

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, active 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.

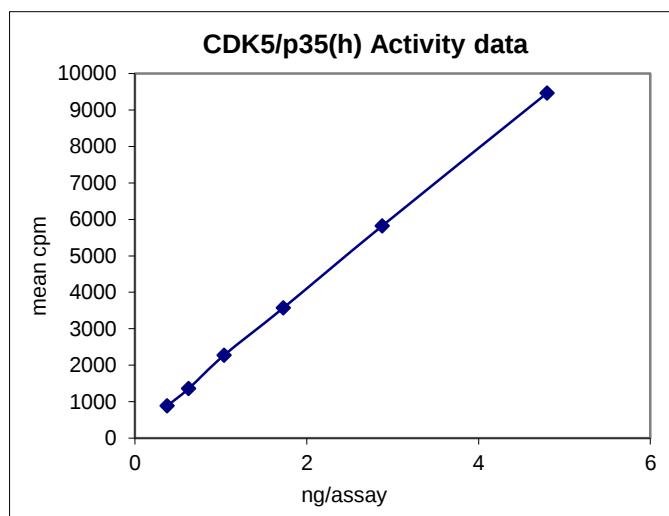
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

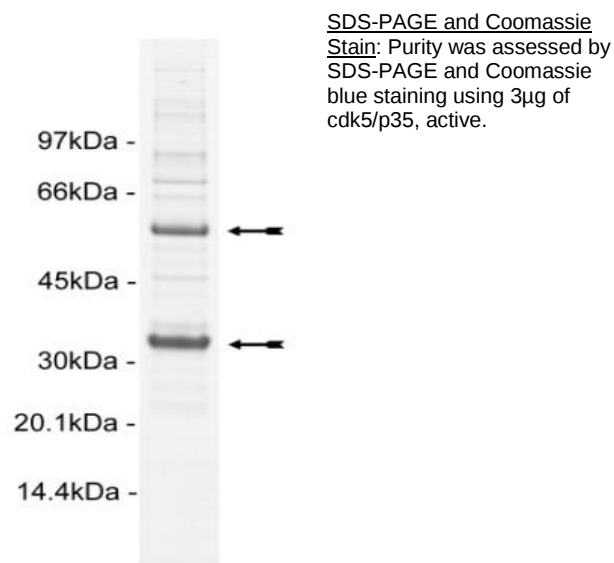
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.4–4.8ng 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.



Certificate of Analysis

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 -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]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 dH₂O.
5. Add 10µl of diluted [γ -³³P]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.

Certificate of Analysis

cdk5/p35 Sequence Information

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

Recombinant cdk5 amino acid sequence:

```

1  MHHHHHHMQK YEKLEKIGEG TYGTVFKAKN RETHEIVALK RVRLLLLDDEG VPSSALREIC
61  LLKELKHKNI VRLHDVLHSD KKLTLVFEFC DQDLKKYFDS CNGDLDP EIV KSFLFQLLKG
121 LGFCHSRNVL HRDLKPQNLL INRNGELKLA DFGLARAFGI PVRCSAEVV TLWYRPPDVL
181 FGAKLYSTSI DMWSAGCIFA ELANAGRPLF PGNDVDDQLK RIFRLLGTPT EEQWPSMTKL
241 PDYKPYPMYP ATTSILNVNVP KLNATGRDLL QNLLKCNPVQ RISAEELQH 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 tcttcagct actaaaaggg
361 ctgggattct gtcatagccg caatgtgcta cacagggacc tgaagcccca gaacctgcta
421 ataaacagga atggggagct gaaattggct gattttggcc tggctcgagc ctttgggatt
481 cccgtccgct gttactcagc tgagggtggtc aactgtggt accgcccacc ggatgtcctc
541 tttggggcca agctgtactc cacgtccatc gacatgtggt cagccggctg tatctttgca
601 gagctggcca atgctgggcg gcctcttttt cccggcaatg atgtcgatga ccagttgaag
661 agaactctcc gactgctggg gacgcccacc gaggagcagt ggccctctat gaccaagctg
721 ccagactata agccctatcc gatgtaccgg gccacaacat ccctggtgaa cgtcgtgcc
781 aaactcaatg ccacaggag ggatctgctg cagaaccttc tgaagtgtaa ccctgtccag
841 cgtatctcag cagaagaggc cctgcagcac ccctacttct ccgacttctg tccgccctag

```

Certificate of Analysis

cdk5/p35 Sequence Information

<u>Protein</u>	Human p35
<u>Accession number</u>	GenBank X80343
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	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  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPEF  MGTVLSLSPS
241  YRKATLFEDG  AATVGHYTAV  QNSKNAKDKN  LKRHSIISVL  PWKRIVAVSA  KKKNSKKVQP
301  NSSYQNNITH  LNNENLKKSL  SCANLSTFAQ  PPPAQPPAPP  ASQLSGSQTG  GSSSVKKAPH
361  PAVTSAGTPK  RVIVQASTSE  LLRCLGEFLC  RRCYRLKHL  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  tgcaaccac  tgcatttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtgtga  attgggtttg  gagtttccca  atcttcctta  ttatatattg
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atatactga  caagcacaca
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcgggtttg
301  gatatttagat  acggtgtttc  gagaattgca  tatagtaaa  actttgaaac  tctcaaagtt
361  gatttttcta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatatta  atggtgatca  tgtaacccat  cctgacttca  tggttgatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagtgc  tgttccaggg  gcccgaaatt  atgggcacgg  tgctgtccct  gtctcccagc
721  taccggaagg  ccacgctgtt  tgaggatggc  gcggccaccg  tgggccacta  tacggccgta
781  cagaacagca  agaacgcaa  ggacaagaac  ctgaagcgcc  actccatcat  ctccgtgctg
841  ccttgaaga  gaatcgtggc  cgtgtcggcc  aagaagaaga  actccaagaa  ggtgcagccc
901  aacagcagct  accagaacaa  catcacgcac  ctcaacaatg  agaacctgaa  gaagtcgctg
961  tcgtgcgcca  acctgtccac  attcgccag  cccccaccgg  ccagccgccc  tgcacccccg
1021  gccagccagc  tctcgggttc  ccagaccggg  ggctcctcct  cagtcaagaa  agccccctac
1081  cctgccgtca  cctccgcagg  gacgcccaca  cgggtcatcg  tccaggcgct  caccagttag
1141  ctgcttcgct  gcttgggtga  gtttctctgc  cgccggtgct  accgctgaa  gcacctgtcc
1201  cccacggacc  ccgtgctctg  gctgcgcagc  gtggaccgct  cgctgcttct  gcagggtggt
1261  caggaccagc  gcttcatcac  gccggccaac  gtggtcttcc  tctacatgct  ctgcagggat
1321  gttatctcct  ccgagggtgg  ctccgatcac  gagctccagg  ccgtcctgct  gacatgcctg
1381  tacctctcct  actcctacat  gggcaacgag  atctcctacc  cgctcaagcc  cttcctggtg
1441  gagagctgca  aggaggcctt  ttgggaccgt  tgctctctct  tcatcaacct  catgagctca
1501  aagatgctgc  agataaatgc  cgaccacac  tacttcacac  aggtcttctc  cgacctgaag
1561  aacgagagcg  gccaggagga  caagaagcgg  ctctccttag  gcctggatcg  gtga

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.