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ProductInformation

GST-p30
bovine, recombinant
expressed in *E. coli*

Product Number **P 1246**
Storage Temperature –20 °C

Synonym: GST-Neuronal Cdk5 Activator Protein p30

Product Description
p30 is an endogenous truncated form of p39, one of two isoforms of the Neuronal Cdk5 (cyclin-dependent kinase 5) activator protein (NCKa).[1] These activator proteins are structurally distinct from cyclins, which activate checkpoint kinases in the Cdk family. Further difference from cyclins lies in the absence of phosphorylation in the activation by NCKa proteins. Together, the brain specific activator:catalytic subunit pair (CNK5a:Cdk5) form the holoenzyme Nclk (Neuronal Cdc2-like Kinase, also known as Brain Proline-directed Protein Kinase, BPDK), which is a unique member of the Cdk family and a key regulator in neurotransmission, axon guidance, central nervous system (CNS) architecture, and pathogenesis in neurodegenerative diseases such as Alzheimer's, Amyotrophic Lateral Sclerosis, and Parkinson's Disease.[2]

Although Cdk5 shows wide distribution in most tissues, the NCK5a proteins are restricted to the CNS. They complex with Cdk5 and recruit it to the plasma membrane, where Nclk can interact with cell surface receptors (such as neurotrophic factors and extracellular matrix molecules) to mediate a downstream signaling cascade. While p39 is closely related to p35 by sequence homology (57%),[1] differential expression patterns by region, cell type, and developmental stages suggest that the two isoforms have distinct functional roles.[3,4,5] The level of p35 or p39 in post-mitotic neurons is dynamic.[6] By analogy to p35, phosphorylation and proteolysis are likely regulatory mechanisms for p39; however, details concerning p39 are subjects of ongoing investigation.

Similar to p35, p39 is also processed giving rise to a truncated form, p30 which can associate with Cdk5 to form an active Nclk[1].

Although much less is known about p39 or its truncated form p30, it is clear that NCKa proteins are developmentally regulated and have a role in normal CNS physiology as well as a contributor to neuronal pathology.

GST-p30 is a purified functional recombinant protein that can be used in further investigations. It is supplied in a solution of 25 mM Tris, pH7.4, 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
GST-p30 can be used to reconstitute the kinase activity of Cdk5 (Product Code C 0490, GST-Cdk5) to form the holoenzyme. One µl of GST-Cdk5 is incubated for one hour at room temperature with 2 µl of GST-p30 activator protein with 30.4 µl of reconstitution buffer

The reconstitution buffer consists of 20 mM MOPS, pH 7.2, 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 (reconstitution buffer with 0.1 mM ^{32}P-γ-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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2. Shelton, S.B., and Johnson, G.V., Cyclin-dependent kinase-5 in neurodegeneration. J Neurochem., **88(6)**,1313-26 (2004).
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