

Anti-Hsc70/HSPA8 Antibody Picoband™

Catalog Number: PB9641

About HSPA8

HSPA8 (heat shock 70kDa protein 8) also known as HSC70, HSC71, HSP73, HSPA10, FORMERLY, LAP1 or LPS-ASSOCIATED PROTEIN 1, is a heat shock protein that in humans is encoded by the HSPA8 gene. The HSPA8 gene contains 9 exons and spans 5 kb. The deduced HSPA8 protein has 646 amino acids and a predicted molecular mass of 70,899 Da. And the HSPA8 gene is mapped on 11q24.1. HSPA8 plays an important role in cells by transiently associating with nascent polypeptides to facilitate correct folding. HSP73 also functions as an ATPase in the disassembly of clathrin-coated vesicles during transport of membrane components through the cell. Rapid decay involves AU-rich binding protein AUF1, which complexes with heat-shock proteins HSC70 and HSP70, translation initiation factor EIF4G, and poly (A)-binding protein. In the absence of Ii3, Hsc70 formed a complex with Hsp40 and Hip, and this complex, in association with Eif4g and Pabp, formed a high-stability complex with Bim mRNA that protected it from ribonucleases.

Overview

Product Name	Anti-Hsc70/HSPA8 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Hsc70/HSPA8 Antibody Picoband™ catalog # PB9641. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 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	Rabbit
Uniprot ID	P11142

Technical Details

Immunogen	E.coli-derived human Hsc70 recombinant protein (Position: Q520-A614). Human Hsc70 shares 98.9% amino acid (aa) sequence identity with both mouse and rat Hsc70.
Predicted Reactive Species	Hamster
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
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, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry, 1-3ug/1x10⁶ cells, Human

Anti-Hsc70/HSPA8 Antibody Picoband™ (PB9641) Images

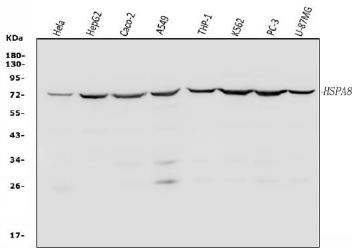


Figure 1. Western blot analysis of Hsc70 using anti-Hsc70 antibody (PB9641).

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 HepG2 whole cell lysates,
Lane 3: human CACO-2 whole cell lysates,
Lane 4: human A549 whole cell lysates,
Lane 5: human THP-1 whole cell lysates,
Lane 6: human K562 whole cell lysates,
Lane 7: human PC-3 whole cell lysates,
Lane 8: human U87MG 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 rabbit anti-Hsc70 antigen affinity purified polyclonal antibody (Catalog # PB9641) 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-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for Hsc70 at approximately 71 kDa. The expected band size for Hsc70 is at 71 kDa.

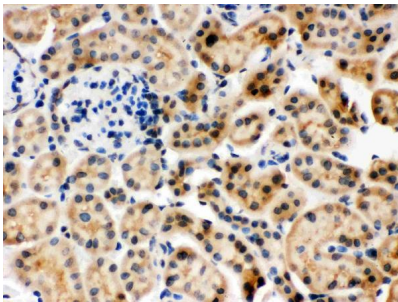


Figure 2. IHC analysis of Hsc70 using anti-Hsc70 antibody (PB9641).

Hsc70 was detected in a paraffin-embedded section of mouse kidney tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 ug/ml rabbit anti-Hsc70 Antibody (PB9641) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

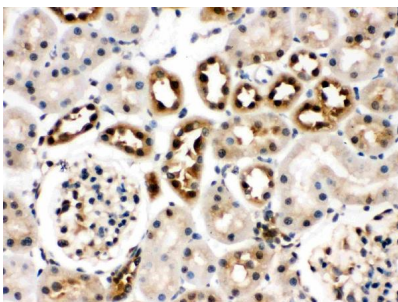


Figure 3. IHC analysis of Hsc70 using anti-Hsc70 antibody (PB9641).

Hsc70 was detected in a paraffin-embedded section of rat kidney tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 ug/ml rabbit anti-Hsc70 Antibody (PB9641) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

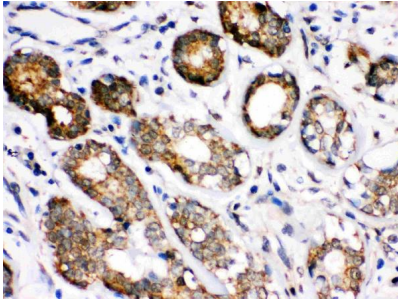


Figure 4. IHC analysis of Hsc70 using anti-Hsc70 antibody (PB9641).

Hsc70 was detected in a paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 ug/ml rabbit anti-Hsc70 Antibody (PB9641) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

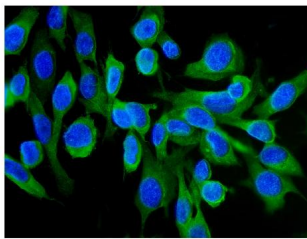


Figure 5. IF analysis of Hsc70 using anti-Hsc70 antibody (PB9641).

Hsc70 was detected in immunocytochemical section of U20S 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-Hsc70 Antibody (PB9641) 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.

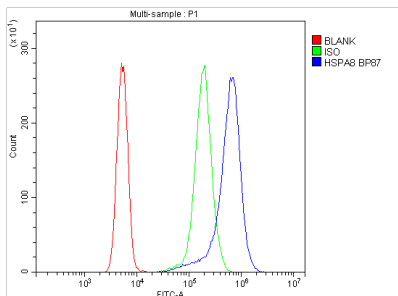


Figure 6. Flow Cytometry analysis of K562 cells using anti-Hsc70 antibody (PB9641).

Overlay histogram showing K562 cells stained with PB9641 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Hsc70 Antibody (PB9641, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

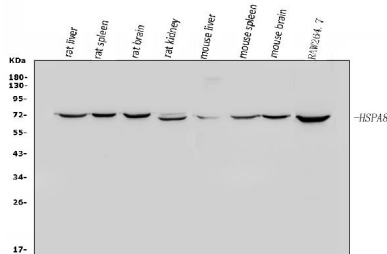


Figure 7. Western blot analysis of Hsc70 using anti-Hsc70 antibody (PB9641).

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: rat liver tissue lysates,
Lane 2: rat spleen tissue lysates,
Lane 3: rat brain tissue lysates,
Lane 4: rat kidney tissue lysates,
Lane 5: mouse liver tissue lysates,
Lane 6: mouse spleen tissue lysates,
Lane 7: mouse brain tissue lysates,
Lane 8: mouse RAW264.7 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 rabbit anti-Hsc70 antigen affinity purified polyclonal antibody (Catalog # PB9641) 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-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for Hsc70 at approximately 71 kDa. The expected band size for Hsc70 is at 71 kDa.

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