HSP60 Chicken Polyclonal Antibody

CPCA-HSP60

Ordering Information
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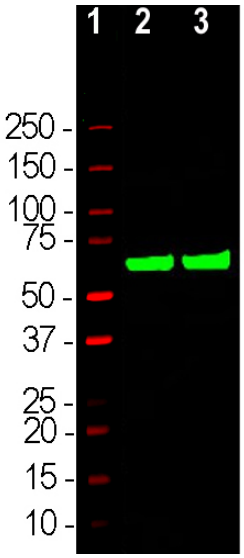
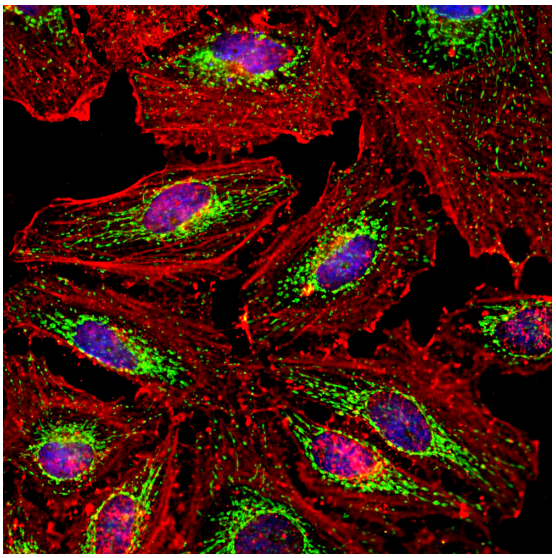
HGNC Name: *HSBD1*
UniProt: *P10809*
RRID: *AB_2572330*
Immunogen: *Recombinant full length human HSP60 expressed in and purified from E. coli*
Format: *Concentrated IgY preparation in PBS plus 0.02% NaN_3*
Storage: *Store at 4°C*
Recommended dilutions:
WB: 10,000-20,000. IF/ICC: 1: 2,000-5,000. IHC: 1:15,000

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



Western blot analysis of different cell lysates using chicken pAb to HSP60, CPCA-HSP60, dilution 1:20,000, in green. [1] protein standard (red), [2] SH-SY5Y cells, [3] HeLa cells. The strong single strong band corresponds to the HSP60 protein with an apparent SDS-PAGE molecular weight of 60kDa.



Immunofluorescent analysis of of HeLa cells stained with chicken pAb to HSP60, dilution 1:5,000 in green and costained with mouse mAb to actin, MCA-5J11, dilution 1:500 in red. Blue is DAPI staining of nuclear DNA. The CPCA-HSP60 antibody produces strong and specific staining of mitochondria, while the actin antibody labels the submembranous cytoskeleton, stress fibers and bundles of actin associated with cell adhesion sites.

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. 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 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 to another protein, which suggested HSP60 as an obvious candidate. We made recombinant full length human HSP60 which MCA-1C7 bound to strongly. The mouse had likely developed autoantibodies to HSP60 since this molecule appears to unusually immunogenic, frequently generating autoantibodies in humans and other species (6). The recombinant protein was then used to generate polyclonal rabbit anti HSP60 RPCA-HSP60 and this chicken polyclonal antibody to HSP60. Like MCA-1C7, these antibodies are excellent markers of mitochondria and recognize HSP60 cleanly on western blots. The antibody also identifies metabolically active cells in IHC, see data under the "Additional Info" tab.

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.