Order online now!

www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for 200-301-A27

Hsp70 Antibody

Overview

Description:	Anti-HSP70 (MOUSE) Monoclonal Antibody - 200-301-A27
Item No.:	200-301-A27
Size:	200 µg
Applications:	ELISA, FC, IHC, WB, Other
Reactivity:	Human, Mouse, Rat, Bovine, C. elegans, Carp, Chicken, D. melanogaster, Dog, Guinea Pig, Hamster, Monkey, Pig, Rabbit, Sheep
Host Species:	Mouse

Product Details

Background:	Anti-Hsp 70 antibody is routinely used in cancer and signal transduction research. Hsp70 genes encode abundant heat-inducible 70-kDa hsps (hsp70s). In most eukaryotes, hsp70 genes exist as part of a multigene family. Hsp70s are found in most cellular compartments of eukaryotes, including nuclei, mitochondria, chloroplasts, the endoplasmic reticulum and the cytosol, as well as in bacteria. The genes show a high degree of conservation, having at least 50% identity (2). The N-terminal two-thirds of hsp70s are more conserved than the C-terminal one-third. Hsp70 binds ATP with high affinity and possesses a weak ATPase activity which can be stimulated by binding to unfolded proteins and synthetic peptides (3). When hsc70 (constitutively expressed) present in mammalian cells was truncated, ATP binding activity was found to reside in an N-terminal fragment of 44 kDa which lacked peptide binding capacity. Polypeptide binding ability therefore resided within the C-terminal half (4). The structure of this ATP binding domain displays multiple features of nucleotide binding proteins (5). All hsp70s, regardless of location, bind proteins, particularly unfolded ones. The molecular chaperones of the hsp70 family recognize and bind to nascent polypeptide chains as well as partially folded intermediates of proteins, preventing their aggregation and misfolding. The binding of ATP triggers a critical conformational change leading to the release of the bound substrate protein (6). The universal ability of hsp70s to undergo cycles of binding to and release from hydrophobic stretches of partially unfolded proteins determines their role in a great variety of vital intracellular functions such as protein synthesis, protein folding and oligomerization, and protein transport.
Synonyms:	Heat shock 70 kDa protein 1 antibody, heat shock 70kDa protein 1A antibody, Heat shock 70kDa protein 1B antibody, Heat shock induced protein antibody, heat shock protein 70 antibody, HSP70 1 antibody
Host Species:	Mouse

Clonality:	Monoclonal
Clone ID:	C92F3A-5
Format:	IgG1

Target Details

Gene Name:	HSPA1A
Reactivity:	Human, Mouse, Rat, Bovine, C. elegans, Carp, Chicken, D. melanogaster, Dog, Guinea Pig, Hamster, Monkey, Pig, Rabbit, Sheep
Immunogen Type:	Conjugated Peptide
Immunogen:	Hsp70 monoclonal antibody was prepared using conventional hybridoma technology after repeated immunizations with a synthetic peptide from the region of amino acid residues 436-503 of human Hsp70 protein.
Purity/Specificity:	This Protein G purified monoclonal antibody reacts with human and mouse Hsp70 protein. A BLAST analysis was used to suggest cross-reactivity with Hsp70 from human, bovine, mouse, rat, C.elegans, dog, chicken, Drosophila, carp, guinea pig, hamster, monkey, pig, rabbit and sheep sources based on 100% homology with the immunizing sequence. No cross-reactivity occurs with hsc70 (hsp73). Cross-reactivity with Hsp70 from other sources has not been determined.
Relevant Links:	• NCBI - NP_005336.3 • UniProtKB - P0DMV8 • GeneID - 3304

Application Details

Tested Applications:	ELISA, FC, IHC, WB
Suggested Applications:	Other (Based on references)
Application Note:	This Protein G purified antibody has been tested for use in ELISA, immunofluorescence microscopy, western blotting, FACS, immunoelectron microscopy, immunohistochemistry and immunoprecipitation. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 70 kDa in size corresponding to Hsp70 by western blotting in the appropriate cell lysate or extract. In general, a 1:1,000 dilution is suggested for most applications and is suitable to detect Hsp70 in 20 µg of heat shocked HeLa cell lysate by western blotting.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IF:	1:200-1:1000

