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Anti-ISG15 Antibody Picoband®

Catalog Number: PB9951

About Isg15

Interferon-stimulated gene 15 (ISG15) is a 17 kDA secreted protein that in humans is encoded by the ISG15 gene. The protein encoded by this gene is a ubiquitin-like protein that is conjugated to intracellular target proteins upon activation by interferon-alpha and interferon-beta. Several functions have been ascribed to the encoded protein, including chemotactic activity towards neutrophils, direction of ligated target proteins to intermediate filaments, cell-to-cell signaling, and antiviral activity during viral infections. While conjugates of this protein have been found to be noncovalently attached to intermediate filaments, this protein is sometimes secreted.

Overview

Product Name	Anti-ISG15 Antibody Picoband®
Reactive Species	Mouse
Description	Boster Bio Anti-ISG15 Antibody Picoband® catalog # PB9951. Tested in IHC, WB applications. This antibody reacts with Mouse. 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 antibody formulated with stabilizing components, 0.9 mg NaCl, 0.2 mg Na2HPO4, and 0.05 mg NaN3. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
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	Q64339

Technical Details

Immunogen	E.coli-derived mouse ISG15 recombinant protein (Position: A2-G155). Mouse ISG15 shares 65.8% amino acid (aa) sequence identity with human ISG15.
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).

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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	Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Mouse Western blot, 0.1-0.5ug/ml, Mouse

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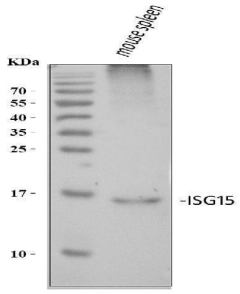
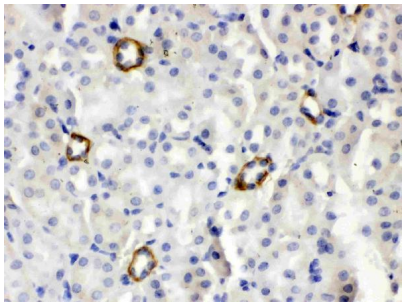
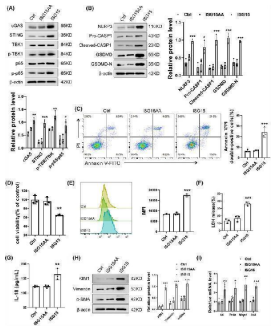
Anti-ISG15 Antibody Picoband® (PB9951) Images



Western blot analysis of ISG15/Ucrp using anti-ISG15/Ucrp antibody (PB9951). 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: mouse spleen 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-ISG15/Ucrp antigen affinity purified polyclonal antibody (Catalog # PB9951) 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 ISG15/Ucrp at approximately 17 kDa. The expected band size for ISG15/Ucrp is at 18 kDa.



IHC analysis of ISG15/Ucrp using anti-ISG15/Ucrp antibody (PB9951). ISG15/Ucrp was detected in paraffin-embedded section of mouse kidney 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 1ug/ml rabbit anti-ISG15/Ucrp Antibody (PB9951) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



ISG15 contributed to TECs injury in an ISGylation-dependent manner. (A) Western blot analysis and densitometric quantification of cGAS, STING, TBK1, p-TBK1, p65, p-p65 expression in TECs (n = 3). (B) Western blot analysis and densitometric quantification of NLRP3, Pro-CASP1, Cleaved-CASP1, GSDMD, GSDMD-N expression in TECs (n = 3). (C) Flow cytometry analysis and quantitative data depicting the Annexin V/PI double-positive cells rate (n = 3). (D) CCK-8 activity assay quantified cell viability (n = 3). (E–G) Levels of ROS (E), LDH (F), IL-18 (G) in TECs (n = 3). (H) Western blot analysis and densitometric quantification of KIM1, alpha-SMA and Vimentin expression in TECs (n = 3). (I) Relative mRNA level of pro-inflammatory factors (Il6 , Tnfa , Mcp1 and Il18) in TECs (n = 3). TECs were transfected with empty vector, ISG15AA or ISG15 (4 μg). Results are expressed as the mean ± SD. * p

ISG15 promoted STING signalling via cytosolic mtDNA and established a positive feedback loop. (A) Western blot

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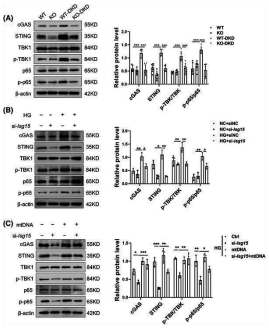
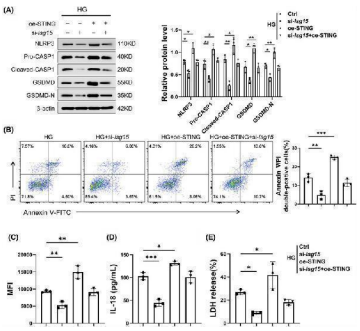
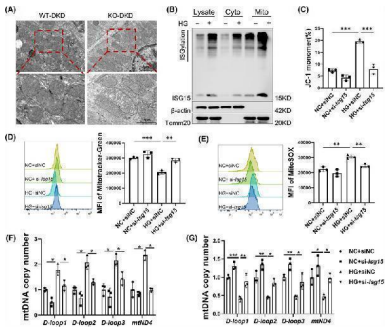


