

Certificate of Analysis

Casein Kinase 1 γ 2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-712, 14-712-K, 14-712M

Parent Lot # D8EN012U

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 6His-tagged, recombinant, human Casein Kinase 1 γ 2, amino acids 18–end, expressed by baculovirus in Sf21 cells. Purified using Ni²⁺/NTA agarose. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 49.7kDa.

Specific Activity (Parent lot# D8EN012U): 10339U/mg, where one unit of Casein Kinase 1 γ 2 activity is defined as 1nmol phosphate incorporated into 200 μ M (KRRRALS(p)VASLPGL) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 2.091mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

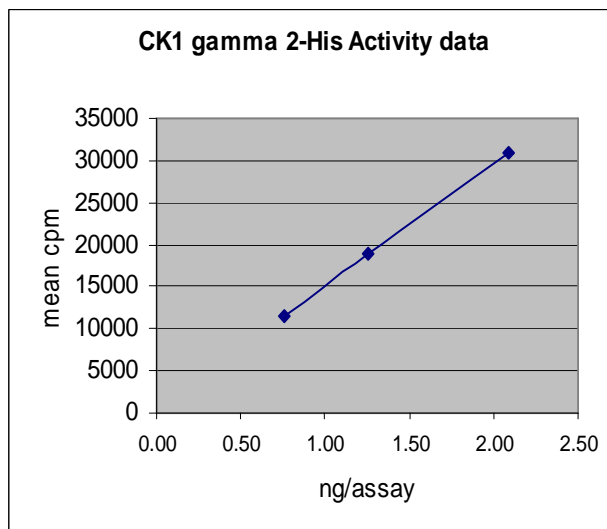
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

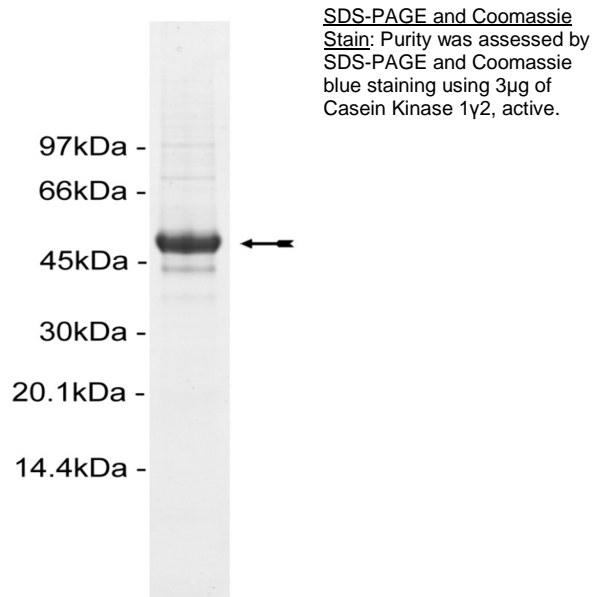
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.7–2.0ng 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 identity as Casein Kinase 1 γ 2 with the translated native sequence listed on page three.



Certificate of Analysis

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. Make up a 2mM stock. Add 2.5 μ l of stock per assay point.
3. **Casein Kinase 1 γ 2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.7–2.0ng 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 (0.7–2.0ng) Casein Kinase 1 γ 2, 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.

Certificate of Analysis

Casein Kinase 1 γ 2 Sequence Information

<u>Protein</u>	Human Casein Kinase 1 γ 2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M34 of the recombinant protein is equivalent to M18 of human Casein Kinase 1 γ 2
<u>Accession number</u>	GenBank NM_001319

Recombinant Casein Kinase 1 γ 2 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDFEF MSTMSKAGGG RSSHGIRSSG TSSGVLVMPG
61 NFRVGGKKIGC GNFGELRLGK NLYTNEYVAI KLEPIKSRAP QLHLEYRFYK QLSATEGVPQ
121 VYFPGPCGNY NAMVLELLGP SLEDLFDLCD RTFTLKTVLM IAIQLITRME YVHTKSLIYR
181 DVKPENFLVG RPGTKRQHAI HIIDFGLAKE YIDPETKKHI PYREHKSLTG TARYMSINTH
241 LGKEQSRRDD LEALGHMFMY FLRGLSPWQG LKADTLKERY QKIGDTKRAT PIEVLCENFP
301 EEMATYLRYV RRLDFFEKPD YDYLRKLFVD LFDRSGFVFD YEYDWAGKPL PTPIGTVHTD
361 LPSQPQLRDK TQPHSKNQAL NSTNGELNAD DPTAGHSNAP ITAPAEVEVA DETKCCCFK
421 RRRKSLQRH K

```

Recombinant Casein Kinase 1 γ 2 nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tattttcagg ggcctatgga tccggaattc atgtcgacta tgtccaaggc cggcgggggc
121 cggagcagcc acggcatccg gagctcgggg accagctcgg gggctctgat ggtgggcccc
181 aacttccgcg tcggcaagaa gatcggctgc ggcaacttcg gggagctccg cctaggaaag
241 aatctctata caaatgaata cgtggctatc aaattggagc cgatcaagtc ccgggccccg
301 cagctgcacc tggagtaccg gttctacaag cagctcagcg ccacagaggg cgtccctcag
361 gtctactact tcgggtccgtg cgggaattac aacgccatgg tgctggagct gctggggccc
421 agcctggagg acctgttcga cctgtgcgac cggaccttca cgctcaagc ggtgctgatg
481 atcgccatcc agctgatcac gcgcatggag tatgtgcaca ccaagagcct aatctaccgg
541 gacgtgaagc ccgagaactt cctgggtgggc cgcccgggga ccaagcggca gcatgccatc
601 cacatcatcg acttcgggct ggccaaggag tacatcgacc ccgagaccaa gaagcacatc
661 ccgtaccgcg agcacaagag cctgacgggc acggcgcgct acatgagcat caacacgcac
721 ctgggcaagg agcagagccg ccgcgacgac ctggaggcgc tgggccacat gttcatgtac
781 ttctctgcgc gcagcctccc ctggcagggg ctcaaggccg acacgctcaa ggagcggtac
841 cagaagatcg gggacaccaa acgcgccacg cccatcgagg tgctctgcga gaacttcca
901 gaggagatgg ccacgtacct gcgctatgtg cggcgccctg acttcttcga gaagcccgc
961 tatgactacc tgcggaagct cttcaccgac ctcttcgacc gcagtggctt cgtgttcgac
1021 tatgagtacg actgggcccg gaagcccctg ccgaccccca tcggcaccgt ccacaccgac
1081 ctgccctccc agcctcagct ccgggacaaa acccagccgc acagcaaaaa ccaggcggtg
1141 aactccacca acggggagct gaatgcggac gacccacggy ccggccactc caacgccccg
1201 atcacagcgc ctgcagaggt ggaggtggcc gatgaaacca aatgctgctg tttcttcaag
1261 aggagaaaga gaaaatcgct gcagcgacac aagtga

```

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.