IHC:	10 µg/ml
WB:	1:500-1:2000

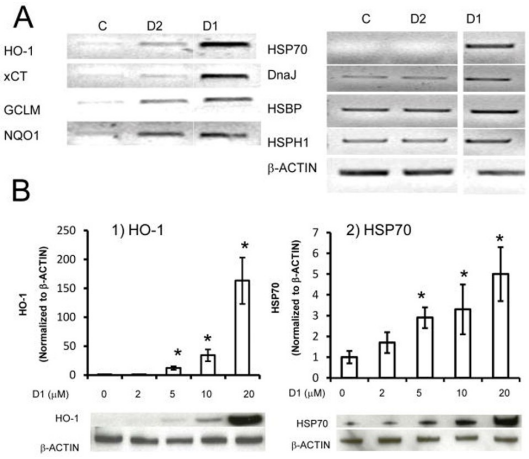
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm
Buffer:	1X PBS, pH 7.4
Preservative:	0.1% (w/v) Sodium Azide
Stabilizer:	50% (v/v) Glycerol

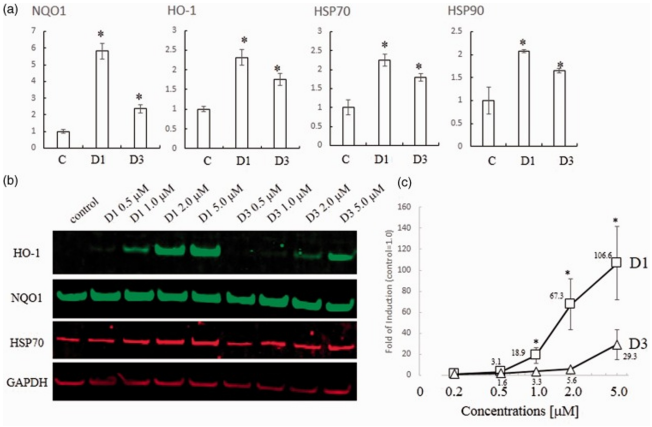
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Hsp70 antibody is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



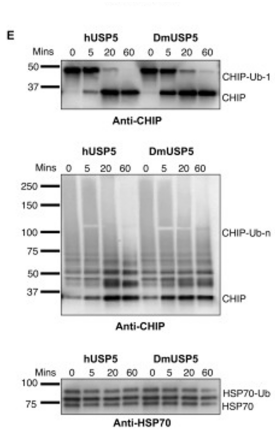
Western Blot
(A) PCR analysis of phase 2 and HSP genes induced by 20 µM D1. Total RNAs were extracted from ARPE cells after treatment with 20 µM D1 or D2 for 24 h in serum-free medium. RT-PCR was performed with the specific primers listed in the Materials and Methods. (B) Western blot analysis to show dose-dependent induction of HO-1 and HSP70 proteins by D1. Lysates were prepared from cells after treatment with various concentrations of D1; 10 µg protein samples were loaded per lane and subjected to SDS-PAGE prior to immunoblotting. The experiments were repeated three times with similar results. Lower panel: Cell lysates (10 µg protein/lane) were subjected to SDS-PAGE and immunoblotted for HO-1 and HSP70. Upper panel: Analysis of HO-1 and HSP70 normalized to β-actin by taking the ratio of their densitometric values on immunoblots. * $p < 0.05$ from control cells.
Fig1 PMID 21883218



