Certificate of Analysis

REACTION BIOLOGY

ProQinase™ CDK5

cyclin dependent kinase 5

Recombinant Human Protein Kinase

HGNC Symbol: CDK5

Synonyms: PSSALRE

Product No.: 0356-0000-1

Lot: 002

Description: Human CDK5, amino acids M_1-P_{292} (as in NCBI/Protein entry NP_004926.1), N-terminal GST-His_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CDK5, Lot 002, was confirmed as CDK5 by Western Blotting, using a CDK5 specific antibody

Theoretical $MW_{Fusion\ Protein}$: 62,841 Da

Expression host: Sf9 insect cells

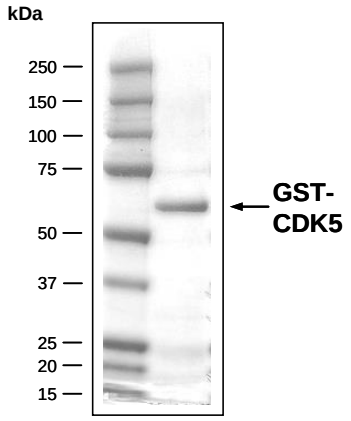
Purification: GST-Affinity Chromatography

Storage buffer: 50 mM Tris pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

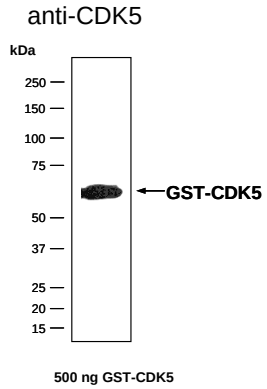
Protein concentration: 0.940 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

CDK5 Lot 002:
Coomassie stain



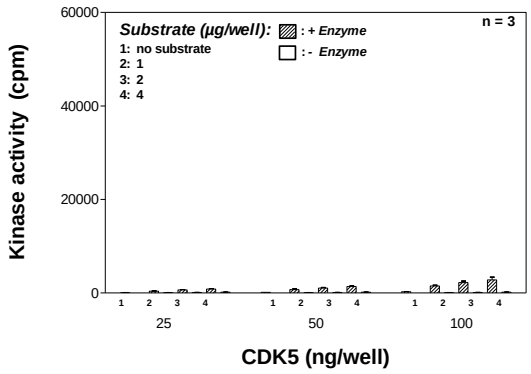
2.0 µg GST-CDK5

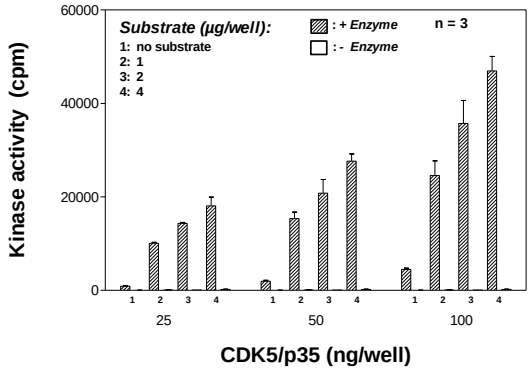
CDK5 Lot 002:
Western Blot Analysis



500 ng GST-CDK5

Kinase activity CDK5 vs CDK5/p35 complexes:





Final assay concentrations:
- 60 mM HEPES-NaOH, pH 7.5
- 3 mM $MgCl_2$
- 3 mM $MnCl_2$
- 3 µM Na-orthovanadate
- 1.2 mM DTT
- 50 µg / ml $PEG_{20.000}$
- 1 µM ATP (798,000 cpm ^{33}P-γ-ATP)
- Substrate (variable): RB-CTF
- Recombinant CDK5 or CDK5/p35 (variable)

Assay: ^{33}PanQinase® Assay

Recombinant Proteins

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ProQinase™ CDK5

Product No.: 0356-0000-1

GST-CDK5 Recombinant Fusion Protein Amino Acid Sequence

```
1    MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID 60
61   GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241  LVPRGSPGLD GIYARPMQKY EKLEKIGEGT YGTVFKAKNR ETHEIVALKR VRLDDDDEGV 300
301  PSSALREICL LKELKHKNIV RLHDVLHSDK KLTLVFEFCD QDLKKYFDSC NGDLDPEIVK 360
361  SFLFQLLKGL GFCHSRNVLH RDLKPQNLLI NRNGELKLAD FGLARAFGIP VRCYSAEVVT 420
421  LWYRPPDVLF GAKLYSTSID MWSAGCIFAE LANAGRPLFP GNDVDDQLKR IFRLLGTPTE 480
481  EQWPSMTKLP DYKPYPMYPA TTSLVNVVPK LNATGRDLLQ NLLKCNPVQR ISAEEALQHP 540
541  YFSDFCPP                                                          600
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDK4 fragment

CDK5 wt[1] Amino Acid Sequence

```
1    MQKYEKLEKI GEGTYGTVFK AKNRETHEIV ALKRVRLDDD DEGVPSSALR EICLLKELKH 60
61   KNIVRLHDVL HSDKKLTLVF EFCDQDLKKY FDSCNGDLDP EIVKSFLFQL LKGLGFCHSR 120
121  NVLHRDLKPQ NLLINRNGEL KLADFGLARA FGIPVRCYSA EVVTLWYRPP DVLFGAKLYS 180
181  TSIDMWSAGC IFAELANAGR PLFPGNDVDD QLKRIFRLLG TPTEEQWPSM TKLPDYKPYP 240
241  MYPATTSLVN VVPKLNATGR DLLQNLLKCN PVQRISAEEA LQHPYFSDFC PP         300
```

blue: CDK5 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_004926.1

Recombinant Proteins

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