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Certificate of Analysis

Cdk5/p35, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-477, 14-477-K, 14-477M
Parent Lot # 2293611

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Complex of recombinant full length human cdk5 containing and *N*-terminal 6His-tag and recombinant full length human p35 containing an *N*-terminal GST-tag. Both are expressed by baculovirus in Sf21 insect cells. Purified using Ni-NTA agarose. Purity 85.9% by SDS-PAGE and Coomassie blue staining.
CDK5 MW= 34.3kDa, p35 MW= 61kDa.

Specific Activity (Parent lot# 2293611): 2271U/mg, where one unit of cdk5/p35 activity is defined as 1nmol phosphate incorporated into 0.1mg/ml histone H1 per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.279mg/ml of enzyme in Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

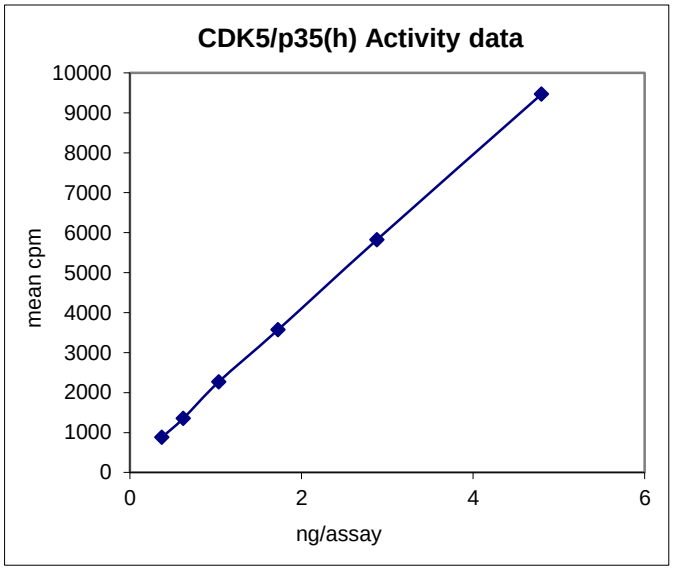
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

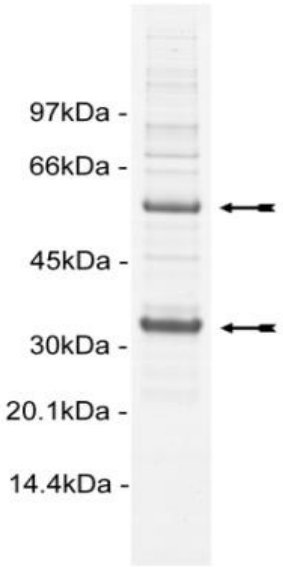
Quality Control Testing

Kinase Assay: 0.4–4.8ng of this lot of enzyme phosphorylated 0.1mg/ml histone H1 in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as cdk5/p35 with the translated sequence of cdk5 listed on pages three and four.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active cdk5/p35.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH7.0, 1mM EDTA.
2. **Histone H1:** Use at a final assay concentration of 0.1mg/ml. Make up a 1mg/ml stock in 20mM MOPS pH7.0. Add 2.5µl of stock per assay point.
3. **cdk5/p35, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–4.8ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **histone H1**.
3. Add **2.5µl (0.4–4.8ng) cdk5/p35, active**.
4. Add 5µl dH_20.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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cdk5/p35 Sequence Information

Protein	Human cdk5
Accession number	GenBank X66364
Tags	*N*-terminal 6His
Native sequence	M1 of native protein is equivalent to M8 of recombinant protein

Recombinant cdk5 amino acid sequence:

```
  1 MHHHHHHMQK YEKLEKIGEG TYGTVFKAKN RETHEIVALK RVRLDDDDEG VPSSALREIC
 61 LLKELKHKNI VRLHDVLHSD KKLTLVFEFC DQDLKKYFDS CNGDLDPEIV KSFLFQLLKG
121 LGFCHSRNVL HRDLKPQNLL INRNGELKLA DFGLARAFGI PVRCYSAEVV TLWYRPPDVL
181 FGAKLYSTSI DMWSAGCIFA ELANAGRPLF PGNDVDDQLK RIFRLLGTPT EEQWPSMTKL
241 PDYKPYPMYP ATTSLVNVVP KLNATGRDLL QNLLKCNPVQ RISAEEALQH PYFSDFCPP
```

Recombinant cdk5 nucleotide sequence:

```
  1 atgcatcatc accatcacca tatgcagaaa tacgagaaac tggaaaagat tggggaaggc
 61 acctacggaa ctgtgttcaa ggccaaaaac cgggagactc atgagatcgt ggctctgaaa
121 cgggtgaggc tggatgacga tgatgagggt gtgccgagtt ccgccctccg ggagatctgc
181 ctactcaagg agctgaagca caagaacatc gtcaggcttc atgacgtcct gcacagcgac
241 aagaagctga ctttggtttt tgaattctgt gaccaggacc tgaagaagta ttttgacagt
301 tgcaatggtg acctcgatcc tgagattgta aagtcattcc tcttccagct actaaaaggg
361 ctgggattct gtcatagccg caatgtgcta cacagggacc tgaagcccca gaacctgcta
421 ataaacagga atggggagct gaaattggct gattttggcc tggctcgagc ctttgggatt
481 cccgtccgct gttactcagc tgaggtggtc acactgtggt accgcccacc ggatgtcctc
541 tttggggcca agctgtactc cacgtccatc gacatgtggt cagccggctg tatctttgca
601 gagctggcca atgctgggcg gcctcttttt cccggcaatg atgtcgatga ccagttgaag
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781 aaactcaatg ccacagggag ggatctgctg cagaaccttc tgaagtgtaa ccctgtccag
841 cgtatctcag cagaagaggc cctgcagcac ccctacttct ccgacttctg tccgccctag
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cdk5/p35 Sequence Information

Protein Human p35

Accession number GenBank X80343

Tags *N*-terminal GST

Native sequence M1 of native protein is equivalent to M231 of recombinant protein

Recombinant p35 amino acid sequence:

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MGTVLSLSPS
241 YRKATLFEDG AATVGHYTAV QNSKNAKDKN LKRHSIISVL PWKRIVAVSA KKKNSKKVQP
301 NSSYQNNITH LNNENLKKSL SCANLSTFAQ PPPAQPPAPP ASQLSGSQTG GSSSVKKAPH
361 PAVTSAGTPK RVIVQASTSE LLRCLGEFLC RRCYRLKHLS PTDPVLWLRS VDRSLLLQGW
421 QDQGFITPAN VVFLYMLCRD VISSEVGSDH ELQAVLLTCL YLSYSYMGNE ISYPLKPFLV
481 ESCKEAFWDR CLSVINLMSS KMLQINADPH YFTQVFSDLK NESGQEDKKR LLLGLDR
```

Recombinant p35 nucleotide sequence:

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
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1021 gccagccagc tctcgggttc ccagaccggg ggctcctcct cagtcaagaa agcccctcac
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1561 aacgagagcg gccaggagga caagaagcgg ctcctcctag gcctggatcg gtga
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Reviewed and approved by site quality representative.