Product Datasheet

HSP60 Antibody - BSA Free
NBP1-77397

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NBP1-77397

HSP60 Antibody - BSA Free

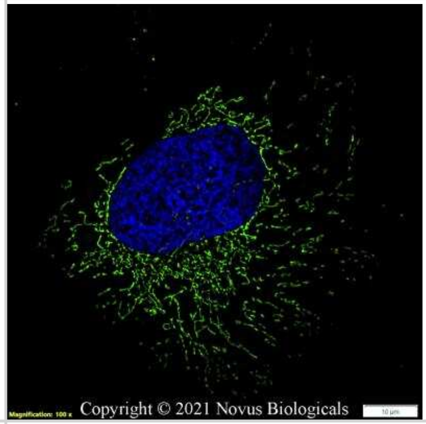
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol

Product Description	
Host	Rabbit
Gene ID	3329
Gene Symbol	HSPD1
Species	Human, Mouse
Marker	Mitochondria Marker
Immunogen	A synthetic peptide made to an internal region of the human Hsp60 protein (within residues 70-150). [Swiss-Prot P10809]

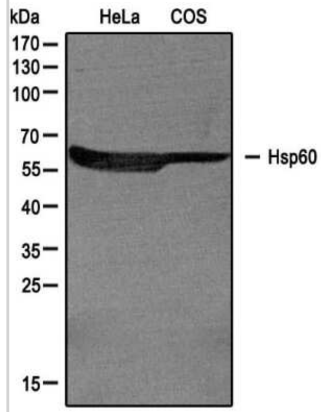
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 0.5 ug/ml, Simple Western 1:5000, Flow Cytometry, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:50-1:1000, Immunohistochemistry-Paraffin 1:100, Immunoblotting, Knockdown Validated reported in scientific literature (Shi et al)
Application Notes	In Western Blot, a band is seen ~61 kDa representing Hsp60. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In Simple Western only 10-15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HepG2 lysate 0.05 mg/mL, separated by Size, antibody dilution of 1:5000, apparent MW was 62 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

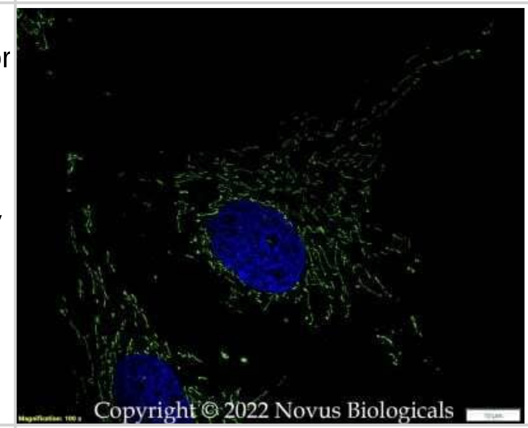
Immunocytochemistry/Immunofluorescence: HSP60 Antibody [NBP1-77397] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with HSP60 Antibody (NBP1-77397) at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



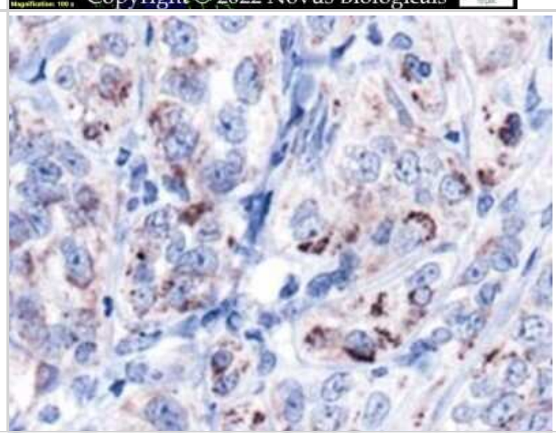
Western Blot: HSP60 Antibody [NBP1-77397] - Analysis of extracts from HeLa and COS cells using NBP1-77397 Hsp60 antibody at 1:1000

