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Anti-Hsp90 alpha Antibody Picoband™ (monoclonal, 6B5)

Catalog # ABO15059

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P07900
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Hsp90 alpha Antibody Picoband™ (monoclonal, 6B5) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	3320
Other Names	Heat shock protein HSP 90-alpha, 3.6.4.10, Heat shock 86 kDa, HSP 86, HSP86, Heat shock protein family C member 1, Lipopolysaccharide-associated protein 2, LAP-2, LPS-associated protein 2, Renal carcinoma antigen NY-REN-38, HSP90AA1 (HGNC:5253), HSP90A, HSPC1, HSPCA
Calculated MW	84660
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat, Monkey Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 6B5
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Hsp90 alpha, identical to the related mouse and rat sequences.
Purification	Immunogen affinity purified.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six

months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	HSP90AA1 (HGNC:5253)
Synonyms	HSP90A, HSPC1, HSPCA
Function	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity which is essential for its chaperone activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function (PubMed:11274138, PubMed:12526792, PubMed:15577939, PubMed:15937123, PubMed:27353360, PubMed:29127155). Engages with a range of client protein classes via its interaction with various co-chaperone proteins or complexes, that act as adapters, simultaneously able to interact with the specific client and the central chaperone itself (PubMed:29127155). Recruitment of ATP and co-chaperone followed by client protein forms a functional chaperone. After the completion of the chaperoning process, properly folded client protein and co- chaperone leave HSP90 in an ADP-bound partially open conformation and finally, ADP is released from HSP90 which acquires an open conformation for the next cycle (PubMed:26991466, PubMed:27295069). Plays a critical role in mitochondrial import, delivers preproteins to the mitochondrial import receptor TOMM70 (PubMed:12526792). Apart from its chaperone activity, it also plays a role in the regulation of the transcription machinery. HSP90 and its co-chaperones modulate transcription at least at three different levels (PubMed:25973397). In the first place, they alter the steady-state levels of certain transcription factors in response to various physiological cues (PubMed:25973397). Second, they modulate the activity of certain epigenetic modifiers, such as histone deacetylases or DNA methyl transferases, and thereby respond to the change in the environment (PubMed:25973397). Third, they participate in the eviction of histones from the promoter region of certain genes and thereby turn on gene expression (PubMed:25973397). Binds bacterial lipopolysaccharide (LPS) and mediates LPS-induced inflammatory response, including TNF secretion by monocytes (PubMed:11276205). Antagonizes STUB1-mediated inhibition of TGF-beta signaling via inhibition of STUB1-mediated SMAD3 ubiquitination and degradation (PubMed:24613385). Mediates the association of TOMM70 with IRF3 or TBK1 in mitochondrial outer membrane which promotes host antiviral response (PubMed:20628368, PubMed:25609812).
Cellular Location	Nucleus {ECO:0000250|UniProtKB:P07901}. Cytoplasm {ECO:0000250|UniProtKB:P07901}. Melanosome. Cell membrane. Mitochondrion. Note=Identified by mass spectrometry in melanosome fractions from stage I to stage IV

Background

Heat shock protein HSP 90-alpha is a protein that in humans is encoded by the HSP90AA1 gene. The gene, HSP90AA1, encodes the human stress-inducible 90-kDa heat shock protein alpha (Hsp90A). Complemented by the constitutively expressed paralog Hsp90B which shares over 85% amino acid sequence identity, Hsp90A expression is initiated when a cell experiences proteotoxic stress. Once expressed Hsp90A dimers operate as molecular chaperones that bind and fold other proteins into their functional 3-dimensional structures. This molecular chaperoning ability of Hsp90A is driven by a cycle of structural rearrangements fueled by ATP hydrolysis. Current research on Hsp90A focuses in its role as a drug target due to its

interaction with a large number of tumor promoting proteins and its role in cellular stress adaptation.

Images

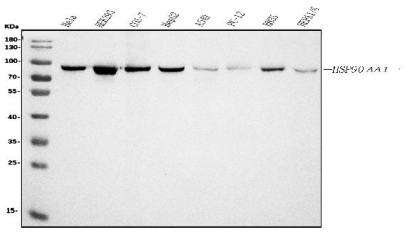


Figure 1. Western blot analysis of Hsp90 alpha using anti-Hsp90 alpha antibody (M01103-4).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human HEK293 whole cell lysates,
Lane 3: monkey COS-7 whole cell lysates,
Lane 4: human HepG2 whole cell lysates,
Lane 5: human A549 whole cell lysates,
Lane 6: rat PC-12 whole cell lysates,
Lane 7: rat RH35 whole cell lysates,
Lane 8: mouse HEPA1-6 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Hsp90 alpha antigen affinity purified monoclonal antibody (Catalog # M01103-4) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Hsp90 alpha at approximately 90KD. The expected band size for Hsp90 alpha is at 90KD.

