Anti-Phospho-PKR (T446) Antibody [SY230]

ET1607-20

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IP, IF-Cell
Molecular Wt:	Predicted band size: 62 kDa
Clone number:	SY230

Description: An interferon-inducible, RNA-dependent protein serine/threonine kinase, PKR has various designations. Mouse PKR is known as DAI, dsJ, PI kinase, p65, p67 or TIK, whereas human PKR is known as p68 or p69. PKR phosphorylates its substrate, a subunit of protein synthesis initiation factor eIF-2 on Ser 51 to inhibit translation. PKR contains two dsRNA binding motifs required for its activation by dsRNA. Three kinds of regulation of PKR enzymatic activity occur, and these include transcriptional regulation in response to interferon, an autoregulatory mechanism controlling PKR expression at the level of translation, and posttranslational regulation by RNA mediated autophosphorylation. Human PKR contains at least 15 autophosphorylation sites, but only Thr-446 and Thr-451 in the activation loop are critical for its kinase activity. Thr-446 is the in vivo autophosphorylation site of PKR. Mutation of threonine to alanine at position 446 substantially reduces PKR function, and mutant kinase containing Ala-451 is completely inactive.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr446 of Human PKR aa 421-470 / 551.

Positive control: Jurkat treated with 100nM Calyculin A for 30 minutes cell lysate, Hela treated with Calyculin A and TNF-alpha whole cell lysate, human tonsil tissue, human spleen tissue, human breast tissue, human breast carcinoma tissue, human kidney tissue, human small intestine tissue, SiHa.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P19525 Human

Recommended Dilutions:

WB	1:500-1:5,000
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.
IF-Cell	1:50

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

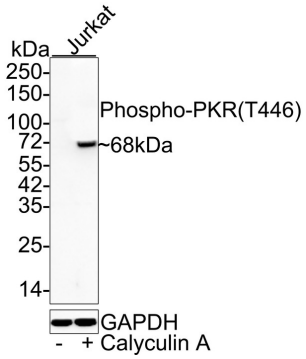

Fig1: Western blot analysis of Phospho-PKR (T446) on different lysates with Rabbit anti-Phospho-PKR (T446) antibody (ET1607-20) at 1/5,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: Jurkat treated with 100nM Calyculin A for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 62 kDa
Observed band size: 68 kDa

Exposure time: 30 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1607-20) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

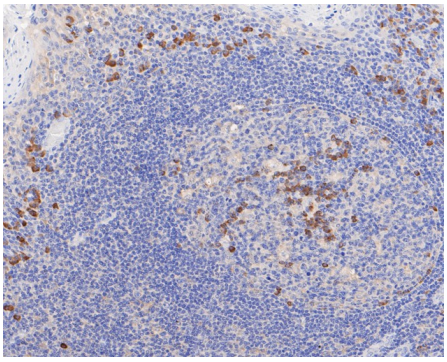

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Phospho-PKR (T446) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-20, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

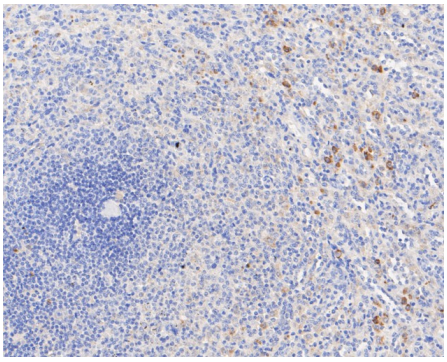

Fig3: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-Phospho-PKR (T446) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-20, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

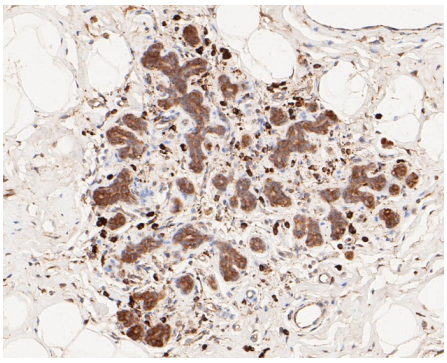

Fig4: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-Phospho-PKR (T446) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-20, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

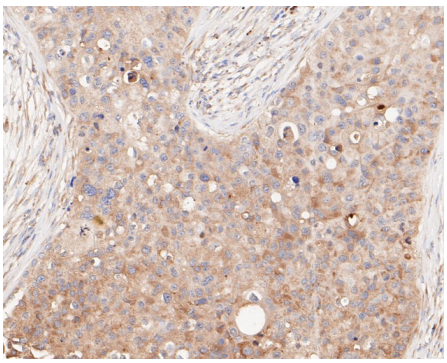

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-PKR (T446) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-20, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

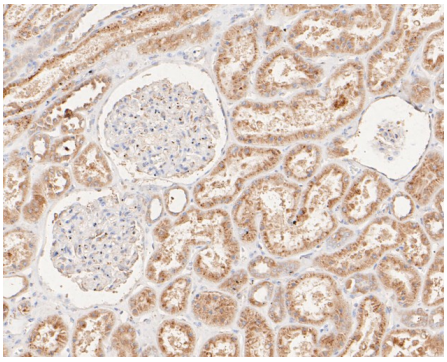

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Phospho-PKR (T446) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-20, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

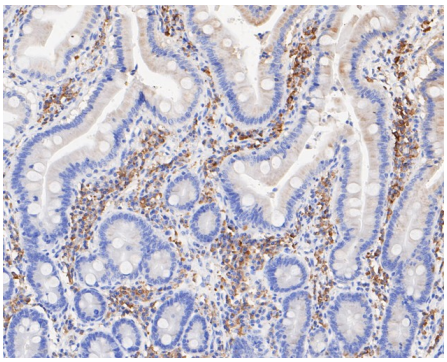

Fig7: Immunohistochemical analysis of paraffin-embedded human small intestine tissue using anti-Phospho-PKR (T446) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-20, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

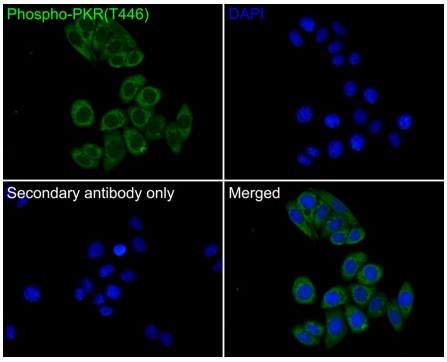

Fig8: Immunocytochemistry analysis of SiHa cells labeling Phospho-PKR (T446) with Rabbit anti-Phospho-PKR (T446) antibody (ET1607-20) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-PKR (T446) antibody (ET1607-20) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Background References

1. Pfaller CK et al. Measles virus C protein impairs production of defective copyback double-stranded viral RNA and activation of protein kinase R. J Virol 88:456-68 (2014).
2. Nejepinska J et al. Reporters transiently transfected into mammalian cells are highly sensitive to translational repression induced by dsRNA expression. PLoS One 9:e87517 (2014).