

Certificate of Analysis

Casein Kinase 1 δ , active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-520, 14-520-K, 14-520M

Parent Lot # D8KN032U

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: N-terminal, GST-tagged, recombinant human casein kinase 1 delta, amino acids 1–294, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 56.4% by SDS-PAGE and Coomassie blue staining. MW = 61kDa.

Formulation: 2.265mg/ml of enzyme in 50mM Tris/HCl, pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1 % 2-mercaptoethanol. Liquid at -20°C.

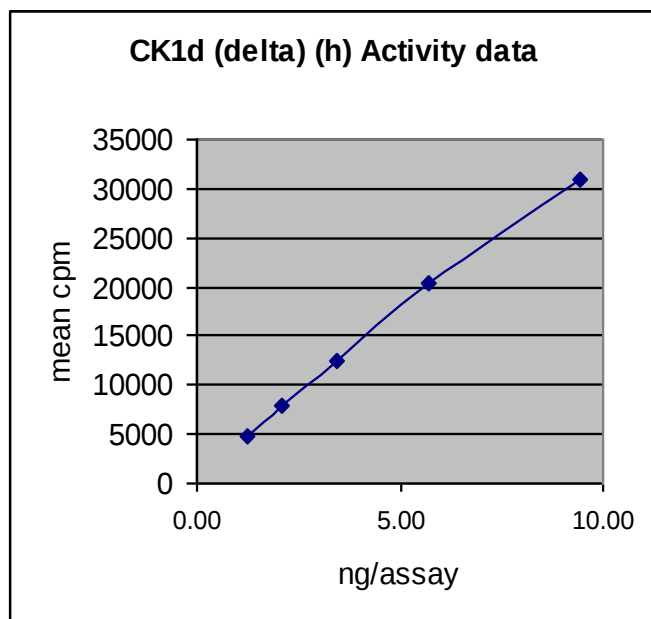
Specific Activity (Parent lot# D8KN032U): 2407U/mg, where one unit of CK1delta, active activity is defined as 1nmol phosphate incorporated into 200 μ M (KRRRALS(p)VASLPGL) (CK1tide) per minute at 30°C with a final ATP concentration of 100 μ M.

Storage and Stability: On receipt of material store at -20°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.

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

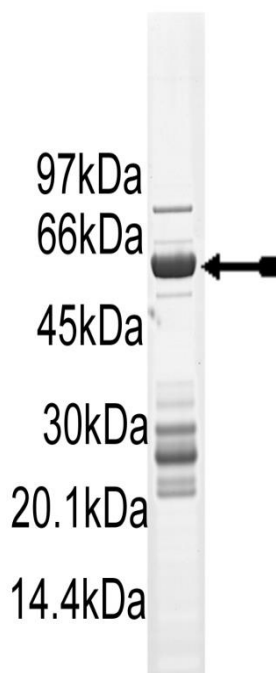
Quality Control Testing

Kinase Assay: 1.2–9.4ng of this lot of enzyme phosphorylated 200 μ M (KRRRALS(p)VASLPGL) 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 Casein Kinase 1 delta with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of casein kinase 1 delta, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KRRRALS(p)VASLPGL):** Use at a final assay concentration of 200 μ M. Prepare a 2mM stock. Use 2.5 μ l of stock per assay point.
3. **Casein Kinase 1 δ active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–9.4ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KRRRALS(p)VASLPGL)**.
3. Add **2.5 μ l (1.2–9.4ng) casein kinase 1 δ , active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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Casein kinase 1 delta Sequence Information

Protein	human casein kinase 1 delta (1–294)
Tags	N-terminal GST
Native sequence	M232 of the recombinant protein is equivalent to M1 of human casein kinase 1 delta
Accession number	GenBank AB063114. This is a rat cDNA that is 100% identical to human casein kinase 1 delta over 1-294 of the predicted amino acid sequence.

Recombinant casein kinase 1 delta amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMa  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPGIR  LMELRVGNRY
241 RLGRKIGSGS  FGDIYLGTDI  AAGEEVAIKL  ECVKTKHPQL  HIESKIYKMM  QGGVGIIPTIR
301 WCGAEGDYNV  VMELLGPSL  EDLFNFCSRK  FSLKTVLLLA  DQMISRIEYI  HSKNFIHRDV
361 KPDNFLMGLG  KKGNLVYIID  FGLAKKYRDA  RTHQHIPPYR  NKNLTGTARY  ASINTHLGIE
421 QSRRDDLESL  GYVLMYFNLG  SLPWQGLKAA  TKRQKYERIS  EKKMSTPIEV  LCKGYPSEFA
481 TYLNFCRSLR  FDDKPDYSYL  RQLFRNLFHR  QGFSYDYVFD  WNMLK

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Recombinant casein kinase 1 delta nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgatg
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atataagctg  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttta  atggtgatca  tgtaacccat  cctgacttca  tggttgatga  cgctcttgat
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1561  ttggaacatgc  tcaaatga

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