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PKR Recombinant Rabbit Monoclonal Antibody (23H52L96)

Catalog Number 700286

Product data sheet

Details

Size	100 µg
Host/Isotope	Rabbit / IgG
Class	Recombinant Monoclonal
Type	Antibody
Clone	23H52L96
Immunogen	A recombinant protein corresponding to amino acids 54-158.
Conjugate	Unconjugated
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS
Contains	0.09% sodium azide
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Species Reactivity

Species reactivity	Human
Published species	Hamster, Not Applicable

Tested Applications	Dilution *
Western Blot (WB)	0.5-3 µg/mL
Immunocytochemistry (ICC/IF)	5 µg/mL

Published Applications

Western Blot (WB)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

This antibody is predicted to react with Rhesus monkey based on sequence homology. Intact IgG appears on a non-reducing gel as ~150 kDa band and upon reduction generating a ~25 kDa light chain band and a ~50 kDa heavy chain. Recombinant rabbit monoclonal antibodies are produced using in vitro expression systems. The expression systems are developed by cloning in the specific antibody DNA sequences from immunoreactive rabbits. Then, individual clones are screened to select the best candidates for production. The advantages of using recombinant rabbit monoclonal antibodies include: better specificity and sensitivity, lot-to-lot consistency, animal origin-free formulations, and broader immunoreactivity to diverse targets due to larger rabbit immune repertoire.

Background/Target Information

EIF2AK2 (PKR) is one of 4 kinases that specifically phosphorylate Ser51 of translation initiation factor eIF2-alpha in response to various environmental stresses. EIF2AK2 plays a key role in antiviral defense mediated through its direct activation by double-stranded RNA produced by viral infection. PKR plays a key role in IFN induced-innate antiviral response, virus-induced apoptosis, cell growth & differentiation. PKR induces apoptosis by up-regulating Fas expression and mediates FADD/Caspase-8 death signaling pathway. Upon viral infection, it functions as a dual protein, sequentially activating both cell survival & cell death pathways using kinase independent and dependent strategies. PRKR regulates multiple pathways which include NF-kappaB activation, p53, p38, & PDGF signaling pathway. PKR has been implicated in tumor suppression & malignancy. To evade the antiviral effects of PKR, viruses have evolved multiple mechanisms, such as the inhibition of PKR by the non-structural protein (NS1) of the influenza virus. More recently, PKR has been implicated in several neurodegenerative diseases including Alzheimer, Huntington, and amyotrophic lateral sclerosis.

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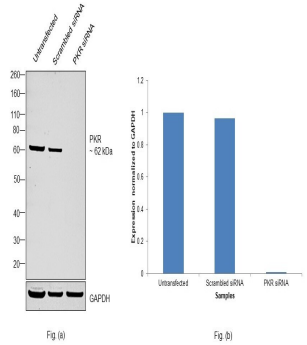
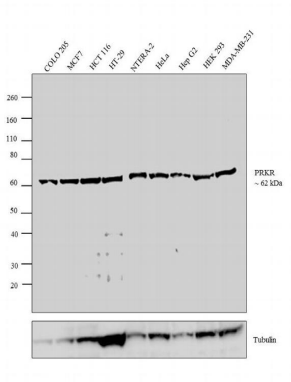
Product Images For PKR Recombinant Rabbit Monoclonal Antibody (23H52L96)



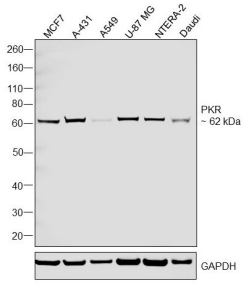
PKR Antibody (700286)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. MCF7 cells were transfected with Interferon-induced, double-stranded RNA-activated protein kinase siRNA and decrease in signal intensity was observed in Western Blot application using Anti-PKR Recombinant Rabbit Monoclonal Antibody (23H52L96) (Product # 700286). {KD}



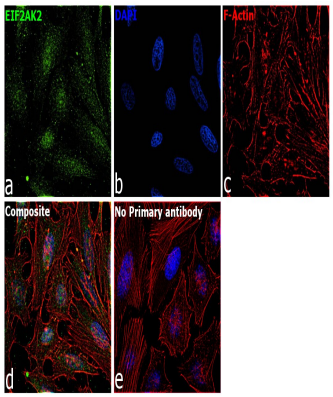
PKR Antibody (700286) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of COLO 205 (Lane 1), MCF7 (Lane 2), HCT 116 (Lane 3), HT-29 (Lane 4), NTERA-2 (Lane 5), HeLa (Lane 6), Hep G2 (Lane 7), HEK 293 (Lane 8) and MDA-MB-231 (Lane 9). The blot was probed with Anti-PRKR Rabbit Monoclonal Antibody (Product # 700286, 1-3 µg/mL) and detected by chemiluminescence using Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.4 µg/mL, 1:2500 dilution). A 62 kDa band corresponding to PRKR was observed across the cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 10 % Bis-Tris gel (Product # NP0302BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody using iBind™ Flex Western Starter Kit (Product # SLF2000S). Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



