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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.

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

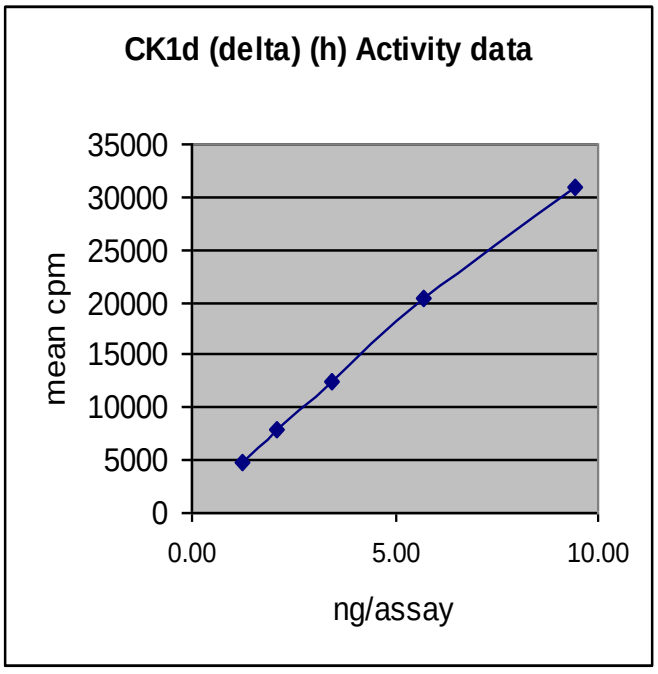
Formulation: 2.265mg/ml of enzyme in 50mM Tris/HCl, pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1 % 2-mercaptoethanol. Liquid at -20°C.

Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 6 months 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

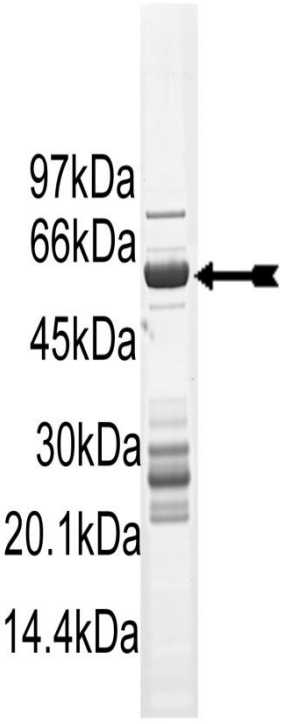
Quality Control Testing

Kinase Assay: 1.2–9.4ng of this lot of enzyme phosphorylated 200µM (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 3µg 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 200µM. Prepare a 2mM stock. Use 2.5µl 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-^{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 **(KRRRALS(p)VASLPGL)**.
3. Add **2.5µl (1.2–9.4ng) casein kinase 1 δ, 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.

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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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPGIR LMELRVGNRY
241 RLGRKIGSGS FGDIYLGTDI AAGEEVAIKL ECVKTKHPQL HIESKIYKMM QGGVGIPTIR
301 WCGAEGDYNV MVMELLGPSL EDLFNFCSRK FSLKTVLLLA DQMISRIEYI HSKNFIHRDV
361 KPDNFLMGLG KKGNLVYIID FGLAKKYRDA RTHQHIPYRE 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 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
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1021 gaccaaatga taagtcgtat tgagtacatt cattcgaaga attttatcca ccgagacgtg
1081 aaaccagata acttcctcat ggggctggga aagaaaggca acctggtcta catcattgac
1141 tttgggctgg ccaagaagta tcgggatgcc cgcacccacc agcatatccc ctatcgagag
1201 aacaagaacc tcacagggac agcacgctat gcctccatca acacacacct tggcattgaa
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1381 gagaagaaga tgtccactcc aattgaagtg ctgtgcaagg gctatccttc tgaatttgcc
1441 acatacctga atttctgccg ttccttacgt tttgatgaca aacctgacta ctcctacctg
1501 agacagctct tcagaaatct gttccatcgc cagggcttct cctacgacta tgtgttcgac
1561 tggaacatgc tcaaatga
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Reviewed and approved by site quality representative.