Western Blot

Induction of phase 2 enzymes and HSPs. (a) PCR analysis of phase 2 and HSP genes induced by D1 or D3. Total RNA was extracted from ARPE cells treated with 5 μM D1 or D3 for 24 hr in serum-free medium. RT-PCR was performed using cDNA template with the specific primers listed in the Materials and Methods section mRNA levels were quantified by densitometry of qPCR band intensity after 28 cycles (n = 3). All genes were normalized to β-actin expression. *Significantly different ($p < .05$) from control. (b) Western blot analysis of dose-dependent induction of HO-1, NQO1, HSP70, and GAPDH proteins by the indicated concentrations of D1 or D3. Cell lysates were prepared, and 10 μg protein/lane of protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, after which the proteins were detected by use of specific antibodies. (c) HO-1 was normalized to GAPDH by taking the ratio of their densitometric values on immunoblots. *Significantly different ($p < .05$) between D1 and D3. HSP = heat-shock protein; RT-PCR = reverse transcription-polymerase chain reaction; ARPE = human apical retinal pigment epithelial cell line; HO-1 = heme oxygenase-1; NQO1 = NADPH quinone oxidoreductase1; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

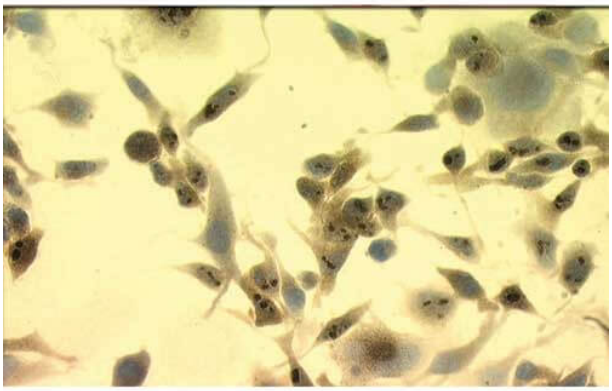
Fig 4 PMID: 26243592



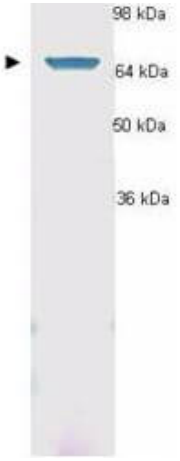
Western Blot

E). Western blots of in vitro deubiquitinating reactions with ubiquitinated proteins. CHIP and HSP70 were ubiquitinated as described under “Experimental Procedures.” The ubiquitination reaction was stopped with excess EDTA, and then 50 nm (final concentration) recombinant human (hUSP5) or Drosophila (Dm) USP5 was added to the mix. Samples were collected at the indicated time points after the addition of the DUB, resolved in 4–20% SDS-PAGE gels, and blotted as indicated. The blots are representative of deubiquitination reactions conducted at least three independent times with similar results.

Fig 1. PMID: 26917723



Immunohistochemistry
Immunohistochemistry of Mouse anti-Hsp70 monoclonal antibody. Tissue: Heat-shocked mouse melanoma. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Hsp70 monoclonal antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase mouse secondary antibody at 1:10,000 for 45 min at RT. Staining: Hsp70 monoclonal as precipitated red signal with hematoxylin purple nuclear counterstain.



Western Blot
Western blot using Rockland's Protein G purified anti-Hsp70 antibody shows detection of Hsp70 in whole cell lysates from heat shocked HeLa cells. The band marked by the arrowhead corresponds to Hsp70 at an approximate molecular weight of 70 kDa. The primary antibody was used at a 1:1000 dilution.

References

- Ristic et al. USP5 Is Dispensable for Monoubiquitin Maintenance in Drosophila. *Journal of Biological Chemistry* (2016)
- Satoh et al. Nrf2 and HSF-1 Pathway Activation via Hydroquinone-Based Proelectrophilic Small Molecules is Regulated by Electrochemical Oxidation Potential. *ASN Neuro* (2015)
- Satoh T et al. Dual neuroprotective pathways of a pro-electrophilic compound via HSF-1-activated heat-shock proteins and Nrf2-activated phase 2 antioxidant response enzymes. *J Neurochem.* (2011)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.