PKR/EIF2AK2 (Human), Active

Catalytic domain, recombinant protein expressed in Sf9 cells

Cat# CY-SPP80

Lot No.		
	5 µg	0.05 µg/µl

Background:

The double-stranded RNA-activated protein kinase (PKR), also known as eukaryotic translation initiation factor 2-alpha kinase 2 (EIF2AK2), is a protein kinase that has been shown to be involved in HIV/gp120-associated neurodegeneration (1). PKR/EIF2AK2 acts as a critical mediator of gp120 neurotoxicity and is a substrate for a family of protein kinases that respond to various forms of environmental stress. Activation of PKR/EIF2AK2 leads to its autophosphorylation and then phosphorylation of its natural substrate, the alpha subunit of eukaryotic protein synthesis initiation factor-2. PKR/EIF2AK2 plays a critical role in mRNA translation, cell proliferation and apoptosis. A novel cross-talk between the PKR/EIF2AK2 and p53 has been shown that has implications in cell proliferation and tumorigenesis (2).

Product Description:

Recombinant human PKR/EIF2AK2 (252-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002759.

Gene Aliases:

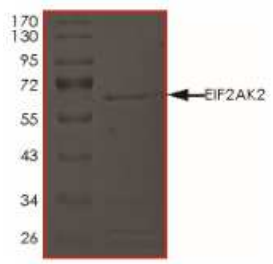
PKR, EIF2AK2, PRKR, EIF2AK1

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >70% by densitometry. Approx. MW 64kDa.



Storage:

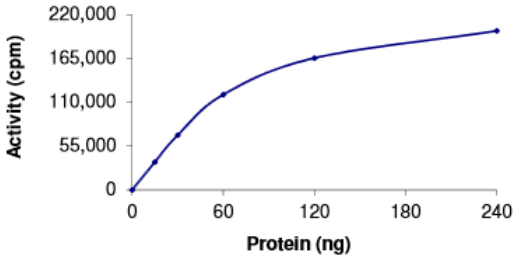
Store product at -70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at -70 °C, 1 year from date of shipment.

Specific Activity:

The specific activity was determined to be 68 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{33}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

Myelin basic protein (MBP) diluted in distilled H2O to a final concentration of 1mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1. Baltzis, D. et al: The eIF2alpha kinases PERK and PKR activate glycogen synthase kinase 3 to promote the proteasomal degradation of p53. J. Biol Chem. 2007; 282(43):31675-87.
2. Alirezaei, M. et al: Human immunodeficiency virus-1/surface glycoprotein 120 induces apoptosis through RNA-activated protein kinase signaling in neurons. J. Neurosci. 2007;27(41):11047-55.

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