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ProductInformation

ANTI-PKR (KT-16)
Developed in Rabbit, Affinity isolated Antibody

Product Number **P9993**

Product Description

Anti-Interferon-induced, double-stranded RNA-activated protein kinase (PKR) is developed in rabbit using as immunogen a synthetic peptide near the C-terminus of human PKR (amino acids 522-537), conjugated to KLH. This sequence is highly conserved (>80%) in rat PKR. The antiserum is affinity purified using the immunizing peptide immobilized on agarose. Anti-PKR specifically recognizes 522-537 amino acids PKR (68 kDa). The antibody detects human and rat PKR. It has been used in immunoblotting applications.

PKR is a ubiquitously expressed serine/threonine protein kinase (68 kDa in human) that is induced by IFN-γ and activated by dsRNA and stress signals.[1] It is a major mediator of the antiviral and anti-proliferative activities of interferons.[1,2,3,4,5] PKR binding to dsRNA, induces a conformational change that leads to PKR autophosphorylation and activation. Activated PKR phosphorylates its substrates, which include among others the α-subunit of translation initiator factor eIF-2 (eIF-2α), thereby inhibiting translation and protein synthesis.[3,6] The antiviral activity of PKR is in part mediated through phosphorylation of eIF-2α, which results in the sequestration of the recycling factor eIF-2B in an inactive complex with eIF-2-GDP.

In addition to its role as a regulator of translation, PKR is involved in the control of cell proliferation, differentiation, tumor suppression, apoptosis and cell cycle progression.[3,6,7,8] PKR has been identified as the signal transducer in cell stress signaling pathways leading to NFκB activation.[9,10] PKR mediates the activation of signal transduction pathways by pro-inflammatory stimuli, including bacterial lipopoly-saccharide (LPS), TNF-α and interleukin-1. In response to dsRNAs, PKR activates IκB kinase (IKK), leading to the degradation of the inhibitors IκBα and IκBβ and the concomitant release of NFκB. PKR activation of NFκB in cells by TNF-α and IFN-γ has been shown to be mediated by both NFκB-inducing kinase (NIK) and IKK.[11] Cells derived from PKR knockout mice or expressing dominant negative forms of PKR, display a defective induction of interferon regulatory factor 1 (IRF-1) and/or NFκB by IFN-γ or dsRNA, implicating PKR in these signaling pathways.[12] PKR interacts with p53 and phosphorylates it on Ser[392] to restrict cell proliferation, thus enhancing the transcriptional activity of this tumor suppressor protein.[13] In addition, overexpression of PKR causes induction of p53, resulting in apoptosis in mammalian cells, suggesting a role for PKR in tumor suppression. Peel et al., showed that human PKR preferentially bound mutant huntingtin RNA transcripts suggesting a role for PKR activation in the Huntington disease process.[14]

Reagent

The Anti-PKR is provided at approximately 1.0 mg/mL in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at -20 °C. For extended storage, upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Samples at working dilution should be discarded if not used within 12 hours.

Product Profile

A recommended working dilution of 1:2,000 is determined by immunoblotting, using a whole cell extract of the human epitheloid carcinoma HeLa cell line induced with interferon-γ.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

1. Williams, B.R.G., A sentinel kinase for cellular stress. Oncogene, **18**, 6112-6220 (1999).
2. Meurs, E.F., et al., Molecular cloning and characterization of human double-stranded RNA-activated protein kinase induced by interferon. Cell, **62**, 379-390 (1990).
3. Meurs, E.F., et al., Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. Proc. Natl. Acad. Sci. USA, **90**, 232-236 (1993).
4. Feng, G-S., et al., Identification of double-stranded RNA-binding domains in the interferon-induced double-stranded RNA-activated p68 kinase. Proc. Natl. Acad. Sci. USA, **89**, 5447-5451 (1992).
5. Tanaka, H., and Samuel, C.E., Mechanism of interferon action: structure of the mouse PKR gene encoding the interferon-inducible RNA-dependent protein kinase. Proc. Natl. Acad. Sci. USA, **91**, 7995-7999 (1994).
6. Chong, K.L., et al., Human p68 kinase exhibits growth suppression in yeast and homology to the translational regulator GCN2. EMBO J., **11**, 1553-1562 (1992).
7. Der, S.D., et al., A double-stranded RNA-activated protein kinase-dependent pathway mediating stress-induced apoptosis. Proc. Natl. Acad. Sci. USA, **94**, 3279-3283 (1997).
8. Gil, J., and Esteban, M., Induction of apoptosis by the ds-RNA-dependent protein kinase (PKR): mechanism of action. Apoptosis, **5**, 107-114 (2000).
9. Zamanian-Daryoush, M., et al., Cell cycle regulation of the double stranded RNA activated protein kinase, PKR Oncogene, Oncogene, **18**, 315-326 (1999).
10. Kumar, S., et al., Double-stranded RNA-dependent protein kinase activates transcription factor NF-kappa B by phosphorylating I kappa B. Proc. Natl. Acad. Sci. USA, **91**, 6288-6292 (1994).
11. Zamanian-Daryoush, M., et al., NF-kappaB activation by double-stranded-RNA-activated protein kinase (PKR) is mediated through NF-kappaB-inducing kinase and IkappaB kinase. Mol. Cell. Biol., **20**, 1278-1290 (2000).
12. Kumar, A., et al., Deficient cytokine signaling in mouse embryo fibroblasts with a targeted deletion in the PKR gene: role of IRF-1 and NF-kappaB. EMBO J., **16**, 406-416 (1997).
13. Cuddihy, A.R., et al., The double-stranded RNA-activated protein kinase PKR physically associates with the tumor suppressor p53 protein and phosphorylates human p53 on serine 392 in vitro. Oncogene, **18**, 2690-2702 (1999).
14. Peel, A. L., et al., Double-stranded RNA-dependent protein kinase, PKR, binds preferentially to Huntington's disease (HD) transcripts and is activated in HD tissues. Hum. Molec. Genet., **10**, 1531-1538 (2001)

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