HSP60 Mouse Monoclonal Antibody

MCA-1C7

Ordering Information
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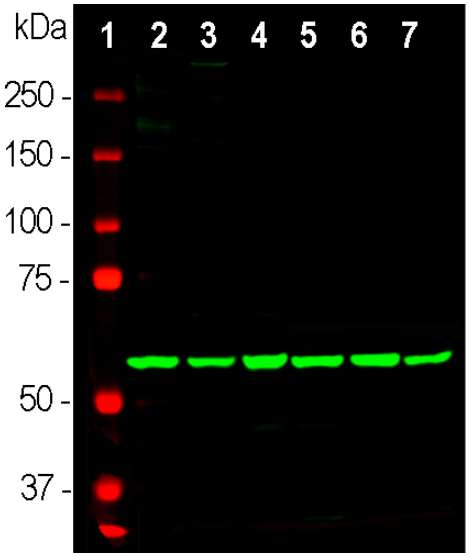
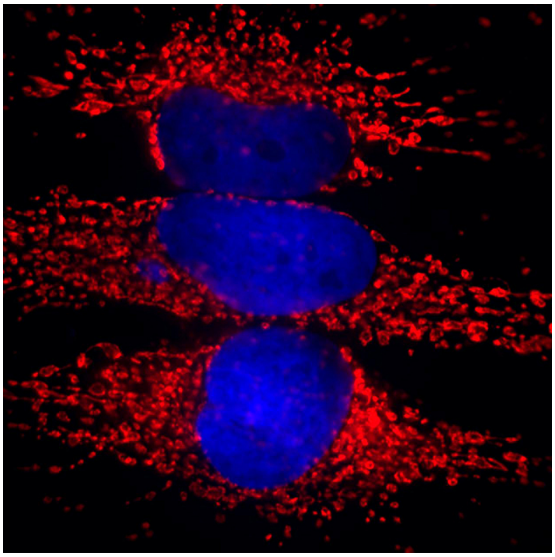
HGNC Name: *HSBD1*
UniProt: *P10809*
RRID: *AB_2572331*
Immunogen: *Spontaneous autoantibody, binds LNERLAKLSDGVAVLKVGGT, amino acids 390-409 of the human sequence*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Store at 4°C for short term, for longer term at -20°C*
Recommended dilutions:
WB: 1:10,000. ICC/IF and IHC: 1:5,000.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG1	60kDa	Human, Rat, Mouse, Cow, Pig, Horse, Dog, Monkey



Western blot analysis of tissue or whole cell lysates using mouse mAb to HSP60, MCA-1C7, dilution 1:10,000, in green. [1] protein standard (red), [2] rat brain, [3] mouse brain, [4] NIH-3T3, [5] HEK293, [6] HeLa, [7] SH-SY5Y cells. Strong single band corresponds to HSP60 protein with apparent SDS-PAGE molecular weight of 60kDa.



Immunofluorescent analysis of HeLa cells stained with mouse mAb to heat shock protein 60 (HSP60), MCA-1C7, dilution 1:5,000, in red. Blue is DAPI staining of nuclear DNA. MCA-1C7 antibody produces strong and specific staining of mitochondria.

Background:

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. HSP60 is a homologue of the well studied bacterial chaperone GroEL and both are ATPases (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).

Our antibody was discovered during screens for antibodies produced by hybridomas from a mouse injected with an unrelated protein. We noted beautiful staining of mitochondria and clean staining of a single band of 60kDa on western blots, suggesting HSP60 as an obvious candidate. We made recombinant full length human HSP60 which MCA-1C7 bound to strongly and specifically. The mouse had likely developed autoantibodies to HSP60 since this molecule appears to be unusually immunogenic, frequently generating autoantibodies in humans and other species (e.g. 6). The HSP60 protein was presumably released from damaged or degenerated cells and is a strong inducer of the innate immune system (7). We mapped the epitope to within the peptide LNERLAKLSDGVAVLKVGGT, amino acids 390-409 of the human sequence which is highly conserved in all vertebrates so we can predict that MCA-1C7 will be widely applicable (see here) for full sequence information). The antibody has a KD of 4.736 X 10^{-10} M. The antibody works well for western blotting and for IF, ICC and IHC (for IHC see data under "Additional Info" tab). The same full length recombinant protein was also used to generate polyclonal rabbit RPCA-HSP60 and polyclonal chicken CPCA-HSP60 antibodies. Like MCA-1C7, these antibodies are excellent markers of mitochondria and recognize HSP60 cleanly on western blots.

References:

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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.