BOSTER BIOLOGICAL TECHNOLOGY
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Anti-GRP78 BiP/HSPA5 Antibody Picoband®

Catalog Number: PA1815

About HSPA5

HSPA5 (heat shock 70kDa protein 5) also known as glucose-regulated protein, 78kD (GRP78) or BiP, is a member of the heat-shock protein-70 (HSP70) family and is involved in the folding and assembly of proteins in the endoplasmic reticulum. BiP is also an essential component of the translocation machinery, as well as playing a role in retrograde transport across the ER membrane of aberrant proteins destined for degradation by the proteasome. The HSPA5 gene is mapped on 9q33.3. Shen et al. (2002) concluded that HSPA5 retains ATF6 in the ER by inhibiting its Golgi localization signals and that dissociation of HSPA5 during ER stress allows ATF6 to be transported to the Golgi. The findings of Shen et al. (2002) demonstrated that HSPA5 is a key element in sensing the folding capacity within the ER.

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Overview

Product Name	Anti-GRP78 BiP/HSPA5 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-GRP78 BiP/HSPA5 Antibody catalog # PA1815. Tested in IHC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
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	P11021

Technical Details

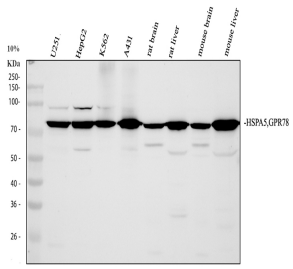
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human GRP78 BiP, identical to the related mouse and rat sequences.

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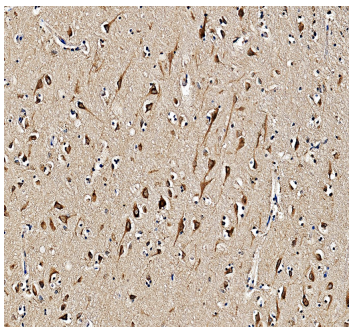
Recommended Detection Systems	Boster recommends ECL Plus Western Blotting Substrate (Catalog # AR1196-200) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.025-0.25ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human

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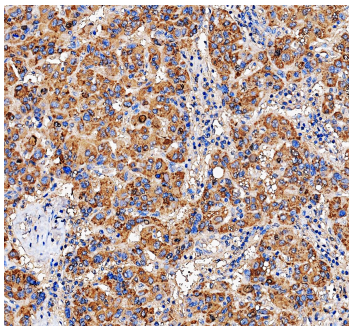
Anti-GRP78 BiP/HSPA5 Antibody Picoband® (PA1815) Images



Western blot analysis of HSPA5 using anti-HSPA5 antibody (PA1815). Electrophoresis was performed on a 10% SDS-PAGE gel at 80V (StacHSPA5g gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human U251 whole cell lysates, Lane 2: human HepG2 whole cell lysates, Lane 3: human K562 whole cell lysates, Lane 4: human A431 whole cell lysates, Lane 5: rat brain tissue lysates, Lane 6: rat liver tissue lysates, Lane 7: mouse brain tissue lysates, Lane 8: mouse liver tissue 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-HSPA5 antigen affinity purified polyclonal antibody (PA1815) at 0.17 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 ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for HSPA5 at approximately 78 kDa. The expected band size for HSPA5 is at 72 kDa.



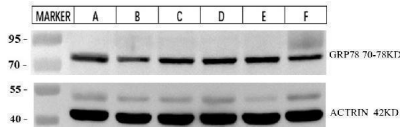
IHC analysis of HSPA5 using anti-HSPA5 antibody (PA1815). HSPA5 was detected in a paraffin-embedded section of human cerebral cortex tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-HSPA5 Antibody (PA1815) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



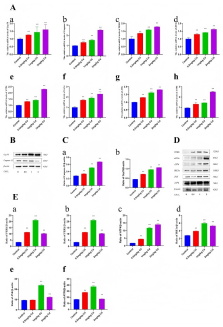
IHC analysis of HSPA5 using anti-HSPA5 antibody (PA1815). HSPA5 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-HSPA5 Antibody (PA1815) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

