10320 Camino Santa Fe, Suite G
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Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3)

Catalog # ABO14869

Specification

Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P04792
Host	**Mouse**
Isotype	**Mouse IgG1**
Reactivity	**Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) - Additional Information

Gene ID 3315

Other Names
Heat shock protein beta-1, HspB1, 28 kDa heat shock protein, Estrogen-regulated 24 kDa protein, Heat shock 27 kDa protein, HSP 27, Heat shock protein family B member 1, Stress-responsive protein 27, SRP27, HSPB1, HSP27, HSP28

Calculated MW
27 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml
 Immunocytochemistry/Immunofluorescence, 2 µg/ml
 Flow Cytometry, 1-3 µg/1x10^6 cells

Subcellular Localization
Nucleus. Spindle. Cytoplasm.

Tissue Specificity
Detected in all tissues tested: skeletal muscle, heart, aorta, large intestine, small intestine, stomach, esophagus, bladder, adrenal gland, thyroid, pancreas, testis, adipose tissue, kidney, liver, spleen, cerebral cortex, blood serum and cerebrospinal fluid. Highest levels are found in the heart and in tissues composed of striated and smooth muscle.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

Immunogen
E.coli-derived human Hsp27/HSPB1 recombinant protein (Position: M1-K205). Human Hsp27 shares 83% amino acid (aa) sequence identity with mouse Hsp27.

Cross Reactivity
No cross-reactivity with other proteins.

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.**

Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) - Protein Information

Name HSPB1

Synonyms HSP27, HSP28

Function
Small heat shock protein which functions as a molecular chaperone probably maintaining denatured proteins in a folding- competent state (PubMed:10383393, PubMed:20178975). Plays a role in stress resistance and actin organization (PubMed:19166925). Through its molecular chaperone activity may regulate numerous biological processes including the phosphorylation and the axonal transport of neurofilament proteins (PubMed:23728742).

Cellular Location
Cytoplasm. Nucleus Cytoplasm, cytoskeleton, spindle Note=Cytoplasmic in interphase cells. Colocalizes with mitotic spindles in mitotic cells. Translocates to the nucleus during heat shock and resides in sub-nuclear structures known as SC35 speckles or nuclear splicing speckles.

Tissue Location
Detected in all tissues tested: skeletal muscle, heart, aorta, large intestine, small intestine, stomach, esophagus, bladder, adrenal gland, thyroid, pancreas, testis, adipose tissue, kidney, liver, spleen, cerebral cortex, blood serum and cerebrospinal fluid. Highest levels are found in the heart and in tissues composed of striated and smooth muscle.

Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) - Images

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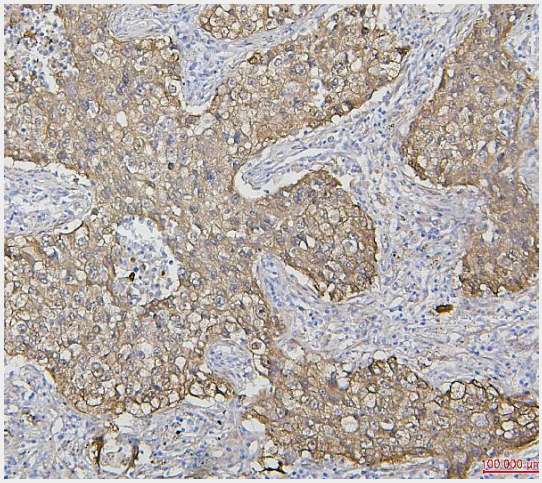

Figure 2. IHC analysis of HSP27 using anti-HSP27 antibody (M00676-5).
HSP27 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSP27 Antibody (M00676-5) 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.

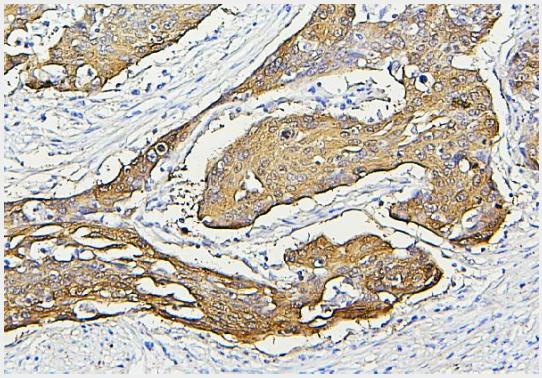

Figure 3. IHC analysis of HSP27 using anti-HSP27 antibody (M00676-5).
HSP27 was detected in paraffin-embedded section of human oesophagus squama cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSP27 Antibody (M00676-5) 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.

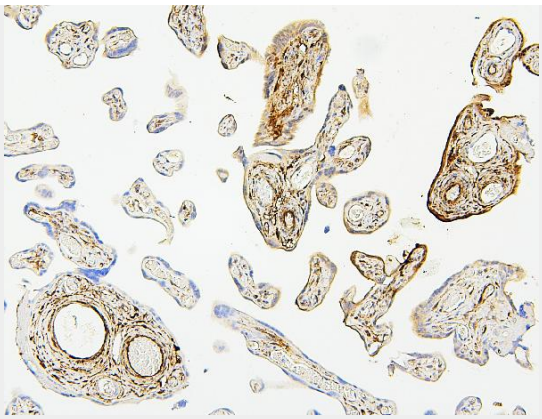

Figure 4. IHC analysis of HSP27 using anti-HSP27 antibody (M00676-5).
HSP27 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSP27 Antibody (M00676-5) 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.

