

# Agrisera

This product is **for research use only** (not for diagnostic or therapeutic use)

contact: [support@agrisera.com](mailto:support@agrisera.com)

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

product **AS08 371S**

## HSP70 | Positive control/quantitation standard

### product information

#### Background

**Heat-shock protein 70 (Hsp70)** is the major stress-inducible protein in vertebrates and is highly conserved throughout evolution. It plays a role as a molecular chaperone and is important for allowing cells to cope with acute stressor insult, especially those affecting the protein machinery. Heat shock cognate protein 70 (HSC70), is a highly conserved protein and a member of the family of molecular chaperones.

**Source of HSP70 standard:** recombinant Hsp70 of *Arabidopsis thaliana* UniProt: [Q9LHA8](#), TAIR: [AT3G12580](#)

#### Format

Lyophilized in glycerol

#### Reconstitution

For reconstitution add 82  $\mu$ l of steril water. Please notice that this product contains glycerol and might appear as liquid but is provided lyophilized. Final standard concentration is going to be 0.15 pmol/ $\mu$ l.

#### 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 antibody matching HSP70 standard:

[AS08 371](#) | anti-HSP70 | heat shock protein 70 cytoplasmic, rabbit antibody

[collection of other protein standards](#)

[collection of matching global antibodies](#)  
[Plant and algal protein extraction buffer](#)

#### Additional information

The HSP70 protein standard can be used in a combination with Agrisera global antibodies ([AS08 371](#)) to quantitate HSP70 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 (eg 0.1, 0.2, 0.3 pmol). Adjust range to fit your samples and your experiment.

For most applications a sample load of 0.2  $\mu$ g of chlorophyll/well will give a HSP70 signal in this range.

Positive control: a 2  $\mu$ 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 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

70 kDa

#### Additional information

**Concentration:** after re-constitution with sterile milliQ water final concentration of the standard is 0.15 pmoles/ $\mu$ 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,**

# Agrisera

This product is **for research use only** (not for diagnostic or therapeutic use)

contact: [support@agrisera.com](mailto:support@agrisera.com)

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

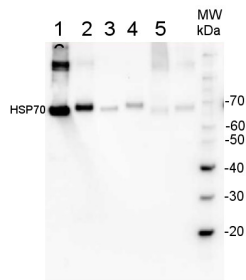
**briefly pulse in a microcentrifuge to collect material from cap.**

**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



(1), product number AS08 371S, 1 µg of total protein from samples such as *Lycopersicum esculentum* leaf (2), *Nicotiana tabaccum* leaf, (3), *Zea mays* leaf (4), *Hordeum vulgare* leaf (5), *Arabidopsis thaliana* leaf (6) were extracted with 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 kept 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 reagent 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 AS10 1489, 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 chemiluminescence detection reagent of extreme femtogram range, 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. unantitiated 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.](#)