

CDK5/p35, Active

Full-length recombinant proteins expressed in Sf9 cells

Catalog # C33-10BG

Lot # S299-9

Product Description

Recombinant full-length human CDK5 and human p35 were co-expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession numbers for CDK5 and p35 are [NM_004935](#) and [NM_003885](#), respectively.

Gene Aliases

CDK5: PSSALRE

P35: CDK5R1; CDK5P35, CDK5R, NCK5A, p35, p35nck5a

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.

Storage and Stability

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.

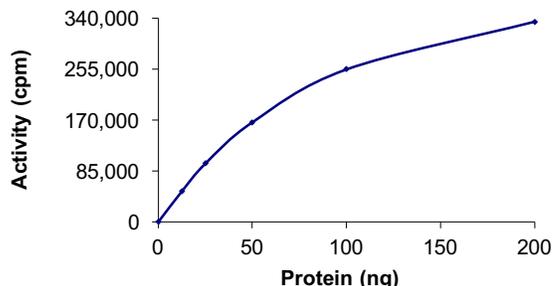
Scientific Background

CDK5 is a member of the Cyclin-Dependent Kinase family that is most abundant in the mammalian brain. Active form of CDK5, which has also been called neuronal cdc2-like kinase, is a heterodimer of CDK5 and a 25 kDa protein which is derived proteolytically from a 35 kDa brain and neuron-specific protein and is essential for the kinase activity of CDK5 (1). CDK5 has emerged as a crucial regulator of neuronal migration in the developing central nervous system. CDK5 phosphorylates a diverse list of substrates, implicating it in the regulation of a range of cellular processes - from adhesion and motility, to synaptic plasticity and drug addiction (2).

References

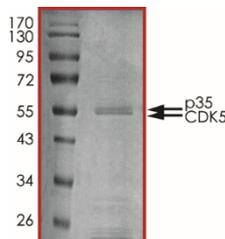
1. Tang, D. et al: Cyclin-dependent kinase 5 (Cdk5) and neuron-specific Cdk5 activators. *Prog Cell Cycle Res.* 1996;2:205-16.
2. Dhavan, R. et al: A decade of CDK5. *Nat Rev Mol Cell Biol.* 2001 Oct;2(10):749-59.

Specific Activity



The specific activity of CDK5/p35 was determined to be **210 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. CDK5 Approx. MW **59kDa** and p35 Approx. MW **60kDa**.

CDK5/p35, Active

Human recombinant proteins expressed in Sf9 cells

Catalog #	C33-10BG
Specific Activity	210 nmol/min/mg
Lot #	S299-9
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C33-10BG)

Active CDK5/p35 (0.1µg/µl) diluted with Kinase Dilution Buffer I (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active CDK5/p35 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: H10-54N)

Histone H1 diluted in 50mM Tris-HCl, pH 7.5, and 150mM NaCl buffer to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active CDK5/p35, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active CDK5/p35 (Catalog # C33-10BG)
 - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog # H10-54N)
 - Component 3.** 5µl distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity (cpm) on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³³P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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