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Certificate of Analysis

CDK5/p25, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-516, 14-516-K, 14-516M
Parent Lot # 1575904

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 *N*-terminal 6His-tagged, recombinant, full length human CDK5 and *N*-terminal GST-tagged, recombinant, full length human p25. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 88.1% by SDS-PAGE and Coomassie blue staining. CDK5 MW = 34.4kDa, p25 MW = 49.4kDa.

Specific Activity (Parent lot# 1575904): 3040U/mg, where one unit of CDK5/p25 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: 2.357mg/ml of 50mM 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.

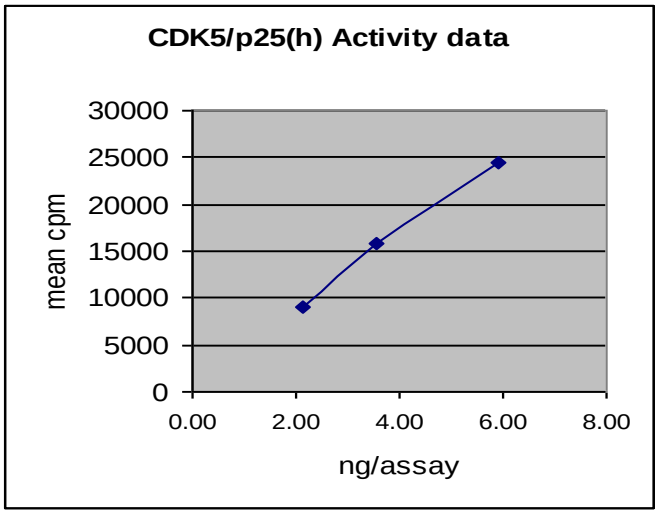
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

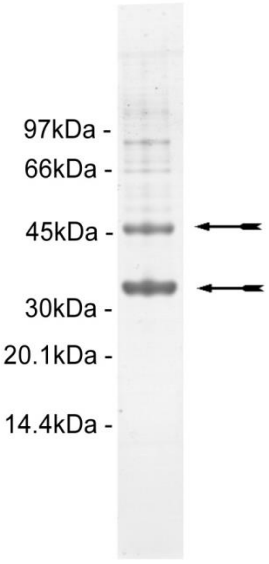
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.8–5.9ng 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 with the translated sequence listed on pages three and four.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CDK5/p25.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Histone H1:** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Use 2.5µl of stock per assay point.
3. **CDK5/p25, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.8–5.9ng 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 (0.8–5.9ng) CDK5/p25, active**.
4. Make up to 15µl with dH_2O.
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 of 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 Sequence Information

Protein human CDK5

Tags *N*-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human CDK5

Accession number GenBank X66364

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
661 agaatcttcc gactgctggg gacgcccacc gaggagcagt ggccctctat gaccaagctg
721 ccagactata agccctatcc gatgtacccg gccacaacat ccctggtgaa cgtcgtgccc
781 aaactcaatg ccacagggag ggatctgctg cagaaccttc tgaagtgtaa ccctgtccag
841 cgtatctcag cagaagaggc cctgcagcac ccctacttct ccgacttctg tccgccctag
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p25 Sequence Information

Protein human p25

Tags N-terminal GST

Native sequence F223 of the recombinant protein is equivalent to F98 of human p35 (see below)

Accession number p25 is an *in vivo* cleavage product of the p35 protein described in GenBank X80343. The recombinant product contains the full length p25 protein, equivalent to residues F98–end of p35, as described in Gentry *et al*., Nature 402, 615-622, 1999.

Recombinant p25 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 LEFAQPPPAQ PPAPPASQLS
241 GSQTGGSSSV KKAPHPAVTS AGTPKRVIVQ ASTSELLRCL GEFLCRRCYR LKHLSPTDPV
301 LWLRSVDRSL LLQGWQDQGF ITPANVVFLY MLCRDVISSE VGSDHELQAV LLTCLYLSYS
361 YMGNEISYPL KPFLVESCKE AFWDRCLSVI NLMSSKMLQI NADPHYFTQV FSDLKNESGQ
421 EDKKRLLLGL DR
```

Recombinant p25 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
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggaattcg cccagccccc accggcccag ccgcctgcac ccccggccag ccagctctcg
 721 ggttcccaga ccgggggctc ctcctcagtc aagaaagccc ctcaccctgc cgtcacctcc
 781 gcagggacgc ccaaacgggt catcgtccag gcgtccacca gtgagctgct tcgctgcctg
 841 ggtgagtttc tctgccgccg gtgctaccgc ctgaagcacc tgtcccccac ggaccccgtg
 901 ctctggctgc gcagcgtgga ccgctcgctg cttctgcagg gctggcagga ccagggcttc
 961 atcacgccgg ccaacgtggt cttcctctac atgctctgca gggatgttat ctcctccgag
1021 gtgggctcgg atcacgagct ccaggccgtc ctgctgacat gcctgtacct ctcctactcc
1081 tacatgggca acgagatctc ctacccgctc aagcccttcc tggtggagag ctgcaaggag
1141 gccttttggg accgttgcct ctctgtcatc aacctcatga gctcaaagat gctgcagata
1201 aatgccgacc cacactactt cacacaggtc ttctccgacc tgaagaacga gagcggccag
1261 gaggacaaga agcggctcct cctaggcctg gatcggtga
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