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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 AS08 371

HSP70 | Heat shock protein 70 (cytoplasmic)

Product information

Immunogen	KLH-conjugated synthetic peptide conserved in known higher plant HSC70 proteins including three isoforms of *Arabidopsis thaliana* HSC70-1 UniProt: F4KCE5 , HSC70-2 UniProt: A0A178UTH3 and HSC70-3 Uniprot: O65719 as well as heat shock inducible Hsp70 of *Arabidopsis thaliana* TAIR: AT3g12580/T2E22_110 and At1g16030 and AT3g12580/T2E22_110
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water
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	This product can be sold containing ProClin if requested

Application information

Recommended dilution	1 : 3000 - 1: 10 000, 5 µg protein/well (WB), 2-3 µl/protein extract of concentration 3-5 mg/ml
Expected | apparent MW	70 kDa
Confirmed reactivity	*Arabidopsis thaliana, Brassica juncea, Brassica napus, Brassica oleracea, Chlamydomonas reinhardtii, Chlamydomonas sp. UWO241, Cucumis sativus, E. teft, Fagopyrum esculentum, Hordeum vulgare, Medicago sativa, Oryza sativa, Salicornia* sp., *Silene vulgaris, Solanum lycopersicum, Picrorhiza kurroa, Pinus strobus, Polyscias elegans, Zea mays,* algae: *Desmodesmus subspicatus, Gracilaria vermiculophylla, phycobiont: Trebouxia TR1 and TR9, Plasmodium falciparum, Setaria italica, Triticum aestivum, Ulva prolifera, Vicia faba*
Predicted reactivity	*Ageratina adenophora, Allium sativum, Arabis alpina, Arachis diogoi, Arundo donax, Brassica napus, brassica rapa subsp. pekinensis, Camellia sinensis, Citrus sinensis, Coffea arabica, Eriobotrya japonica, Gossypium arboretum, Glycine max, Glycine soja, Helianthus annuus, Hordeum vulgare var. distichum, Lotus japonicus, Medicago sativa, medicago truncatula, Musa acuminata subsp. malaccensis, Nannochloropsis gaditana, Nicotiana tabacum, Nicotiana bethamiana, Olea europea, Phaseolus vulgaris, Physcomitrium patens, Pinus taeda, Pisum sativum, Populus balsamifera, Populus trichocarpa, Saccharum sp, Salix gilgiana, Saussurea medusa,Solanum tuberosum, Solanum commersonii, Spinacia oleracea, Tragopogon dubius, Tragopogon porrifolius, Triticum aestivum, Vitis vinifera, Volvox sp.* Species of your interest not listed? Contact us
Not reactive in	*Polyscias elegans*
Additional information	Can be sold containing 0.1% ProClin if requested This antibody can be used as a marker of cytoplasmic fraction in tomato (Anfoka et al. 2015). Applied primary antibody dilution in western blot depends upon sensitivity of detection reagents (pico or femtogram for chemiluminescent detection). Immunoprecipitation protocol using Agrisera anti-Hsp70 cytosolic antibodies, see tab: protocols.
Selected references	Vuković et al. (2024). Heat Priming Modifies Heat Stress Response in BPM1-Overexpressing Arabidopsis thaliana (L.) Heynh. J Olant Growth Regulation. https://doi.org/10.1007/s00344-024-11337-4. Llamas et al. (2023). In planta expression of human polyQ-expanded huntingtin fragment reveals mechanisms to prevent disease-related protein aggregation. Nat Aging. 2023 Nov;3(11):1345-1357.doi: 10.1038/s43587-023-00502-1. Chong et al. (2022) The tomato OST1-VOZ1 module regulates drought-mediated flowering. Plant Cell. 2022 Apr 26;34(5):2001-2018. doi: 10.1093/plcell/koac026. PMID: 35099557; PMCID: PMC9048945. Bychkov et al. (2022) The role of PAP4/FSD3 and PAP9/FSD2 in heat stress responses of chloroplast genes. Plant Sci. 2022 Sep;322:111359. doi: 10.1016/j.plantsci.2022.111359. Epub 2022 Jun 20. PMID: 35738478. Cvetkovska et al. (2022) A constitutive stress response is a result of low temperature growth in the Antarctic green alga Chlamydomonas sp. UWO241. Plant, Cell & Environment, 45, 156– 177. https://doi.org/10.1111/pce.14203 Wang et al. (2022) 17-(Allylamino)-17-demethoxygeldanamycin treatment induces the accumulation of heat shock proteins and alleviates senescence in broccoli. Postharvest Biology and Technology,Volume 186, 2022, 111818, ISSN

