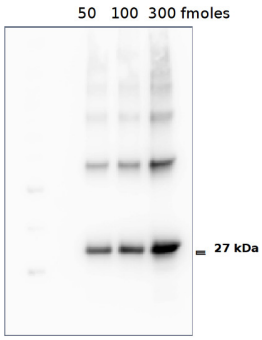
Application example



Following standard western blot procedure this image was obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad) as described below. 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 applied to the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample. Using this 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.