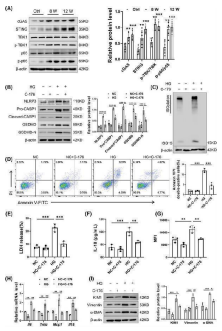
analysis and densitometric quantification of cGAS, STING, TBK1, p-TBK1, p65, p-p65 expression in WT and KO mice treated with vehicle or STZ (n = 6). (B and C) Western blot analysis and densitometric quantification of cGAS, STING, TBK1, p-TBK1, p65, p-p65 expression in TECs (n = 3). TECs were transfected with mtDNA (4 μg) or si- Isg15 (50 nM), and then cultured in HG medium for 48 h. Results are expressed as the mean ± SD. * p



ISG15–STING loop-maintained HG-induced injury in TECs. (A) Western blot analysis and densitometric quantification of NLRP3, Pro-CASP1, Cleaved-CASP1, GSDMD and GSDMD-N expression in TECs (n = 3). (B) Flow cytometry analysis and quantitative data depicting the Annexin V/PI double-positive cells rate (n = 3). (C–E) Levels of ROS (C), IL-18 (D), LDH (E) in TECs (n = 3). TECs were transfected with oe-STING (4 μg) or si- Isg15 (50 nM), and then cultured in HG medium for 48 h. Results are expressed as the mean ± SD. * p



ISG15 was involved in HG-induced mitochondrial impairment and mtDNA release. (A) Representative TEM images of kidney tissues from WT and KO mice treated with STZ (n = 6). (B) Western blot analysis ISG15/ISGylation expression in TECs treated with vehicle or HG (n = 3). (C–E) Flow cytometry analysis and quantitative data depicting the mitochondrial membrane potential (C), mitochondrial mass (D) and mtROS (E) (n = 3). (F and G) qPCR analysis the mtDNA (Loop1-3 and mt-Nd4) copy number in the cytosolic compartments (F) and mitochondria (G) (n = 3). TECs were treated with vehicle or C-176 (10 μM), and then cultured in HG medium for 48 h. Results are expressed as the mean ± SD. * p



The cGAS–STING pathway was activated in the DKD mice. (A) Western blot analysis and densitometric quantification of cGAS, STING, TBK1, p-TBK1, p65, p-p65 expression in DKD mice (n = 6). (B) Western blot analysis and densitometric quantification of NLRP3, Pro-CASP1, Cleaved-CASP1, GSDMD, GSDMD-N expression in TECs (n = 3). (C) Western blot analysis ISG15/ISGylation expression in TECs (n = 3). (D) Flow cytometry analysis and quantitative data depicting the Annexin V/PI double-positive cells rate (n = 3). (E–G) Levels of LDH (E), IL-18 (F), ROS (G) in TECs (n = 3). (H) Relative mRNA level of pro-inflammatory factors (Il6 , Tnfa , Mcp1 , Il18) in TECs (n = 3). (I) Western blot analysis and densitometric quantification of KIM1, alpha-SMA and Vimentin expression in TECs (n = 3). TECs were transfected with vehicle or C-176 (10 μM), and then cultured in HG medium for 48 h. Results are expressed as the mean ± SD. * p

Inhibition of pyroptosis blocked TECs damage and fibrosis

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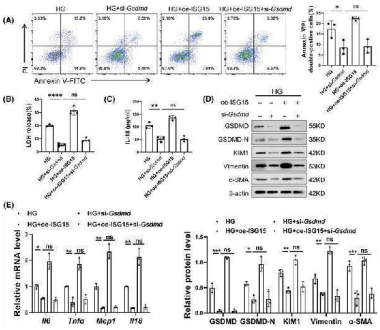
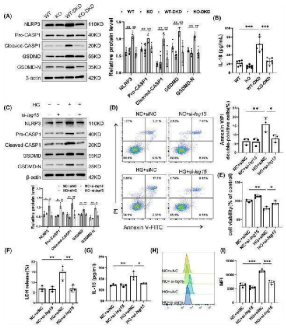
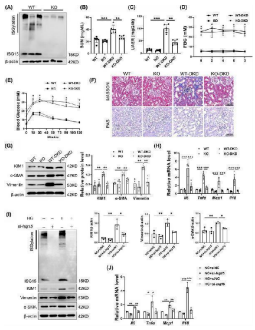


induced by ISG15. (A) Flow cytometry analysis and quantitative data depicting the Annexin V/PI double-positive cells rate (n = 3). (B and C) Levels of LDH (B), IL-18 (C) in TECs (n = 3). (D) Western blot analysis and densitometric quantification of GSDMD, GSDMD-N, KIM1, alpha-SMA and Vimentin expression in TECs (n = 3). (E) Relative mRNA level of pro-inflammatory factors (Il6 , Tnfa , Mcp1 and Il18) in TECs (n = 3). TECs were transfected with si- Gsdmd (50 nM) or oe-ISG15 (4 μg), and then cultured in HG medium for 48 h. Results are expressed as the mean ± SD. * p



