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antibody and ELISA experts

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Anti-PKR/EIF2AK2 Antibody Picoband™

Catalog Number: PB9803

About EIF2AK2

EIF2AK2 (Eukaryotic Translation Initiation Factor 2-Alpha Kinase 2), also called PKR, is an enzyme that in humans is encoded by the EIF2AK2 gene. Activation of EIF2AK2 allows the kinase to phosphorylate its natural substrate, the alpha subunit of eukaryotic protein synthesis initiation factor-2, leading to the inhibition of protein synthesis. By FISH analysis, Squire et al. (1993) assigned the EIF2AK2 gene to the boundary between chromosome 2p22-p21. Ben-Asouli et al. (2002) showed that human gamma-interferon mRNA uses local activation of PKR in the cell to control its own translation yield. IFNG mRNA was found to activate PKR through a pseudoknot in its 5-prime untranslated region. Taylor et al. (1999) studied the mechanism underlying the resistance of hepatitis C virus (HCV) to interferon. They demonstrated that the HCV envelope protein E2 contains a sequence identical with phosphorylation sites of the interferon-inducible protein kinase PKR and the translation initiation factor EIF2-alpha, a target of PKR. E2 inhibited the kinase activity of PKR and blocked its inhibitory effect on protein synthesis and cell growth.

Overview

Product Name	Anti-PKR/EIF2AK2 Antibody Picoband™
Reactive Species	Human
Description	Boster Bio Anti-PKR/EIF2AK2 Antibody Picoband™ catalog # PB9803. Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, ICC, 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	P19525

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human PKR, different from the related mouse sequence by fifteen amino acids, and from the related rat sequence by thirteen amino acids.
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 ICC.
Cross Reactivity	No cross-reactivity with other proteins.

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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 Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Flow Cytometry, 1-3 ug/1x10^6 cells, Human

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Anti-PKR/EIF2AK2 Antibody Picoband™ (PB9803) Images

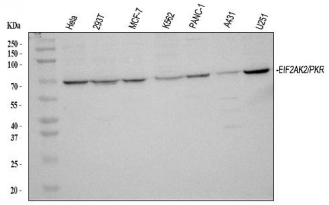

Figure 1. Western blot analysis of PKR/EIF2AK2 using anti-PKR/EIF2AK2 antibody (PB9803).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human 293T whole cell lysates,
Lane 3: human MCF-7 whole cell lysates,
Lane 4: human K562 whole cell lysates,
Lane 5: human PANC-1 whole cell lysates,
Lane 6: human A431 whole cell lysates,
Lane 7: human U251 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-PKR/EIF2AK2 antigen affinity purified polyclonal antibody (Catalog # PB9803) 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 PKR/EIF2AK2 at approximately 69 kDa. The expected band size for PKR/EIF2AK2 is at 62 kDa.

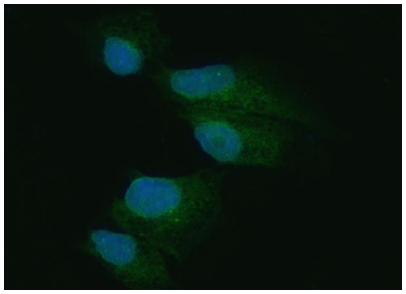

Figure 2. IF analysis of PKR/EIF2AK2 using anti-PKR/EIF2AK2 antibody (PB9803).
PKR/EIF2AK2 was detected in an immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 ug/mL rabbit anti-PKR/EIF2AK2 Antibody (PB9803) overnight at 4°C. DyLight488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:500 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.

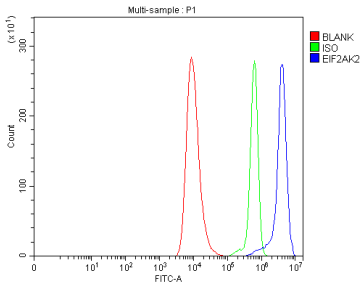

Figure 3. Flow Cytometry analysis of HEL cells using anti-PKR/EIF2AK2 antibody (PB9803).
Overlay histogram showing HEL cells stained with PB9803 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-PKR/EIF2AK2 Antibody (PB9803, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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