CDK5/p25, Active
Recombinant human protein expressed in Sf9 cells

Catalog # C33-10G
Lot # S263-3

Product Description
Recombinant full-length human CDK5 and p25 were co-expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag on both proteins. The gene accession numbers for CDK5 and p25 are NM_004935 and NM_003885, respectively.

Gene Aliases
CDK5: PSSALRE
p25: CDK5R1; CDK5P35, CDK5R, NCK5A, p23, 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

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C33-10G)
Active CDK5/p25 (0.1µg/µl) diluted with Kinase Dilution Buffer III (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/p25 for optimal results).

Kinase Dilution Buffer I (Catalog #: K23-09)
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 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, 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/p25, 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/p25 (Catalog # C33-10G)
   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 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 [P33]-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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