Western blot analysis of HSPA5 using anti-HSPA5 antibody

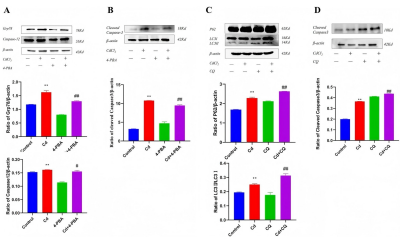
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(PA1815). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1-6: Different segments of the porcine large intestine and small intestine 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-HSPA5 antigen affinity purified monoclonal antibody (Catalog # PA1815) at 1:2000 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 1:10000 for 1 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with ChemiDoc MP system. A specific band was detected for HSPA5 at approximately 70-78 kDa. The expected band size for HSPA5 is at 73 kDa.



Cd induces ERS and activates the PERK, IRE1alpha, and ATF6 signaling pathways in rats. (A) mRNA expression levels of related factors. (a) Grp78; (b) Capase12; (c) PERK; (d) eIF2alpha; (e) ATF4; (f) IRE1alpha; (g) JNK; (h) ATF6. (B) Blots for Grp78 and Capase12. (C) Protein expression levels of related factors. (a) Grp78; (b) Capase12. (D) Blots for PERK, eIF2alpha, ATF4, IRE1alpha, JNK and ATF6. (E) Protein expression levels of related factors. (a) PERK; (b) eIF2alpha; (c) ATF4; (d) IRE1alpha; (e) JNK; (f) ATF6. beta -actin served as an internal reference and quantitative analysis of band intensities was performed using ImageJ software. Compared with the control group * $p < 0.05$ indicates a significant difference; ** $p < 0.01$ indicates a highly significant difference.Source: Chengxiang Guo et al. Licensed under CC BY 4.0. PMID: 42491593.



The impact of ERS and autophagy on apoptosis. (A) Western blot images and quantitative analysis of Grp78 and Caspase-12. (B) Western blot images and quantitative analysis of cleaved Caspase-3 after 4-PBA treatment. (C) Western blot images and quantitative analysis of P62 and LC3. (D) Western blot images and quantitative analysis of cleaved Caspase-3 after CQ treatment. beta -actin served as an internal reference and quantitative analysis of band intensities was performed using ImageJ software. Compared with the control group * $p < 0.05$ indicates a significant difference; ** $p < 0.01$ indicates a highly significant difference. Compared with the Cd group, # $p < 0.05$ indicates a significant difference; ## $p < 0.01$ indicates a highly significant difference.Source: Chengxiang Guo et al. Licensed under CC BY 4.0. PMID: 42491593.

7 Publications Citing This Product

1. PubMed ID: 31500865, Chi L,Jiao D,Nan G,Yuan H,Shen J,Gao Y.miR-9-5p attenuates ischemic stroke through targeting ERMP1-mediated endoplasmic reticulum stress.Acta Histochem.2019 Nov;121(8):151438.doi:10.1016/j.acthis.2019.08.005.Epub 2019 Sep 7.PMID:31500865.

2. PubMed ID: 33819629, Huang Y,Zhao C,Kong Y,Tan P,Liu S,Liu Y,Zeng F,Yuan Y,Zhao B,Wang J.Elucidation of the mechanism of NEFA-induced

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PERK-eIF2alpha signaling pathway regulation of lipid metabolism in bovine hepatocytes.J Steroid Biochem Mol Biol.2021 Apr 2:105893.doi:10.1016/j.jsbmb.2021.105893.Epub ahead of print.PMID:33819629.

3. PubMed ID: -, Pei-pei Fang,Chen-wei Pan,Wei Lin,Jie Li,Shan-shan Huang,Guang-yao Zhou,Wen-jun Du,Qiang Li, "ASK1 Enhances Angiotensin II-Induced Liver Fibrosis In Vitro by Mediating Endoplasmic Reticulum Stress-Dependent Exosomes",Mediators of Inflammation,vol.2020,Art

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