

# Product Datasheet

## HSP27 Antibody - BSA Free

### NBP1-75477

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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**NBP1-75477**

HSP27 Antibody - BSA Free

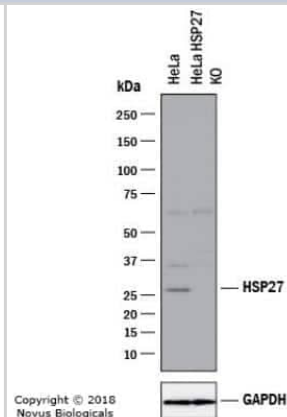
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol

Product Description	
Host	Rabbit
Gene ID	3315
Gene Symbol	HSPB1
Species	Human, Mouse, Rat
Reactivity Notes	Immunogen displays the following percentage of sequence identity for non-tested species: Chicken (80%).
Immunogen	A synthetic peptide made to an internal region of the human Hsp27 protein (within residues 80-150). [Swiss-Prot# P04792]

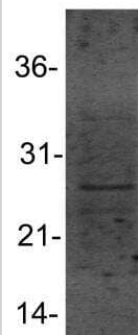
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockout Validated
Recommended Dilutions	Western Blot 2 ug/ml, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:100, Knockout Validated
Application Notes	This HSP27 antibody is useful for ICC/IF, IHC-P, and Western blot where a band is seen ~27 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

**Images**

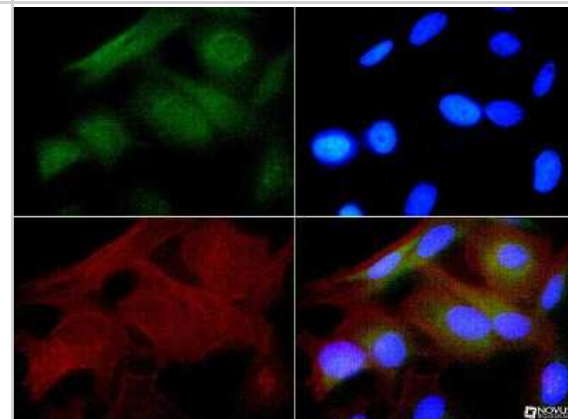
Knockout Validated: HSP27 Antibody [NBP1-75477] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and HSP27 knockout (KO) HeLa cell line. PVDF membrane was probed with 2 ug/ml of Rabbit Anti-Human HSP27 Polyclonal Antibody (Catalog # NBP1-75477) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (catalog number HAF008). Specific band was detected for HSP27 at approximately 27 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.



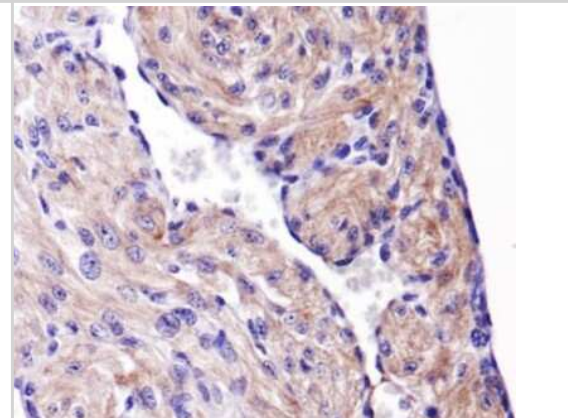
Western Blot: HSP27 Antibody [NBP1-75477] - Analysis of Jurkat cell lysate (30ug) using anti-Hsp27 antibody. Image from verified customer review.



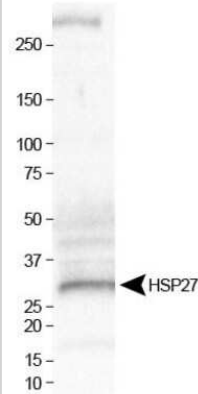
Immunocytochemistry/Immunofluorescence: HSP27 Antibody [NBP1-75477] - Hsp27 Antibody [NBP1-75477] - HSP27 antibody was tested in HeLa cells with FITC (green). Nuclei and actin were counterstained with DAPI (blue) and Phalloidin (red).



Immunohistochemistry: Hsp27 Antibody [NBP1-75477] - Staining of HSP27 in mouse heart using DAB with hematoxylin counterstain.



Western Blot: Hsp27 Antibody [NBP1-75477] - Analysis of HSP27 in human skeletal muscle lysate.



## Publications

Lopez-Gonzalez I, Carmona M, Arregui L et al.  $\alpha$ B-crystallin and HSP27 in glial cells in tauopathies. *Neuropathology* 2014-07-02 [PMID: 24985029] (IHC-P)

### Details:

Hsp27 antibody used for IHC-P for immunostaining HSP27 in glial cells with /without tau deposits in progressive supranuclear palsy, corticobasal degeneration (CBD), argyrophilic grain disease (AGD), Pick's disease (PiD), Alzheimer's disease, frontotemporal lobar degeneration associated with mutations in the tau gene (FTLD-tau), globular glial tauopathy (GGT) and tauopathy in the elderly. Sections boiled in citrate buffer for 20 min to retrieve tau antigenicity, blocked with 1% H<sub>2</sub>O<sub>2</sub> in methanol followed by 3% normal horse serum 1:100 incubated at 4C ON followed by peroxidase-DAB detection with EnVision kit. Antibody also used for double-labeling immunofluorescence and confocal microscopy of paraffin tissue sections.

Stice JP, Chen L, Kim SC et al. 17Beta-Estradiol, aging, inflammation, and the stress response in the female heart *Endocrinology* 2011-04-01 [PMID: 21303943] (WB, Rat)



## Procedures

### Western Blot protocol for HSP27 Antibody (NBP1-75477)

HSP27 Antibody:

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 HSP27 Antibody (NBP1-75477)

HSP27 Antibody:

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 HSP27 Antibody (NBP1-75477)**

HSP27 Antibody:

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 1:25,000 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 NBP1-75477**

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NB820-59253	Human Skeletal Muscle Whole Tissue Lysate (Adult Whole Normal)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **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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