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ProductInformation

GST-p25
bovine, recombinant
expressed in *E. coli*

Product Number **P 1371**
Storage Temperature –20 °C

Synonym: GST-Neuronal Cdk5 Activator Protein p25

Product Description
p25 is an endogenous truncated form of p35, the better studied of two isoforms of the Neuronal Cdk5 (cyclin-dependent kinase 5) activator protein (NCKa). 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 (NCK5a: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.[1]

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. p35 is known to be myristoylated. The level of p35 or p39 in post-mitotic neurons is dynamic.[2] Phosphorylation by Cdk5 targets it for degradation by the proteasome pathway and is developmentally controlled. It is known that phospho-p35 has lower susceptibility to calpain cleavage and predominates in fetal brain, while the unphosphorylated p35 is more abundant in adult brain and is more resistant to proteasome degradation, but is readily cleavable by calpain.[3]

In other experimental models p25 is emerging as an aberrant truncated form of p35 through the activity of calpain.[4]

The association of p25 with Cdk5 leads to hyperactivation, resulting in hyperphosphorylation of pathological substrates (such as Tau[1] and βAPP[5]) and subcellular redistribution. Evidence has accumulated that these events play an essential role in neuronal cell death in experimental models of Alzheimer's and Parkinson's diseases.[1] p25 is elevated in AD brain and its activation of Cdk5 produces a distinct pattern of βAPP phosphorylation and processing than p35.[5] The role of p25 in the phosphorylation of Tau has not yet been determined. It could be direct phosphorylation of Tau by Nclk or indirect via Nclk phosphorylation of other kinases and phosphatases that act on Tau.[1]

The current data suggests that p25 is both a developmentally regulated truncated form of p35 that has a role in normal CNS physiology, as well as a contributor to neuronal pathology. However, it is clear that p25 modifies target specificity of Cdk5.

GST-p25 is a purified functional recombinant protein that can be used for 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-p25 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-p25 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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3. Saito, T. *et al*., Developmental regulation of the proteolysis of the p35 cyclin-dependent kinase 5 activator by phosphorylation. J Neurosci., **23(4)**, 1189-97 (2003).
4. Kusakawa, G. *et al*., Calpain-dependent proteolytic cleavage of the p35 cyclin-dependent kinase 5 activator to p25. J. Biol. Chem., **275(22)**, 17166-72 (2000).
5. Liu, F. *et al*., Regulation of amyloid precursor protein (APP) phosphorylation and processing by p35/Cdk5 and p25/Cdk5. FEBS Lett., **547(1-3)**, 193-6 (2003).
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