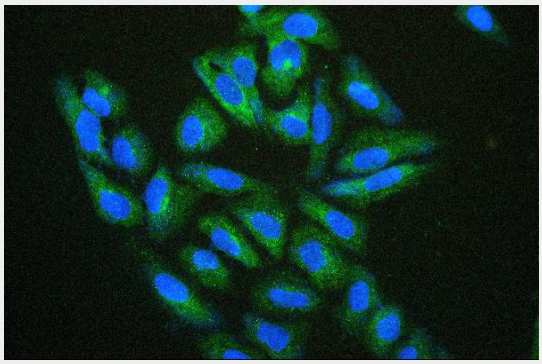

Figure 5. IF analysis of HSP27 using anti- HSP27 antibody (M00676-5).
HSP27 was detected in immunocytochemical section of U20S cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti- HSP27 Antibody (M00676-5) 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.

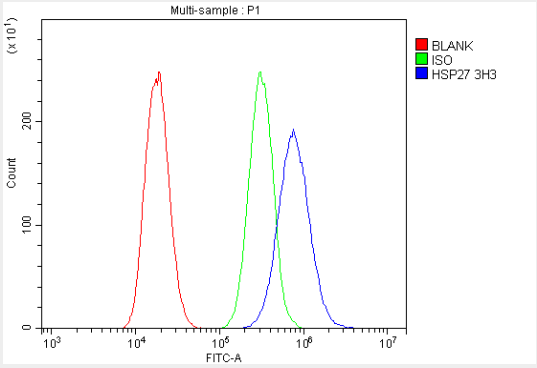

Figure 6. Flow Cytometry analysis of A549 cells using anti-HSP27 antibody (M00676-5).

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Overlay histogram showing A549 cells stained with M00676-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HSP27 Antibody (M00676-5,1 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.

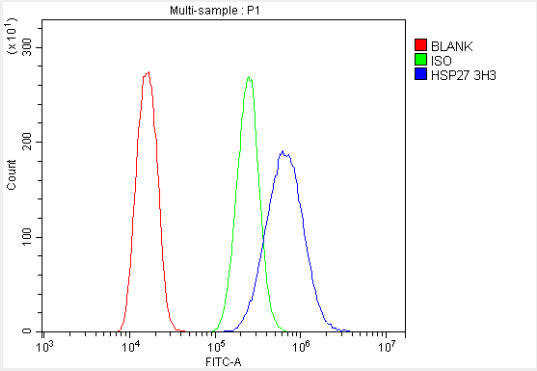

Figure 7. Flow Cytometry analysis of U20S cells using anti-HSP27 antibody (M00676-5).
Overlay histogram showing U20S cells stained with M00676-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HSP27 Antibody (M00676-5,1 µg/1x10^6 cells) for 30 min at 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.

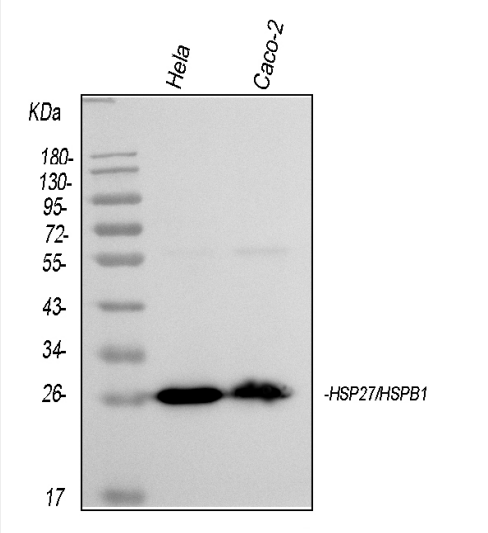

Figure 1. Western blot analysis of HSP27 using anti-HSP27 antibody (M00676-5).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates
Lane 2: human CACO-2 whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-HSP27 antigen affinity purified monoclonal antibody (Catalog # M00676-5) 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 HSP27 at approximately 27 kDa. The expected band size for HSP27 is at 27 kDa.

Anti-Hsp27/HSPB1 Antibody Picoband™ (monoclonal, 3H3) - Background

HSPB1 (Heat shock 27kDa protein 1), also known as HSP27, is a protein that in humans is encoded by the HSPB1 gene. HSP27 gene is mapped to 7q11.23. The protein encoded by this gene is induced by environmental stress and developmental changes. The encoded protein is involved in stress resistance and actin organization and translocates from the cytoplasm to the nucleus upon stress induction. Defects in this gene are a cause of Charcot-Marie-Tooth disease type 2F (CMT2F) and distal hereditary motor neuropathy (dHMN).