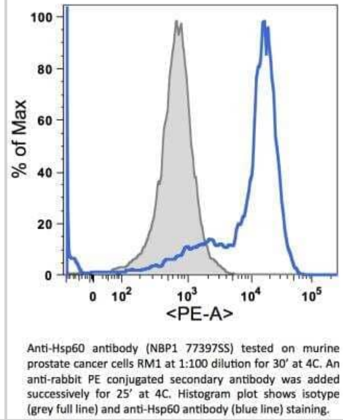
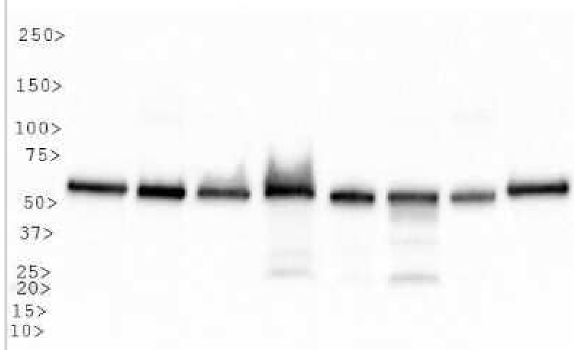
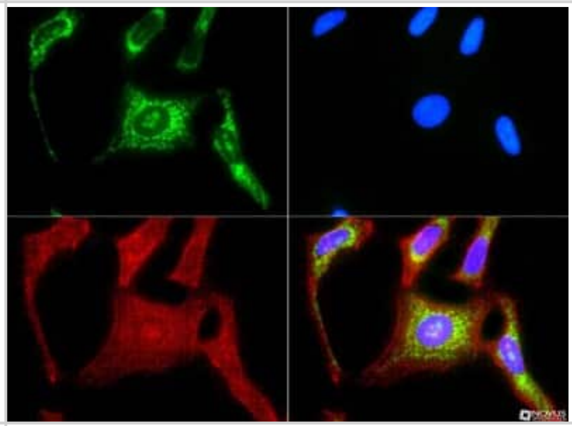
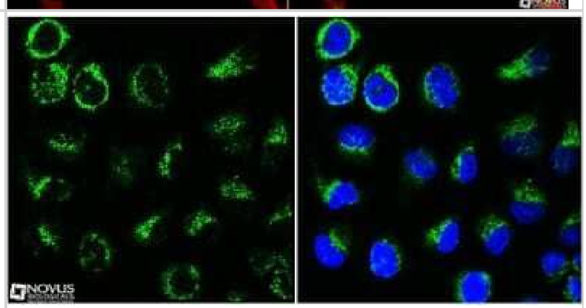


Immunocytochemistry/Immunofluorescence: HSP60 Antibody - BSA Free [NBP1-77397] - Rat FR cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with HSP60 Antibody (NBP1-77397) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

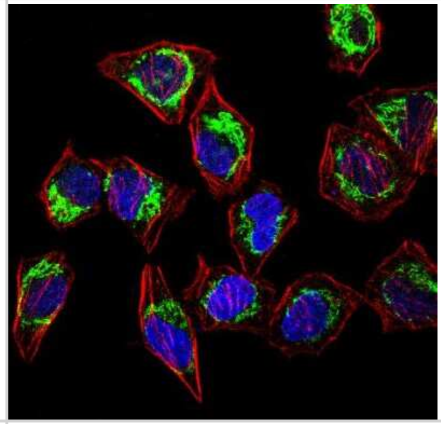


Immunohistochemistry: HSP60 Antibody [NBP1-77397] - Staining of HSP60 in human kidney carcinoma using DAB with hematoxylin counterstain.

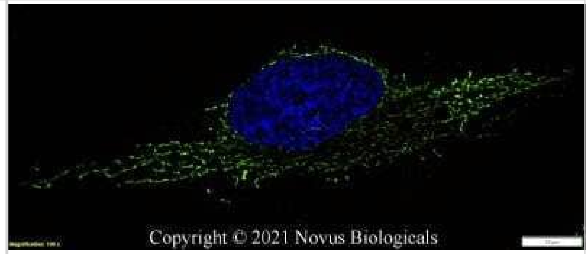


Flow Cytometry: HSP60 Antibody [NBP1-77397] - Analysis of HSP60 in RM1 cells (murine prostate cancer cell line) using anti-HSP60 antibody. The primary antibody was used at a dilution of 1:100. Image from verified customer review.	
Western Blot: HSP60 Antibody [NBP1-77397] - Analysis of HSP60 in: 1) HeLa, 2) HepG2, 3) NIH/3T3, 4) Jurkat, 5) CHO, 6) A431, 7) PC12 and 8) COS7	
Immunocytochemistry/Immunofluorescence: HSP60 Antibody [NBP1-77397] - HSP60 antibody was tested in HeLa cells with FITC (green). Nuclei and actin were counterstained with DAPI (blue) and Phalloidin (red).	
Immunocytochemistry/Immunofluorescence: HSP60 Antibody [NBP1-77397] - Confocal immunofluorescence analysis of HeLa cells using Hsp60 antibody at 1:50 (green). Nuclei were counterstained using DAPI (blue).	

