# **Product Datasheet**

# HSP70/HSPA1A Antibody - BSA Free NBP1-77455

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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## NBP1-77455

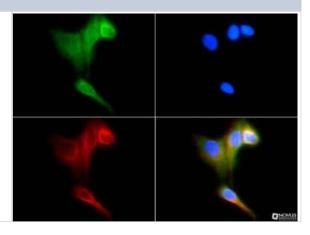
HSP70/HSPA1A Antibody - BSA Free

Product Information			
Unit Size	0.1 ml		
Concentration	1.18 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	3303		
Gene Symbol	HSPA1A		
Species	Human, Mouse		
Immunogen	A synthetic peptide made to an internal portion of the human Hsp70 protein (between residues 550-600) [Uniprot: P08107]		
Product Application Details			
Applications	Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry- Paraffin		

	Parattin
Recommended Dilutions	Simple Western 1:50, Flow Cytometry 1 : 200, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:25, Immunohistochemistry- Paraffin 1:400, Immunohistochemistry-Frozen reported in scientific literature (PMID 26755821)
Application Notes	In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <u>Simple Western Antibody Database</u> for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:50, apparent MW was 67 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

#### Images

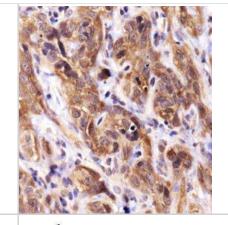
Immunocytochemistry/Immunofluorescence: HSP70/HSPA1A Antibody [NBP1-77455] - HSP70 antibody was tested at 1:25 in HeLa cells with FITC (green). Nuclei were counterstained with DAPI (blue).

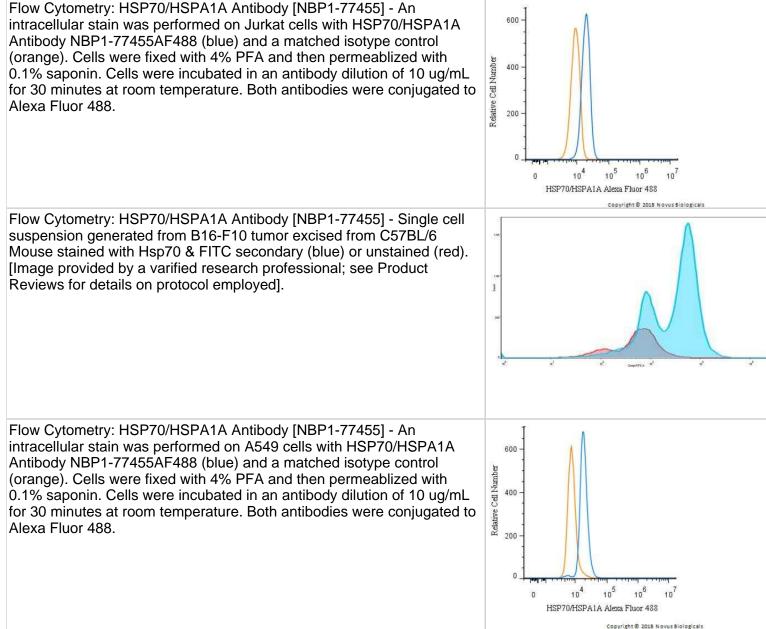


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Immunohistochemistry: HSP70/HSPA1A Antibody [NBP1-77455] -Analysis of Hsp70 in human xenograft kidney cancer using DAB with hematoxylin counterstain.







Simple Western: HSP70/HSPA1A Antibody [NBP1-77455] - Lane view shows a specific band for Hsp70 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

kDa			
230-			
180-			
116-			
66	-		
	_		
40-			
12-			

#### **Publications**

Montes de Oca R, Alavi AS, Vitali N et al. Belantamab Mafodotin (GSK2857916) Drives Immunogenic Cell Death and Immune-mediated Antitumor Responses In Vivo Molecular Cancer Therapeutics 2021-10-01 [PMID: 34253590] (Flow Cytometry)

Kines RC, Thompson CD, Spring S et al. Virus-Like Particle-Drug Conjugates Induce Protective, Long-lasting Adaptive Antitumor Immunity in the Absence of Specifically Targeted Tumor Antigens Cancer Immunology Research 2021-06-01 [PMID: 33853825] (Flow Cytometry, In vivo assay)

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.

Apavaloaei A, Brochu S, Dong M et al. PSMB11 Orchestrates the Development of CD4 and CD8 Thymocytes via Regulation of Gene Expression in Cortical Thymic Epithelial Cells J. Immunol. 2018-12-19 [PMID: 30567730] (ICC/IF, Mouse)

Bandyopadhyay S, Quinn TJ, Scandiuzzi L et al. Low-Intensity Focused Ultrasound Induces Reversal of Tumor-Induced T Cell Tolerance and Prevents Immune Escape. J. Immunol. 2016-01-11 [PMID: 26755821] (FLOW, IHC-Fr, Mouse)

Details:

HSP70 antibody was used for immunofluorescence staining of cryosections from B16-F1 tumor tissues isolated from untreated mice or from mice treated with Low-Intensity Focused Ultrasound/LOFU (FIGURE 4D). The antibody was also used for FLOW on CD452TRP1+ B16 cells from tumors of LOFU-treated and untreated mice (FIGURE 4E).





#### **Procedures**

#### Immunohistochemistry-Paraffin protocol for Hsp70 Antibody (NBP1-77455)

HSP70/HSPA1A 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 Hsp70 Antibody (NBP1-77455)

HSP70/HSPA1A 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 1:25,0000 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 NBP1-77455

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP1-77455AF488	HSP70/HSPA1A Antibody [Alexa Fluor® 488]

#### 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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