eurofins | Discovery Services

Certificate of Analysis

cdk2/cyclin A, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-448, 14-448-K, 14-448M
Parent Lot # 25774U

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: *C*-terminal 6His-tagged human full length cdk2, and *N*-terminal GST-tagged human full length cyclin A. These are expressed individually by baculovirus in Sf21 insect cells. The cdk2 is purified by Ni^{2+}/NTA agarose and then activated using CAK and repurified by Q Sepharose and Ni^{2+}/NTA agarose. The cyclin A is purified using glutathione-agarose. They are then complexed *in vitro*. Combined purity 43% by SDS-PAGE and Coomassie blue staining. cdk2 MW = 35kDa and cyclin A MW = 75kDa.

Specific Activity (Parent lot# 25774U): 184U/mg, where one unit of cdk2/cyclinA, 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: 1.076mg/ml of enzyme in 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.

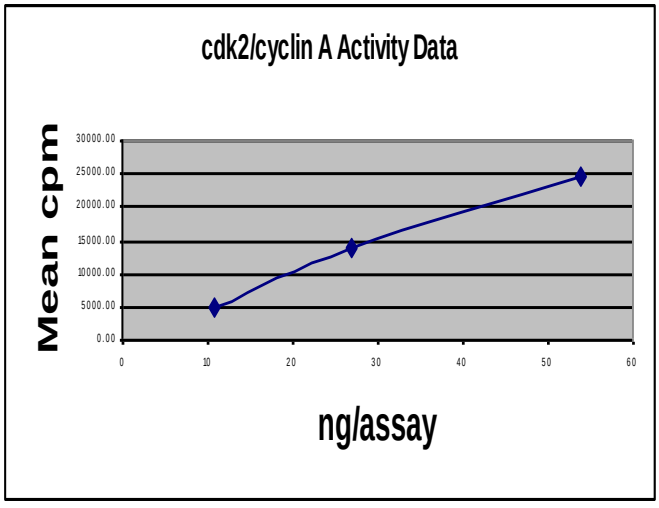
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

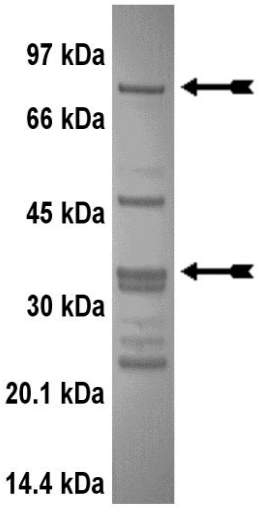
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 11–54ng 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 cdk2 with the translated native sequence listed on page three and cyclin A with the translated native sequence listed on page four



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of cdk2/cyclin A, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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 in 20mM MOPS pH7.0 and add 2.5µl of stock per assay point.
3. **cdk2/cyclin A, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11–54ng 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 (11–54ng) cdk2/cyclin A, active**.
4. Add 5µl of 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.

eurofins | Discovery Services

Certificate of Analysis

cdk2 Sequence Information

Protein human cdk2

Tags *C*-terminal 6His

Native sequence M1 of the recombinant protein is equivalent to M1 of human CDK2

Accession number EMBL M68520

Recombinant cdk2 amino acid sequence:

```
  1 MENFQKVEKI GEGTYGVVYK ARNKLTGEVV ALKKIRLDTE TEGVPSTAIR EISLLKELNH
 61 PNIVKLLDVI HTENKLYLVF EFLHQDLKKF MDASALTGIP LPLIKSYLFQ LLQGLAFCHS
121 HRVLHRDLKP QNLLINTEGA IKLADFGLAR AFGVPVRTYT HEVVTLWYRA PEILLGCKYY
181 STAVDIWSLG CIFAEMVTRR ALFPGDSEID QLFRIFRTLG TPDEVVWPGV TSMPDYKPSF
241 PKWARQDFSK VVPPLDEDGR SLLSQMLHYD PNKRISAKAA LAHPFFQDVT KPVPHLRLHH
301 HHHH
```

