

HSPA9 Monoclonal Antibody (9F8)

Catalog NumberMA1-094

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human, Non-human primate, Rat
Host/Isotope	Mouse / IgG	Published species	Not Applicable
Class	Monoclonal	Tested Applications	Dilution *
Type	Antibody	Immunoprecipitation (IP)	2 µg
Clone	9F8	Western Blot (WB)	1:1,000
Immunogen	Protein expressed in 293T cell transfected with human HSPA9 expression vector	Immunocytochemistry (ICC/IF)	1:50
Conjugate	Unconjugated	Published Applications	
Form	Liquid	Western Blot (WB)	See 1 publications below
Concentration	1 mg/mL	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Purification	Protein A		
Storage buffer	PBS with 1mg/mL BSA, 30% glycerol		
Contains	0.05% sodium azide		
Storage Conditions	-20°C		

Product specific information

MA1-094 has been successfully used in Western blot, immunofluorescence, and immunoprecipitation applications on human, monkey, and rat samples.

Background/Target Information

The HSP70 family is composed of four highly conserved proteins: HSP70, HSC70, GRP75 and GRP78. These proteins serve a variety of roles. GRP75 expression is restricted to the mitochondrial matrix and aids in the translocation and folding of nascent polypeptide chains of both nuclear and mitochondrial origin. GRP75 and GRP78 are unresponsive to heat stress and are induced by glucose deprivation.

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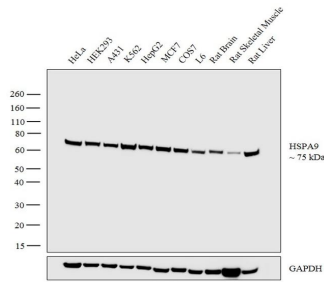
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Product Images For HSPA9 Monoclonal Antibody (9F8)

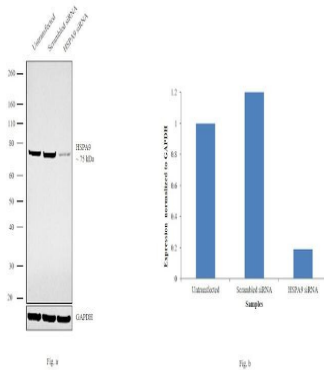
HSPA9 Antibody (MA1-094) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of HeLa (Lane 1), HEK293 (Lane 2), A431 (Lane 3), K562 (Lane 4), HepG2 (Lane 5), MCF7 (Lane 6), COS7 (Lane 7), L6 (Lane 8), tissue extracts of Rat Brain (Lane 9), Rat Skeletal Muscle (Lane 10) and Rat Liver (Lane 11). The blot was probed with Anti-HSPA9 Monoclonal Antibody (Product # MA1-094, 1:1000 dilution) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/ml, 1:4000 dilution). A 75 kDa band corresponding to HSPA9 was observed across all the cell lines and rat tissue extracts tested.



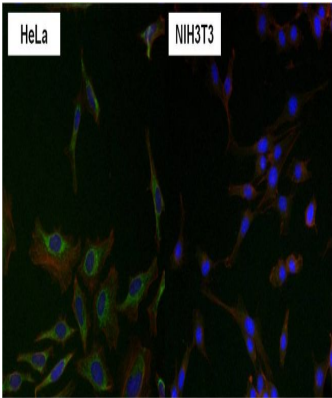
HSPA9 Antibody (MA1-094)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with HSPA9 siRNA and reduction of signal was observed in Western Blot using HSPA9 Monoclonal Antibody (Product # MA1-094). {KD}



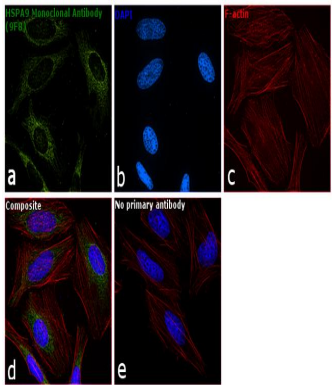
HSPA9 Antibody (MA1-094) in ICC/IF

Immunofluorescent analysis of HSPA9 using anti-HSPA9 monoclonal antibody (Product # MA1-094) shows specific expression in HeLa cells (shown in green) but not in negative control NIH3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature. Cells were blocked with 1% Blocker BSA (Product # 37525) for 15 minutes at room temperature. Cells were probed with a mouse monoclonal antibody recognizing HSPA9 (Product # MA1-094), at a dilution of 1:50 for at least 1 hour at room temperature. Cells were washed with PBS and incubated with DyLight 488 goat-anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. F-Actin (red) was stained with DY-554 phalloidin, nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan at 20X magnification.