PKR Antibody (700286) in WB

Western Blot was performed using Anti-PKR Recombinant Rabbit Monoclonal Antibody (23H52L96) (Product # 700286) and a 62 kDa band corresponding to Interferon-induced, double-stranded RNA-activated protein kinase was observed across tested samples. Whole cell extracts (40 µg lysate) of MCF7 (Lane 1), A-431 (Lane 2), A549 (Lane 3), U-87 MG (Lane 4), NTERA-2 cl.D1 (Lane 5), Daudi (Lane 6) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (0.5 µg/mL concentration) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:20000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using SuperSignal™ West Dura Extended Duration Substrate (Product # 34076).



PKR Antibody (700286) in ICC/IF

Immunofluorescence analysis of Interferon-induced, double-stranded RNA-activated protein kinase was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 5 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 2% BSA for overnight at room temperature. The cells were labeled with PKR Recombinant Rabbit Monoclonal Antibody (23H52L96) (Product # 700286) at 5 µg /mL concentration in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32790), (1:2000 dilution), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b: Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasm and nucleus localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.

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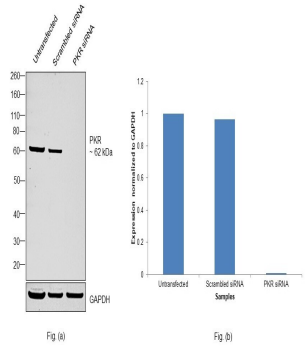
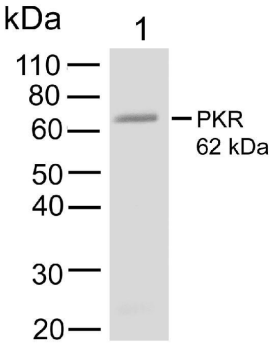


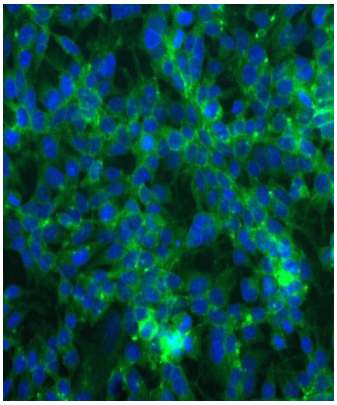
PKR Antibody (700286) in WB

Knockdown of Interferon-induced, double-stranded RNA-activated protein kinase was achieved by transfecting MCF7 with Interferon-induced, double-stranded RNA-activated protein kinase specific siRNAs (Silencer® select Product # S11187, S11185). Western Blot analysis (Fig. a) was performed using Whole cell extracts from the Interferon-induced, double-stranded RNA-activated protein kinase knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with PKR Recombinant Rabbit Monoclonal Antibody (23H52L96) (Product # 700286, 0.5 µg/mL concentration) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:20000 dilution). Densitometric analysis of this Western Blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to Interferon-induced, double-stranded RNA-activated protein kinase.



PKR Antibody (700286) in WB

Western blot analysis of PRKR in MCF-7 cell lysate using a PRKR recombinant rabbit monoclonal antibody (Product # 700286) at a dilution of 1 µg/mL. NBT/BCIP was used as the substrate (Product # WB7105).



PKR Antibody (700286) in ICC/IF

Immunofluorescent analysis of PRKR in HEK293 cells using a PRKR recombinant rabbit monoclonal antibody (Product # 700286) at a dilution of 5 µg/mL followed by detection using an Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody at a dilution of 1:1000. Cells were fixed using 4% paraformaldehyde. Cytoplasmic localization of PKR specific signal is shown in green, while nuclei were stained using SlowFade GOLD with DAPI (Product # S36938) shown in blue.

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PubMed References For PKR Recombinant Rabbit Monoclonal Antibody (23H52L96)

1 Western Blot References

Species / Dilution	Summary
	700286 was used in western blot to utilize an Aestivating Frog Xenopus laevis model to study post-translation regulation of hexokinase function and protein stability
Not Applicable / Not Cited	The protein journal (2016; 35: 61) **"Post-translational Regulation of Hexokinase Function and Protein Stability in the Aestivating Frog Xenopus laevis."** Author(s):Childers CL,Storey KB PubMed Article URL:http://dx.doi.org/10.1007/s10930-016-9647-0

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