Technical Data Sheet

FITC Mouse Anti-Hsp60

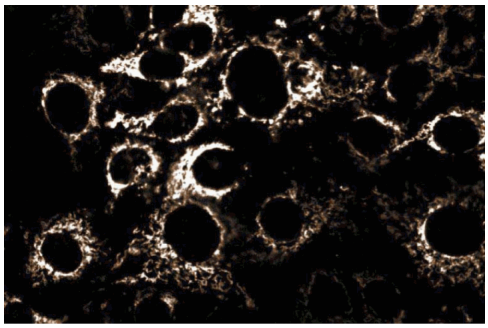
Product Information

Material Number:	**611958**
Size:	50 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.

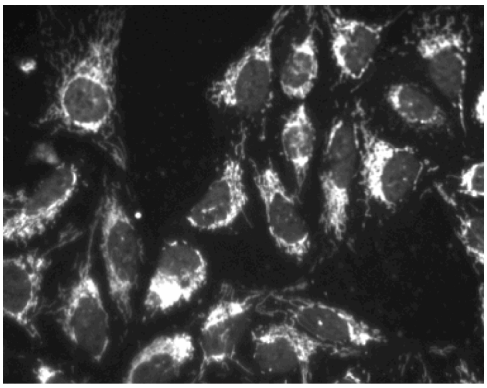
Description

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.

This antibody is routinely tested by immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



***Immunofluorescent staining of NIH-3T3 with** FITC anti-Hsp60 antibody (Cat. No. 611958)*



***Immunofluorescent staining of U2OS 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 X100 fix/perm protocol (see Recommended Assay Procedure) and the FITC anti-Hsp60 antibody (Cat. No. 611958). The image was taken on a Pathway 855 imager using a 20x objective. This antibody also stained U2OS and HeLa cells using both the Triton X100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

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

Store undiluted at -20°C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Tested During Development

BD Biosciences

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Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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)