

Certificate of Analysis

Casein Kinase 1γ1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-711, 14-711-K, 14-711M

Parent Lot # D9BN022U

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 1γ1, amino acids 25–355, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 93% by SDS-PAGE and Coomassie blue staining. MW = 41.9kDa.

Specific Activity (Parent lot# D9BN022U): 19751U/mg, where one unit of Casein Kinase 1γ1, active activity is defined as 1nmol phosphate incorporated into 200μM (KRRRALS(p)VASLPGL) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 2.367mg/ml of enzyme in 50mM Tris/HCl pH8.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

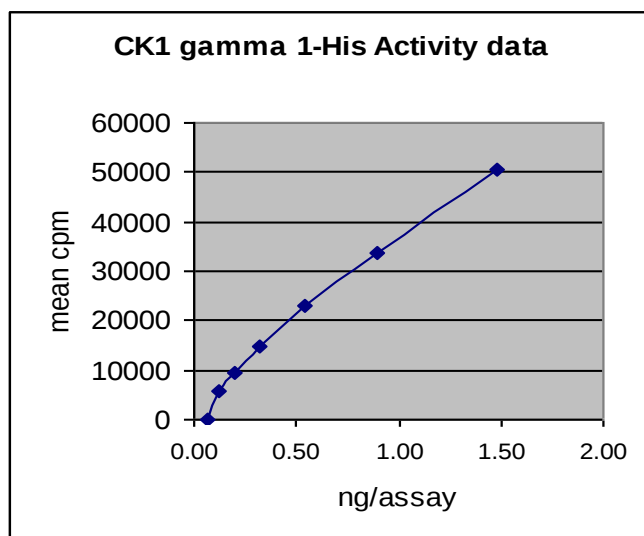
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled 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**

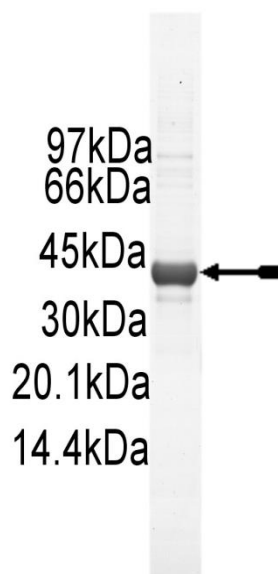
Quality Control Testing

Kinase Assay: 0.54–1.48ng 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 identity as Casein Kinase 1γ1 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of Casein Kinase 1γ1, 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. Make up a 2mM stock. Add 2.5 μ l of stock per assay point.
3. **Casein Kinase 1 γ 1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.54–1.48ng 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 (0.54–1.48ng) Casein Kinase 1 γ 1, 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 1γ1 Sequence Information

<u>Protein</u>	Human Casein Kinase 1γ1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	C31 of the recombinant protein is equivalent to C25 of human Casein Kinase 1γ1
<u>Accession number</u>	GenBank NM_022048

Recombinant Casein Kinase 1γ1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF CSRPSGSSSS SGVLMVGPNF RVGKKIGCGN
61 FGELRLGKNL YTNEYVAIKL EPIKSRAPQL HLEYRFYKQL GSAGEGLPQV YYFGPCGKYN
121 AMVLELLGPS LEDLFDLCDR TFTLKTVLM IAIQLLSRMEY VHSKNLIYRD VKPENFLIGR
181 QGNKKEHVIH IIDFGLAKEY IDPETKKHIP YREHKSALTGT ARYMSINTHL GKEQSRRDDL
241 EALGHMFMYF LRGS LPWQGL KADTLKERYQ KIGDTRKNT IEALCENFPE EMATYLRIVR
301 RLDFFEKPDY EYLRTLTDL FEKKGYTFDY AYDWVGRPIP TPVGSVHVDS GASAITRESH
361 T

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Recombinant Casein Kinase 1γ1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcgcattga tccggaattc tgctctcgac catctggctc ctcctcgtcc
121 tctggggttc ttatggtggg acccaacttc aggggttgga agaagatagg atgtgggaac
181 ttcggagagc tcagattagg taaaaatctc tacaccaatg aatatgtagc aatcaaactg
241 gaaccaataa aatcacgtgc tccacagctt catttagagt acagatttta taaacagctt
301 ggcagtgcag gtgaaggtct cccacaggtg tattactttg gaccatgtgg gaaatataat
361 gccatggtgc tggagctcct tggccctagc ttggaggact tgtttgacct ctgtgaccga
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1081 acataa

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