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Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4)

Catalog # ABO14778

Specification

Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P54652
Host	**Mouse**
Isotype	**Mouse IgG1**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

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

Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) - Additional Information

Gene ID 3306

Other Names
Heat shock-related 70 kDa protein 2, Heat shock 70 kDa protein 2, Heat shock protein family A member 2, HSPA2

Calculated MW
70 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
Cytoplasm, cytoskeleton, spindle

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

Immunogen
A synthetic peptide corresponding to a sequence at the C-terminus of human HSPA2, identical to the related mouse and rat sequences.

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-HSPA2 Antibody Picoband™ (monoclonal, 4A4) - Protein Information

Name HSPA2

Function
Molecular chaperone implicated in a wide variety of cellular processes, including protection of the proteome from stress, folding and transport of newly synthesized polypeptides, activation of proteolysis of misfolded proteins and the formation and dissociation of protein complexes. Plays a pivotal role in the protein quality control system, ensuring the correct folding of proteins, the re-folding of misfolded proteins and controlling the targeting of proteins for subsequent degradation. This is achieved through cycles of ATP binding, ATP hydrolysis and ADP release, mediated by co-chaperones. The affinity for polypeptides is regulated by its nucleotide bound state. In the ATP-bound form, it has a low affinity for substrate proteins. However, upon hydrolysis of the ATP to ADP, it undergoes a conformational change that increases its affinity for substrate proteins. It goes through repeated cycles of ATP hydrolysis and nucleotide exchange, which permits cycles of substrate binding and release (PubMed:26865365). Plays a role in spermatogenesis. In association with SHCBP1L may participate in the maintenance of spindle integrity during meiosis in male germ cells (By similarity).

Cellular Location
Cytoplasm, cytoskeleton, spindle {ECO:0000250|UniProtKB:P17156}. Note=Colocalizes with SHCBP1L at spindle during the meiosis process. {ECO:0000250|UniProtKB:P17156}

Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) - 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-HSPA2 Antibody Picoband™ (monoclonal, 4A4) - Images

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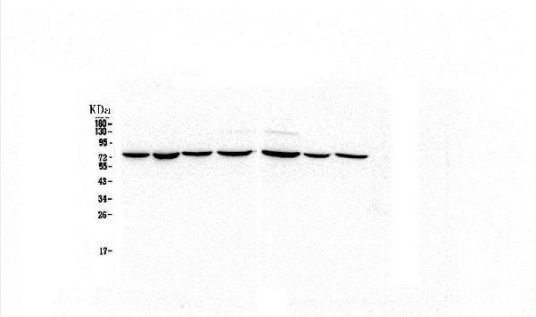

Figure 2. Western blot analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysate,
Lane 2: human MDA-MB-231 whole cell lysate,
Lane 3: human COLO-320 whole cell lysate,
Lane 4: human PANC-1 whole cell lysate.
Lane 5: human HT1080 whole cell lysate,
Lane 6: human MDA-MB-453 whole cell lysate,
Lane 7: human HepG2 whole cell lysate.
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-HSPA2 antigen affinity purified monoclonal antibody (Catalog # M03474-1) 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.

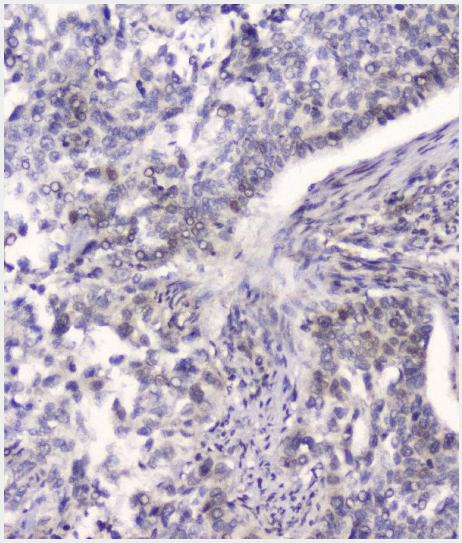

Figure 1. IHC analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
HSPA2 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HSPA2 Antibody (M03474-1) 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.

