BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

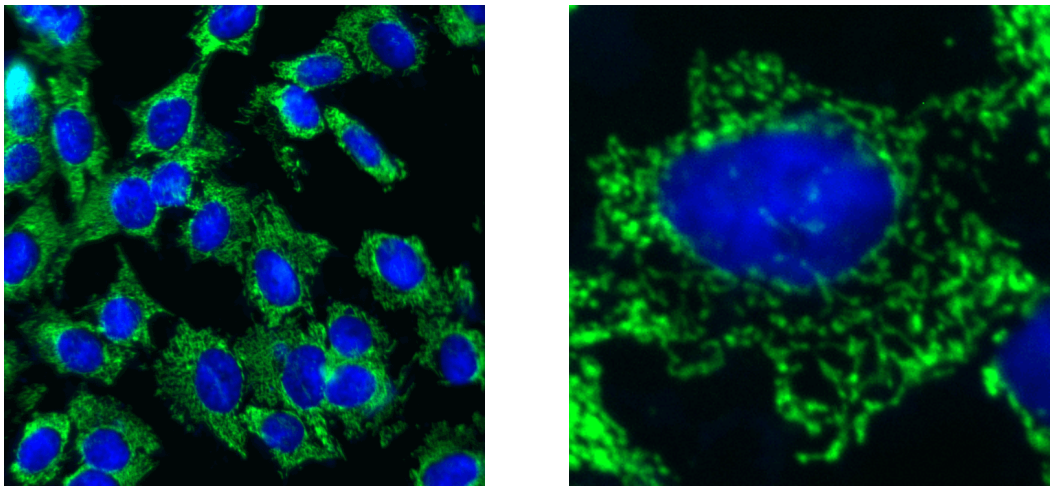
Alexa Fluor® 647 Mouse anti-Hsp60

Product Information

Material Number:	**558684**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	24/HSP60
Immunogen:	Human Hsp60 Recombinant Protein
Isotype:	Mouse IgG1
Reactivity:	Confirmed by Bioimaging: Human Confirmed by western blot using purified antibody (Cat. no. 611562 or 611563): Human, Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA 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.



Immunofluorescent staining of human cell lines. *HeLa cells (ATCC CCL-2) were cultured, fixed, permeabilized with cold methanol, stained with Alexa Fluor® 647 Mouse anti-Hsp60 (pseudo-colored green), and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 855 Bioimager System with 20x (left panel) and 40X (right panel) objectives and merged using BD Attovision™ software. This antibody also stains A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells. Triton X-100 is not recommended as a permeabilization agent with this antibody conjugate.*

Preparation and Storage

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Bioimaging	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

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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 fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells by adding 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubating for 5 minutes at RT.
4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of blocking buffer (3% FBS in 1× PBS) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well and incubating for 30 minutes at RT.
6. Remove the blocking buffer, dilute the antibody conjugate 1:10 in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted antibody conjugate to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody conjugate, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
9. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	620/60	700/75	660 LP
BD Pathway 435	628/40	690/40	FF660

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
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. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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)