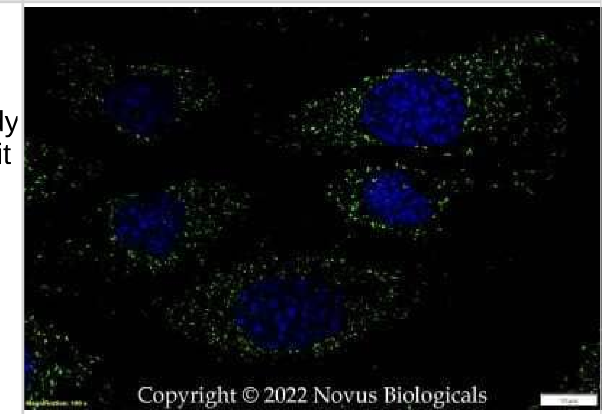
Immunocytochemistry/Immunofluorescence: HSP60 Antibody [NBP1-77397] - Confocal immunofluorescent analysis of HeLa cells using Hsp60 antibody (NBP1-77397, 1:100). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green, A). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red, B). DAPI was used to stain the cell nuclei (blue, C).



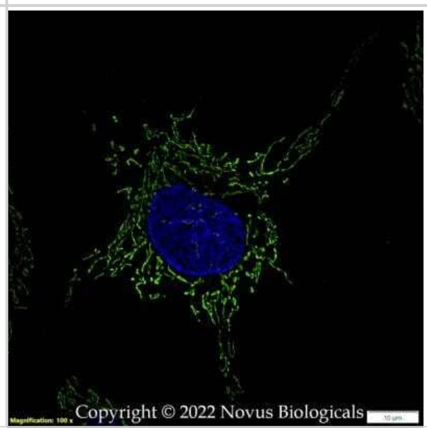
Immunocytochemistry/Immunofluorescence: HSP60 Antibody [NBP1-77397] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with HSP60 Antibody conjugated to Alexa Fluor 488 (NBP1-77397AF488) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



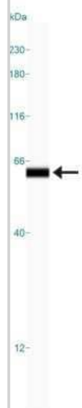
Immunocytochemistry/Immunofluorescence: HSP60 Antibody - BSA Free [NBP1-77397] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with HSP60 Antibody (NBP1-77397) at 2ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



Immunocytochemistry/Immunofluorescence: HSP60 Antibody - BSA Free [NBP1-77397] - HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with HSP60 Antibody (NBP1-77397) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



Simple Western: HSP60 Antibody [NBP1-77397] - Lane view shows a specific band for Hsp60 in 0.05 mg/ml of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Publications

Onay Ucar E, Sengelen A, Mertoglu Kamali E Hsp27, Hsp60, Hsp70, or Hsp90 depletion enhances the antitumor effects of resveratrol via oxidative and ER stress response in human glioblastoma cells Biochemical Pharmacology 2023-02-01 [PMID: 36603687] (WB, Human)

Details:
1:1000 WB dilution

Han Y, Tan L, Zhou T et al. A human iPSC-array-based GWAS identifies a virus susceptibility locus in the NDUFA4 gene and functional variants Cell stem cell 2022-10-06 [PMID: 36206731] (ICC/IF, Human)

Kiyga E, Adıguzel Z, Onay Ucar E Temozolomide increases heat shock proteins in extracellular vesicles released from glioblastoma cells Molecular biology reports 2022-06-25 [PMID: 35752701] (WB, Human)

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

Details:
Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Skalina KA, Singh S, Chavez CG et al. Low Intensity Focused Ultrasound (LOFU)-mediated Acoustic Immune Priming and Ablative Radiation Therapy for in situ Tumor Vaccines Sci Rep. 2019-10-29 [PMID: 31664044] (FLOW, Mouse)

Details:
Citation used the FITC format of this antibody.

Shi G. Characterizing the Function of the Mitochondrial Protease PARL in Mitophagy and Mitochondrial Quality Control Thesis 2017-01-01 (KD, IB, Human)

Procedures

Western Blot protocol for HSP60 Antibody (NBP1-77397)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for HSP60 Antibody (NBP1-77397)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for HSP60 Antibody (NBP1-77397)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NBP1-77397

NB800-PC1	HeLa Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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