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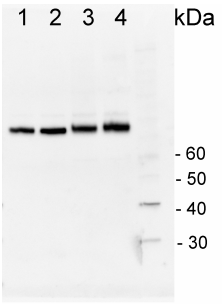
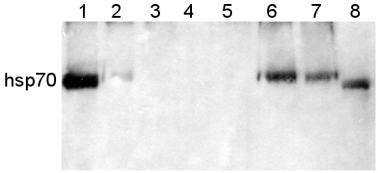
0925-5214, https://doi.org/10.1016/j.postharvbio.2021.111818.
Kumari et al. (2021) In-depth assembly of organ and development dissected Picrorhiza kurroa proteome map using mass spectrometry. BMC Plant Biol. 2021 Dec 22;21(1):604. doi: 10.1186/s12870-021-03394-8. PMID: 34937558; PMCID: PMC8693493.

Application example



1µg of total protein from *Horderum vulgare* pre heat shock leaf **(1),** *Horderum vulgare post* heat shock (2h 40ºC) **(2)**, *Zea mays* pre heat shock total protein leaf **(3)**, *Zea mays* post heat shock (2h 40ºC) **(4)**, total protein leaf extracted with Agrisera Protein Eextraction Buffer (**AS08 300**) were separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF (Milipore)**. Filters were blocked 1h with 2% low-fat **milk powder** in TBS-T (0.1% TWEEN 20) and probed with anti-HSP70 antibody (AS08 371, **1:20 000**, 1h) and secondary anti-rabbit (**1:20 000,** 1 h) antibody (HRP conjugated) in TBS-T containing 2% low fat milk powder. All steps were performed at RT with agitation. Signal was detected with chemiluminescent detection reagent with extreme femtogram range.



Protein from *Solanum lycopersicum* (**1**) total cell extract ca. 30 -50 µg, (**2**) and (**3**) nuclei pellet , (**4**) and (5) ca. 7 µg of nuclei fraction, (**6**) and (**7**) cytoplasmic pellet, (**8**) ca. 7 µg of cytoplasm fraction, were separated on **10% SDS-PAGE** and blotted 1h to **nitrocellulose (Schleicher & Schuell)**. Filters were blocked 1h with 2% low-fat **milk powder** in TBS-T (0.1% TWEEN 20) and probed with anti-HSP70 antibody (AS08 371, **1:5000**, 3h RT). The antibody solution was decanted and the blot was rinsed briefly. Washed 3 times for 15 min in TBS-T at room temperature with agitation. Blot was incubated with a secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1: 5:000. The blot was washed as above and developed for 1 min with ECL detection reagent according to the manufacturers instructions.

Courtesy Dr Rena Gorovits, The Hebrew University of Jerusalem, Israel

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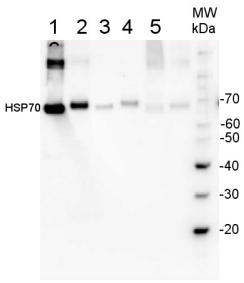

200 fmoles of HSP70 protein standard product number AS08 371S **(1)**, 1 µg of total protein from samples such as *Lycopersicum esculentum* leaf **(2)**, *Nicotiana tabaccum* leaf, **(3)**, *Zea mays* leaf **(4)**, *Hordeum vulgare* leaf **(5)**, *Arabidopsis thaliana* leaf **(6)** were extracted with 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 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/RT 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 in 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.