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

Catalog Number: M03474-1

About HSPA2

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.

Overview

Product Name	Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) catalog # M03474-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 4A4
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	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.
Host	Mouse
Uniprot ID	P54652

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HSPA2, identical to the related mouse and rat sequences.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG1
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

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Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml Immunocytochemistry/Immunofluorescence, 2ug/ml Flow Cytometry, 1-3ug/1x10^6 cells

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Anti-HSPA2 Antibody Picoband™ (monoclonal, 4A4) (M03474-1) Images

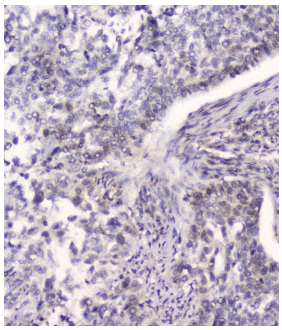

Figure 1. IHC analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
HSPA2 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat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

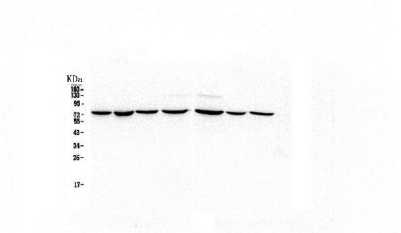

Figure 2. Western blot analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole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

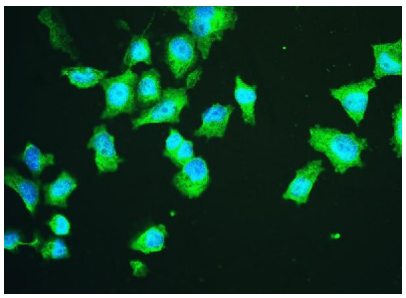

Figure 3. IF analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
HSPA2 was detected in immunocytochemical section of PC-3 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/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.

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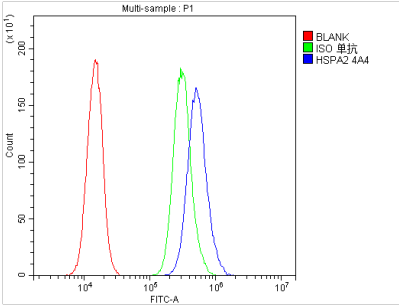

Figure 4. Flow Cytometry analysis of PC-3 cells using anti-HSPA2 antibody (M03474-1).
Overlay histogram showing PC-3 cells stained with M03474-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-HSPA2 Antibody (M03474-1,1ug/1x10^6 cells) for 30 min at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

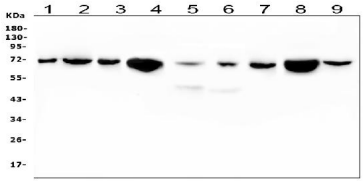

Figure 5. Western blot analysis of HSPA2 using anti-HSPA2 antibody (M03474-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat lung tissue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