Ablation of ISG15 decreased pyroptosis of TECs under HG stimulation. (A) Western blot analysis and densitometric quantification of pyroptosis-related proteins (NLRP3, Pro-CASP1, Cleaved-CASP1, GSDMD, GSDMD-N) expression in kidney tissues from WT and KO mice treated with vehicle or STZ (n = 6). (B) Level of IL-18 in the serum from WT and KO mice treated with vehicle or STZ (n = 6). (C) Western blot analysis of pyroptosis-related proteins (NLRP3, Pro-CASP1, Cleaved-CASP1, GSDMD, GSDMD-N) expression in TECs (n = 3). (D) Flow cytometry analysis and quantitative data depicting the TECs Annexin V/PI double-positive cells rate (n = 3). (E) CCK-8-kit activity assay quantified cell viability (n = 3). (F–I) Level of LDH (F), IL-18 (G), ROS (H and I) in TECs (n = 3). TECs were transfected with sinc (50 nM) or si- Isg15 (50 nM), and then cultured in HG medium for 48 h. Results are expressed as the mean ± SD. * p



ISG15 deletion was protective against renal injury. (A) Western blot analysis and densitometric quantification of ISG15 expression in kidney tissues from WT and KO mice (n = 6). (B–E) BUN (B), UAER (C), FBG (D) and OGTT (E) levels in WT and KO mice treated with vehicle or STZ (n = 6). (F) Representative images of MASSON and PAS staining of the kidney (n = 6). (G) Western blot analysis and densitometric quantification of KIM1, alpha-SMA and Vimentin expression in kidney tissues from WT and KO mice treated with vehicle or STZ (n = 6). (H) Relative mRNA level of pro-inflammatory factors (Il6 , Tnfa, Mcp1 and Il18) in the kidney tissues from WT and KO mice treated with vehicle or STZ (n = 6). (I) Western blot analysis and densitometric quantification of ISG15/ISGylation, KIM1, alpha-SMA and Vimentin expression in TECs (n = 3). (J) Relative mRNA level of pro-inflammatory factors (Il6, Tnfa, Mcp1 and Il18) in TECs (n = 3). TECs were transfected with sinc (50 nM) or si- Isg15 (50 nM), and then cultured in HG medium for 48 h. BUN, blood urea nitrogen; UAER, urinary albumin excretion rates; FBG, fasted blood; OGTT, oral glucose tolerance. Results are expressed as the mean ± SD. * p

High ISG15 expression in DKD mice. (A) Relative mRNA level of Isg15 in the kidney cortical tissues from WT mice and STZ/HFD-induced DKD mice (n = 6). (B) Western blot analysis and quantification of ISG15/ISGylation and KIM-1 expression in kidney cortical tissues from WT mice and

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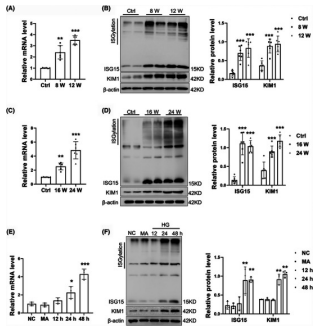

STZ/HFD-induced DKD mice (n = 6). (C) Relative mRNA level of Isg15 in the kidney from db/m, 16 W db/db and 24 W db/db mice (n = 6). (D) Western blot analysis and quantification of ISG15/ISGylation and KIM-1 expression in kidney cortical tissues from db/m, 16 W db/db and 24 W db/db mice (n = 6). (E) Relative mRNA level of Isg15 in the TECs cultured in normal medium, MA or HG for 48 h (n = 3). (F) Western blot analysis and quantification of ISG15/ISGylation and KIM-1 expression in the TECs (n = 3). DKD, diabetic kidney disease; HFD, high-fat diet; HG, high glucose; STZ, streptozotocin; MA, mannitol. Results are expressed as the mean ± SD. * p

1 Publications Citing This Product

1. PubMed ID: 10.1371/journal.ppat.1007235, Interferon-beta expression and type I interferon receptor signaling of hepatocytes prevent hepatic necrosis and virus dissemination in Coxsackievirus B3-infected mice

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Anti-ISG15 Antibody

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