HSPA9 Antibody (MA1-094) in ICC/IF

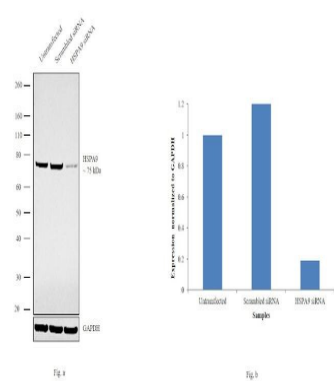
Immunofluorescence analysis of HSPA9 was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with HSPA9 Monoclonal Antibody (9F8) (Product # MA1-094) at 1:50 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing mitochondrial localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



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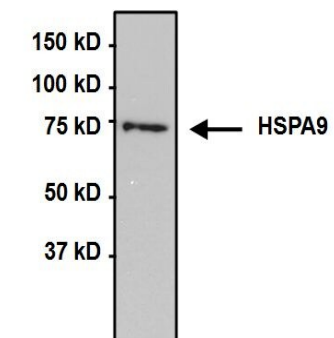


HSPA9 Antibody (MA1-094) in WB

Knockdown of HSPA9 was achieved by transfecting HeLa cells with HSPA9 specific siRNAs (Silencer® select Product # s6990, s6988). Western blot analysis (Fig. a) was performed using whole cell extracts from the HSPA9 knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with HSPA9 Monoclonal Antibody (Product # MA1-094, 1:1000 dilution) and Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25µg/ml, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to HSPA9.

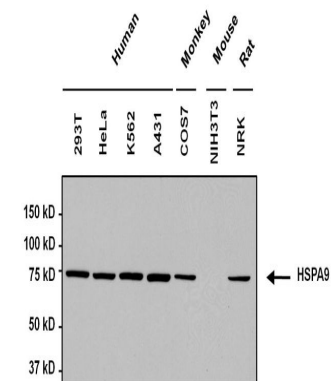
HSPA9 Antibody (MA1-094) in IP

Immunoprecipitation of HSPA9 was performed on HeLa cells. The antigen:antibody complex was formed by incubating 750 µg whole cell lysate with 2 µg of mouse monoclonal antibody recognizing HSPA9 (Product # MA1-094) overnight on a rocking platform at 4°C. The immune-complex was then captured on 50 µL Protein A/G Plus Agarose (Product # 20423). Captured immune-complexes were then washed extensively and proteins eluted with 5X Reducing Sample Loading Dye (Product # 39000). Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to PVDF membrane and blocked with 5% Milk/TBS-0.1%Tween for at least 1 hour. Membranes were then probed with a mouse monoclonal antibody recognizing HSPA9 (Product # MA1-094) at a dilution of 1:1000 overnight rotating at 4°C. Membranes were washed in TBST and probed with Pierce Clean Blot (Product # 21230) at a dilution of 1:1,000 for at least one hour. Membranes were washed and chemiluminescent detection performed using Pierce Super Signal West Dura (Product # 34075).



HSPA9 Antibody (MA1-094) in WB

Western blot analysis of HSPA9 was performed by loading 50 µg whole cell lysate onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. Membranes were then probed with a mouse monoclonal antibody recognizing HSPA9 (Product # MA1-094) at a dilution of 1:1000 overnight at 4°C on a rocking platform. Membranes were then washed in TBS-0.1%Tween 20 and probed with a goat anti-mouse-HRP secondary antibody (Product # 31430) at a dilution of 1:20000 for at least one hour. Membranes were washed and chemiluminescent detection was performed using Super Signal West Pico (Product # 34080).



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PubMed References For HSPA9 Monoclonal Antibody (9F8)

1 Western Blot References

Species / Dilution	Summary
	MA1-094 was used in western blot analysis of HSPA9 in control and PKM2-knockout cells to investigate the effect of methylated PKM2 on InsP3R levels.
Not Applicable / 1:1000	<p>Nature cell biology (2017; 19: 1358)</p> <p>"PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis."</p> <p>Author(s):Liu F, Ma F, Wang Y, Hao L, Zeng H, Jia C, Wang Y, Liu P, Ong IM, Li B, Chen G, Jiang J, Gong S, Li L, Xu W</p> <p>PubMed Article URL:http://dx.doi.org/10.1038/ncb3630</p>

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