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Product Information

Anti-phospho-PKR [pThr^{451}]
produced in rabbit, affinity isolated antibody

Catalog Number **P6493**

Product Description

Anti-phospho-PKR (Interferon-inducible double-stranded RNA-dependent protein kinase) [pThr^{451}] is produced in rabbit using a synthetic phosphorylated peptide derived from the region of PKR that is phosphorylated on Thr^{451} as the immunogen. The antiserum is purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards a non-phosphorylated PKR.

Anti-phospho-PKR [pThr^{451}] specifically recognizes PKR phosphorylated on Thr^{451} (65-68 kDa). The antibody detects human and mouse PKR [pThr^{451}] and has been used in immunoblotting[1,2] and immunohistochemistry.[3]

PKR is a ubiquitously expressed serine/threonine protein kinase (68 kDa in human) that is induced by IFN-γ and activated by double-stranded RNA (dsRNA) and stress signals.[4] It is a major mediator of the antiviral and antiproliferative activities of interferons. PKR binding to dsRNA induces a conformational change that leads to PKR autophosphorylation and activation.[2] Activated PKR phosphorylates its substrates, which include 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 mediated, in part, through phosphorylation of eIF-2α, which results in the sequestration of the recycling factor eIF-2B in an inactive complex with eIF-2-GDP.[5,6] 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.[7-9] 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 NF$\kappa\beta$ by IFN-γ or dsRNA, implicating PKR in these signaling pathways.[10]

Human PKR contains at least 15 autophosphorylation sites, but only Thr^{446} and Thr^{451} in the activation loop were found to be critical for kinase activity in yeast. Phosphorylation of human PKR on Thr^{446} and Thr^{451} in the PKR activation loop is required *in vivo* and *in vitro* for high-level kinase activity. Experiments using the anti-phospho-PKR [pThr^{451}] antibody have shown that dsRNA binding, which is required for dimerization of full-length PKR molecules *in vivo*, leads to autophosphorylation of Thr^{446} and Thr^{451} in the activation loop and stimulation of the eIF-2α kinase function of PKR.[2]

Besides dsRNA, other PKR cellular activators include Protein Activator of Interferon-Induced Protein Kinase (PACT), which heterodimerizes with PKR and activates it by direct protein-protein interaction. Overexpression of PACT in mammalian cells leads also to phosphorylation of the α subunit of the eukaryotic initiation factor 2 (eIF-2α), and to subsequent inhibition of protein synthesis.[11]

Reagent

Anti-phospho-PKR [pThr451], is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 ($\pm$0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier and 0.05% sodium azide. The amount of the reagent is sufficient for 10 blots.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards 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. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

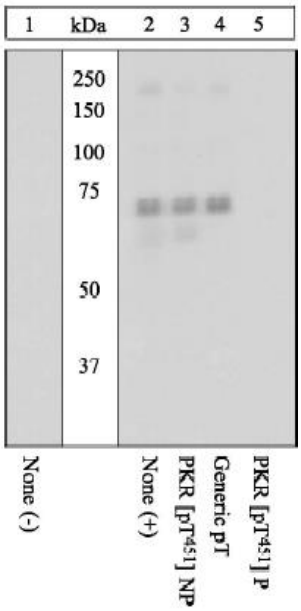
Product Profile
Immunoblotting: is assay dependent.

Note: In order to obtain best results in different techniques and preparations, it is recommended to determine optimal working concentration by titration.

Peptide Competition

1. Extracts prepared from HeLa cells left unstimulated (1) or stimulated with IFN-γ and calyculin (2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
   Lane 1, 2 no peptide
   Lane 3 non-phosphorylated peptide corresponding to the immunogen
   Lane 4 a generic phosphothreonine containing peptide
   Lane 5 immunogen
4. After preincubation membranes were incubated with PKR [pThr^{451}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab′)$_2$ anti-rabbit IgG-HRP and signals were detected using the Pierce SuperSignal® method (see Figure 1).

Figure 1.
Peptide Competition



Only the phosphopeptide corresponding to PKR [pThr^{451}] blocks the antibody signal, demonstrating the antibody specificity; phosphorylation is induced by the addition of IFN-γ and calyculin.

References

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