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ProductInformation

Cdk5
bovine, recombinant
expressed in *E. coli*
as a GST fusion protein

Product Code **C 0490**
Storage Temperature –20 °C

Synonym: Cyclin Dependent Kinase-5

Product Description

Cyclin Dependent Kinase-5 (Cdk5) is a member of the serine/threonine cyclin dependent kinase family. In spite of its name, it is not regulated by cyclins nor is it a checkpoint kinase primarily involved in the regulation of cell cycle progression. Cdk5 is widely expressed in most tissues including ovary, sperm, muscle, and the central nervous system (CNS). Not withstanding this distribution pattern, Cdk5 is predominantly active in the CNS in post-mitotic neurons when it is complexed with brain-specific activator proteins (Neuronal Cdk5 Activator, p35 and p39 isoforms, and NCK5a). The holoenzyme (active heterodimers Cdk5:p35^{NCK5a} or Cdk5:p39^{NCK5a}) is also known as Neuronal Cdc2-like Kinase (Nclk) and Brain Proline-Directed Protein Kinase (BPDK). Current understanding places Nclk as a key regulator in neurotransmission, axon guidance, CNS architecture, and pathogenesis in neurodegenerative diseases such as Alzheimer's, Amyotrophic Lateral Sclerosis, and Parkinson's Disease.[1]

Unlike other Cdks, activation of Cdk5 does not require an activating kinase or autophosphorylation although it has putative phosphorylation sites. In addition, the activator proteins, p35 and p39, are structurally distinct from cyclins. p35 is known to be myristoylated. It binds to Cdk5 and recruits it to the membrane where interactions of the holoenzyme with upstream cell surface signaling receptors (such as neurotrophic factors and extracellular matrix molecules) occur. These regulator proteins are subject to proteolysis generating several truncated forms (p25 and p30) with important physiological consequences in development and pathology.[2] The proteasome mediated proteolysis is itself regulated by phosphorylation of the activator proteins.

Activated Cdk5 has numerous substrates.[1] Some examples are DARPP32 (Dopamine and cAMP Regulated Phosphoprotein,[3] an integrator of neurotransmission), FAK (Focal Adhesion Kinase),[4] Dynamin 1 and Amphiphysin 1 in synaptic vesicle endocytosis,[5] voltage-dependent Ca channel, STAT3,[6] β-catenin, NUDEL,[7] (LIS-1 interacting protein in the growth cone), and Tau in AD brain[1] leading to neuronal apoptosis.

Activated Cdk5 clearly is uniquely important. New understanding continues to emerge on its regulation in normal CNS development and functions and its dysregulation which contributes to a number of devastating diseases.

The product is supplied in a solution of 25 mM Tris, pH 7.4, 1 mM DTT, and 30% glycerol.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –20 °C is recommended. It is stable for at least one year. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

Procedure

For kinase activity, Cdk5 must be reconstituted with neuronal activator proteins p25, a truncated form of p35, (p25, Product Code P 1371) or p30, a truncated form of p39, (p30, Product Code P 1246) to form the holoenzyme. One µl of Cdk5 is incubated for one hour at room temperature with 2 µl of an activator protein with 30.4 µl of reconstitution buffer.

The reconstitution buffer consists of 20 mM MOPS, pH 7.2, with 30 mM $MgCl_2$, 40 µM Na_3VO_4, 50 µM Na/K tartrate, 3.5 mg/ml p-nitrophenyl phosphate, 10 mM NaF, 1 mM DTT, 10 mM β-glycerophosphate, 0.15 µM microcystin, and 0.25 mg/ml BSA.

For the kinase assay, 33.4 µl of the reconsitituted, activated Cdk5 solution is mixed with 16.6 µl of the kinase assay buffer (the reconstitution buffer with 0.1 mM γ-^{32}P-ATP [1,000 cpm/pmole] and 0.1 mM histone H1 peptide substrate). A 30 minute assay at 30 °C results in 30,000 to 70,000 cpm incorporated into the substrate.

One unit will incorporate 1 picomole of phosphate into histone H1 peptide per minute at pH 7.2 at 30 °C.

References

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