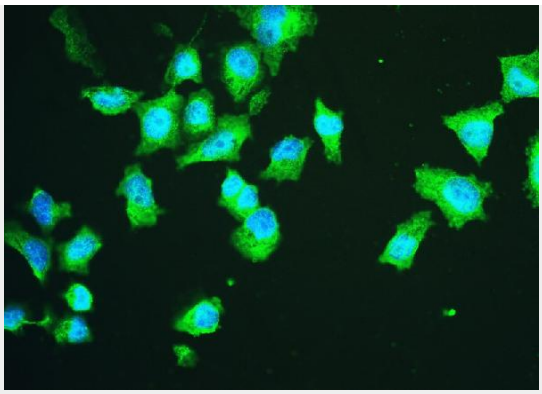

Figure 3. IF analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
HSPA2 was detected in immunocytochemical section of PC-3 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL rabbit anti-HSPA2 Antibody (M03474-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.

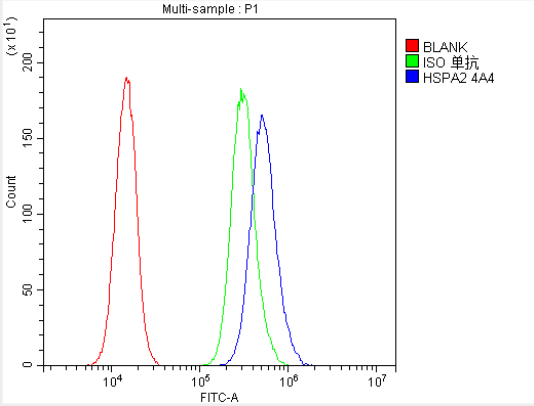

Figure 4. Flow Cytometry analysis of PC-3 cells using anti-HSPA2 antibody (M03474-1).
Overlay histogram showing PC-3 cells stained with M03474-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-HSPA2 Antibody (M03474-1,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

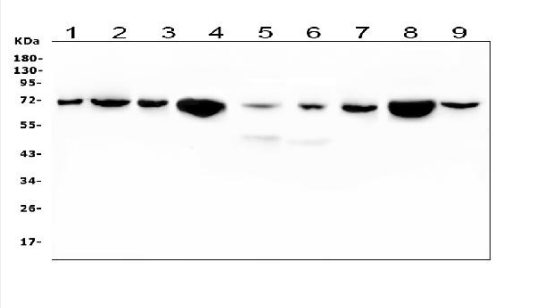

Figure 5. Western blot analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing

conditions.
Lane 1: rat lung tissue lysates,
Lane 2: rat liver tissue lysates,
Lane 3: rat kidney tissue lysates,
Lane 4: rat testicular tissue lysates,
Lane 5: mouse lung tissue lysates,
Lane 6: mouse liver tissue lysates,
Lane 7: mouse kidney tissue lysates,
Lane 8: mouse testicular tissue lysates,
Lane 9: mouse RAW246.7 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-HSPA2 antigen affinity purified monoclonal antibody (Catalog # M03474-1) 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 HSPA2 at approximately 70KD. The expected band size for HSPA2 is at 70KD.

Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) - Background

HSPA2 (heat shock 70kDa protein 2) is also known as HEAT-SHOCK PROTEIN, 70-KD, 2, HSP70-2, HEAT-SHOCK PROTEIN, 70-KD, 3 or HSP70-3. Analysis of the sequence indicated that HSPA2 is the human homolog of the murine Hsp70-2 gene, with 91.7% identity in the nucleotide coding sequence and 98.2% in the corresponding amino acid sequence. HSPA2 has less amino acid homology to the other members of the human HSP70 gene family. HSPA2 is constitutively expressed in most tissues, with very high levels in testis and skeletal muscle. The HSPA2 gene is located on chromosome 14q22-q24. Immunohistochemical analysis detected weak expression of HSPA2 in spermatocytes and stronger expression in spermatids and in the tail of mature sperm. HSPA2 may be critical to sperm maturation through its role as a protein chaperone.