Technical Data Sheet

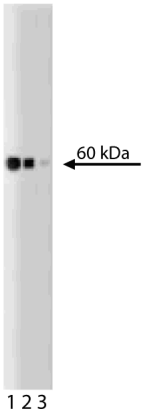
Purified Mouse Anti-Hsp60

Product Information

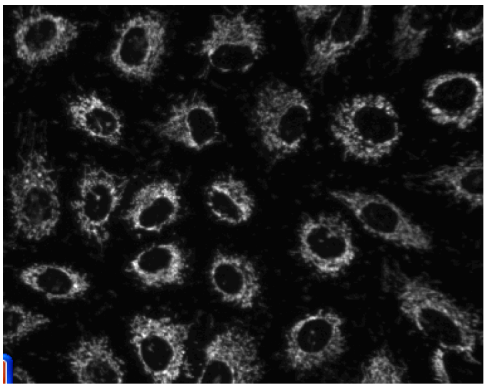
Material Number:	**611563**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	24/HSP60
Immunogen:	Human Hsp60 Recombinant Protein
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat
Target MW:	60 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Heat **s**hock **p**roteins (Hsp) are a set of highly conserved proteins that include constitutively expressed (Hsp60, Hsp70, and Hsp90) and stress-induced (Hsp27 and Hsp72) proteins. Hsp60 is localized to the mitochondria, where it promotes mitochondrial protein folding and facilitates proteolytic degradation of misfolded or denatured proteins. It binds Hsp10, which regulates the substrate binding and ATPase activity of Hsp60. In HeLa and Jurkat mitochondria, Hsp60 associates with caspase-3 to form a complex that dissociates and releases from the mitochondria during apoptosis. In addition, Hsp60 accelerates the maturation of procaspase-3 through its ATP-dependent "foldase" activity. In addition to its role in protein folding, Hsp60 has also been implicated in immune function. In macrophages, its binding to the toll-like receptor-4 complex induces production of TNFα and nitric oxide and stimulation of a proinflammatory response. Thus, the protein folding function of Hsp60 is involved in mitochondrial protein folding in both normal and apoptotic cells, while release of Hsp60 during necrosis is thought to stimulate a proinflammatory response.



Western blot analysis of Hsp60 on a Jurkat lysate (Cat. No. 611451). *Lane 1: 1:5000, lane 2: 1:10000, lane 3: 1:20000 dilution of the Hsp60 antibody.*



Immunofluorescent staining of A549 (ATCC CCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the Triton™ X-100 perm protocol and the anti-Hsp60 antibody. The second step reagent was Alexa Fluor 488 goat anti mouse Ig (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained U-2 OS (ATCC HTB-96) and HeLa (ATCC CCL-2) cells using both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methaol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Ohashi K, Burkart V, Flohe S, Kolb H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol.* 2000; 164(2):558-561. (Biology)

Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S. Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. *EMBO J.* 1999; 18(8):2040-2048. (Biology)

Venner TJ, Singh B, Gupta RS. Nucleotide sequences and novel structural features of human and Chinese hamster hsp60 (chaperonin) gene families. *DNA Cell Biol.* 1990; 9(8):545-552. (Biology)

Xanthoudakis S, Roy S, Rasper D, et al. Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. *EMBO J.* 1999; 18(8):2049-2056. (Biology)