Recombinant cdk2 nucleotide sequence:

```
  1 atggagaact tccaaaaggt ggaaaagatc ggagagggca cgtacggagt tgtgtacaaa
 61 gccagaaaca agttgacggg agaggtggtg gcgcttaaga aaatccgcct ggacactgag
121 actgagggtg tgcccagtac tgccatccga gagatctctc tgcttaagga gcttaaccat
181 cctaatattg tcaagctgct ggatgtcatt cacacagaaa ataaactcta cctggttttt
241 gaatttctgc accaagatct caagaaattc atggatgcct ctgctctcac tggcattcct
301 cttcccctca tcaagagcta tctgttccag ctgctccagg gcctagcttt ctgccattct
361 catcgggtcc tccaccgaga ccttaaacct cagaatctgc ttattaacac agaggggccc
421 atcaagctag cagactttgg actagccaga gcttttggag tccctgttcg tacttacacc
481 catgaggtgg tgaccctgtg gtaccgagct cctgaaatcc tcctgggctg caaatattat
541 tccacagctg tggacatctg gagcctgggc tgcatctttg ctgagatggt gactcgccgg
601 gccctattcc ctggagattc tgagattgac cagctcttcc ggatctttcg gactctgggg
661 accccagatg aggtggtgtg gccaggagtt acttctatgc ctgattacaa gccaagtttc
721 cccaagtggg cccggcaaga ttttagtaaa gttgtacctc ccctggatga agatggacgg
781 agcttgttat cgcaaatgct gcactacgac cctaacaagc ggatttcggc caaggcagcc
841 ctggctcacc ctttcttcca ggatgtgacc aagccagtac cccatcttcg actccatcac
901 catcaccatc attga
```

eurofins | Discovery Services

Certificate of Analysis

cyclin A Sequence Information

Protein human cyclin A

Tags *N*-terminal GST

Native sequence M231of the recombinant protein is equivalent to M1 of human cyclin A

Accession number EMBL X51688

Recombinant cyclinA 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 MLGNSAPGPA
241 TREAGSALLA LQQTALQEDQ ENINPEKAAP VQQPRTRAAL AVLKSGNPRG LAQQQRPKTR
301 RVAPLKDLPV NDEHVTVPPW KANSKQPAFT IHVDEAEKEA QKKPAESQKI EREDALAFNS
361 AISLPGPRKP LVPLDYPMDG SFESPHTMDM SIVLEDEKPV SVNEVPDYHE DIHTYLREME
421 VKCKPKVGYM KKQPDITNSM RAILVDWLVE VGEEYKLQNE TLHLAVNYID RFLSSMSVLR
481 GKLQLVGTAA MLLASKFEEI YPPEVAEFVY ITDDTYTKKQ VLRMEHLVLK VLTFDLAAPT
541 VNQFLTQYFL HQQPANCKVE SLAMFLGELS LIDADPYLKY LPSVIAGAAF HLALYTVTGQ
601 SWPESLIRKT GYTLESLKPC LMDLHQTYLK APQHAQQSIR EKYKNSKYHG VSLLNPPETL
661 NL
```

Recombinant cyclinA 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 ctggaagttc tgttccaggg gcccgaattc atgttgggca actctgcgcc ggggcctgcg
 721 acccgcgagg cgggctcggc gctgctagca ttgcagcaga cggcgctcca agaggaccag
 781 gagaatatca acccggaaaa ggcagcgccc gtccaacaac cgcggacccg ggccgcgctg
 841 gcggtactga agtccgggaa cccgcggggt ctagcgcagc agcagaggcc gaagacgaga
 901 cgggttgcac cccttaagga tcttcctgta aatgatgagc atgtcaccgt tcctccttgg
 961 aaagcaaaca gtaaacagcc tgcgttcacc attcatgtgg atgaagcaga aaaagaagct
1021 cagaagaagc cagctgaatc tcaaaaaata gagcgtgaag atgccctggc ttttaattca
1081 gccattagtt tacctggacc cagaaaacca ttggtccctc ttgattatcc aatggatggt
1141 agttttgagt caccacatac tatggacatg tcaattgtat tagaagatga aaagccagtg
1201 agtgttaatg aagtaccaga ctaccatgag gatattcaca cataccttag ggaaatggag
1261 gttaaatgta aacctaaagt gggttacatg aagaaacagc cagacatcac taacagtatg
1321 agagctatcc tcgtggactg gttagttgaa gtaggagaag aatataaact acagaatgag
1381 accctgcatt tggctgtgaa ctacattgat aggttcctgt cttccatgtc agtgctgaga
1441 ggaaaacttc agcttgtggg cactgctgct atgctgttag cctcaaagtt tgaagaaata
1501 taccccccag aagtagcaga gtttgtgtac attacagatg atacctacac caagaaacaa
1561 gttctgagaa tggagcatct agttttgaaa gtccttactt ttgacttagc tgctccaaca
1621 gtaaatcagt ttcttaccca atactttctg catcagcagc ctgcaaactg caaagttgaa
1681 agtttagcaa tgtttttggg agaattaagt ttgatagatg ctgacccata cctcaagtat
```

Certificate of Analysis

```
1741 ttgccatcag ttattgctgg agctgccttt catttagcac tctacacagt cacgggacaa
1801 agctggcctg aatcattaat acgaaagact ggatataccc tggaaagtct taagccttgt
1861 ctcatggacc ttcaccagac ctacctcaaa gcaccacagc atgcacaaca gtcaataaga
1921 gaaaagtaca aaaattcaaa gtatcatggt gtttctctcc tcaacccacc agagacacta
1981 aatctgtaa
```

Reviewed and approved by site quality representative.