HSP60 Rabbit Polyclonal Antibody

RPCA-HSP60

Ordering Information
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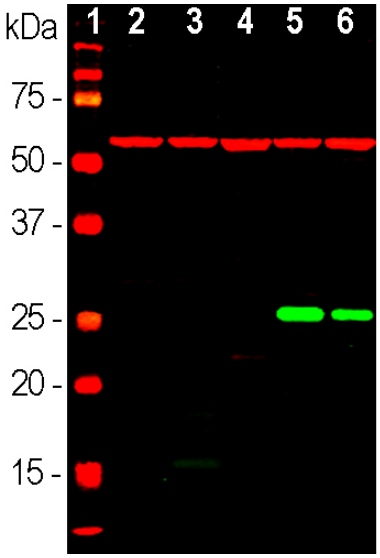
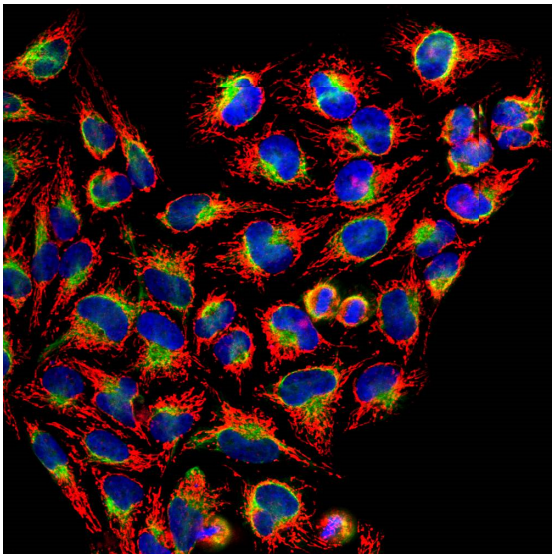
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *HSBD1*
UniProt: *P10809*
RRID: *AB_2572332*
Immunogen: *Recombinant full length human HSP60 expressed in and purified from E. coli*
Format: *Supplied as an aliquot of serum plus 5mM* NaN_3
Storage: *Store at 4°C for short term, for longer term at -20°C. Minimize freeze/thaw cycles*
Recommended dilutions:
WB: 1:5,000-10,000. ICC/IF and IHC: 1:5,000.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Rabbit		60kDa	Hu, Rt, Ms



Western blot analysis of different tissue or cell lysates using rabbit pAb to HSP-60, RPCA-HSP60, dilution 1:5,000 in red. [1] protein standard, [2] rat brain, [3] mouse brain, [4] NIH-3T3, [5] HEK293, [6] HeLa, [7] SH-SY5Y cells. The strong 60kDa band present in all preparations corresponds to HSP60 protein. The blot was simultaneously probed with mouse mAb to HSP27, MCA-6H11, dilution 1:10,000, in green. Strong single band at ~27kDa corresponds to the HSP27 protein, detected only in human cell lines since this particular antibody does not recognize rodent HSP27.



Confocal immunofluorescent analysis of HeLa cells stained with rabbit pAb to HSP60, RPCA-HSP60, dilution 1:1,000, in red, and costained with chicken pAb to vimentin, CPCA-VIM, dilution 1:1,000 in green. The blue is DAPI staining of nuclear DNA. The HSP60 antibody gives strong and specific staining of mitochondria while the vimentin antibody reveals cytoplasmic intermediate filaments.

Background:

The heat shock proteins were discovered, as the name suggests, since they are heavily upregulated when cells are stressed by temperatures above the normal physiological range. They are expressed in unstressed cells also and have a normal function as chaperones, helping other proteins to fold correctly. The need for chaperones is much greater if a cell or tissue is stressed by heat, and so these proteins become heavily up regulated. The different heat shock proteins were originally named based on their SDS-PAGE mobility, so HSP60 has an apparent molecular weight of 60kDa. It is an abundant protein in mitochondria and is typically responsible for the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix 1,2). In addition to its role as a heat shock protein, HSP60 plays an important role in the transport and maintenance of mitochondrial proteins as well as the transmission and replication of mitochondrial DNA (3,4). HSP60 has been implicated in the initiation and/or progression of some subtypes of cardiovascular disease (CVD), implying its potential as a biomarker with applications for diagnosis, assessing prognosis and response to treatment, as well as for preventing and treating CVD (5). HSP60 appears to be unusually immunogenic, frequently generating autoantibodies in humans and other species (e.g. 6). The HSP60 protein presumably released from damaged or degenerated cells is also a strong inducer of the innate immune system (7).

Our original monoclonal antibody to HSP60 MCA-1C7 was discovered during screens of hybridomas from a mouse injected with an unrelated immunogen. The target for one antibody was found to be HSP60. The mouse had likely developed autoantibodies to HSP60 since this molecule appears to unusually immunogenic, frequently generating autoantibodies in humans and other species (6). To prove this we made recombinant HSP60 protein which was then used to generate this polyclonal rabbit antibody to HSP60 and also chicken polyclonal antibody to HSP60, CPCA-HSP60. Like MCA-1C7, these antibodies are excellent markers of mitochondria and recognize HSP60 cleanly on western blots.

References:

1. Radford JC, Coates AR, Henderson B. Chaperonins are cell-signalling proteins: the unfolding biology of molecular chaperones. Expert Rev. Mol. Med. 2:1–17 (2000).
2. Bukau B, Horwich AL.The Hsp70 and Hsp60 Chaperone Machines Cell 92:351-66 (2000).
3. Koll H, et al. Antifolding activity of hsp60 couples protein import into the mitochondrial matrix with export to the intermembrane space. Cell 68:1163–75 (1992).
4. Kaufman BA. Kolesar JE, Perlman PS, Butow RA. A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in Saccharomyces cerevisiae. J. Cell Biol. 163:457-61 (2003).
5. Rizzo M, et al. Heat shock protein-60 and risk for cardiovascular disease. Curr. Pharm. Des. 17:3662-8 (2011).
6. Pockley AG, et al. Identification of human heat shock protein 60 (Hsp60) and anti-Hsp60 antibodies in the peripheral circulation of normal individuals. Cell Stress Chaperones 4:29-35 (1999).
7. Kol A, et al. Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J. Immunol. 164:13-7 (2000).

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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