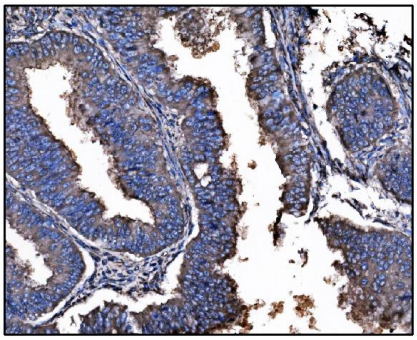


Figure 2. IHC analysis of Hsp90 alpha using anti-Hsp90 alpha antibody (M01103-4).
Hsp90 alpha was detected in paraffin-embedded section of human cervical cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Hsp90 alpha Antibody (M01103-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

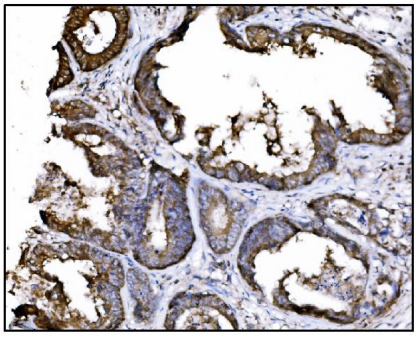


Figure 3. IHC analysis of Hsp90 alpha using anti-Hsp90 alpha antibody (M01103-4).
Hsp90 alpha was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Hsp90 alpha Antibody (M01103-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was

developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

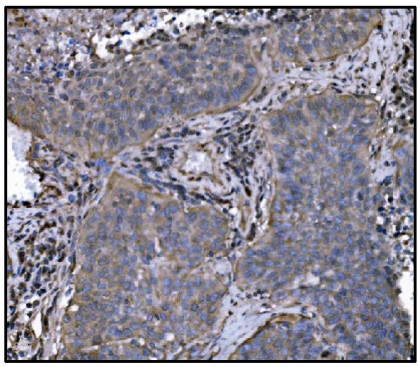


Figure 4. IHC analysis of Hsp90 alpha using anti-Hsp90 alpha antibody (M01103-4).
Hsp90 alpha was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Hsp90 alpha Antibody (M01103-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

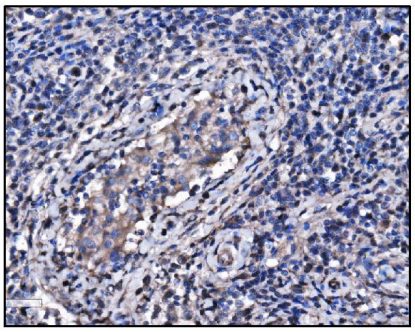


Figure 5. IHC analysis of Hsp90 alpha using anti-Hsp90 alpha antibody (M01103-4).
Hsp90 alpha was detected in paraffin-embedded section of human testis cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Hsp90 alpha Antibody (M01103-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

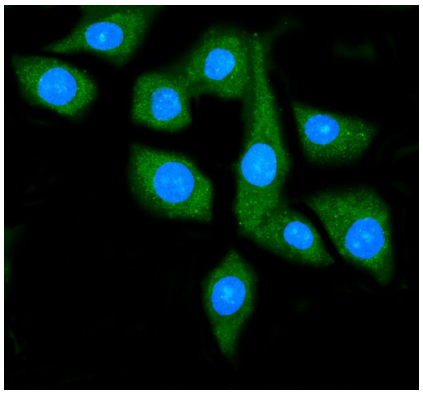


Figure 6. IF analysis of Hsp90 alpha using anti-Hsp90 alpha antibody (M01103-4).
Hsp90 alpha was detected in immunocytochemical section of SiHa cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Hsp90 alpha Antibody (M01103-4) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

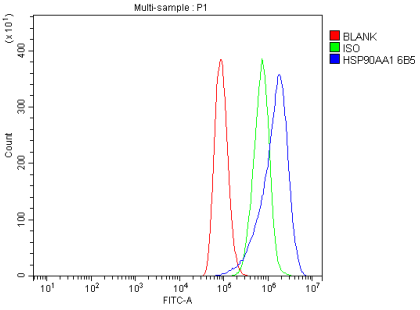


Figure 7. Flow Cytometry analysis of A549 cells using anti-Hsp90 alpha antibody (M01103-4).
Overlay histogram showing A549 cells stained with M01103-4 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hsp90 alpha Antibody (M01103-4, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.