

## Certificate of Analysis

### Casein Kinase 1 $\gamma$ 3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-713, 14-713-K, 14-713M

Parent Lot # 31115U

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 $\gamma$ 3, amino acids 1–330, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 97% by SDS-PAGE and Coomassie blue staining. MW = 42.2kDa.

**Specific Activity (Parent lot# 31115U):** 20408U/mg, where one unit of Casein Kinase 1 $\gamma$ 3, active activity is defined as 1nmol phosphate incorporated into 200 $\mu$ M (KRRRALS(p)VASLPGL) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 3.81mg/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.

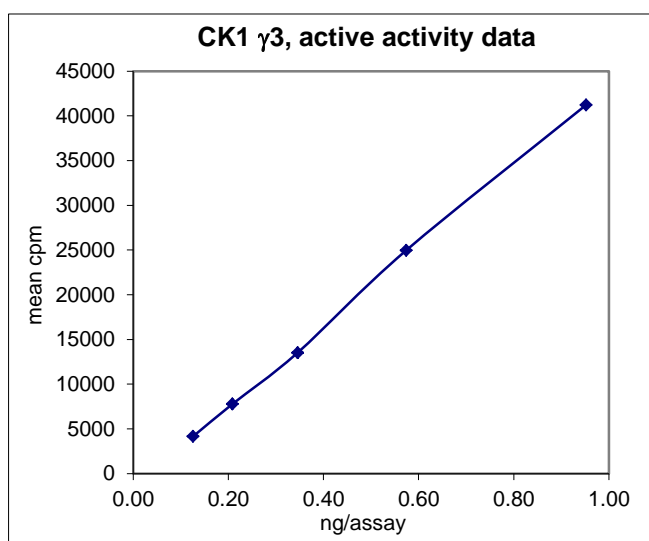
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

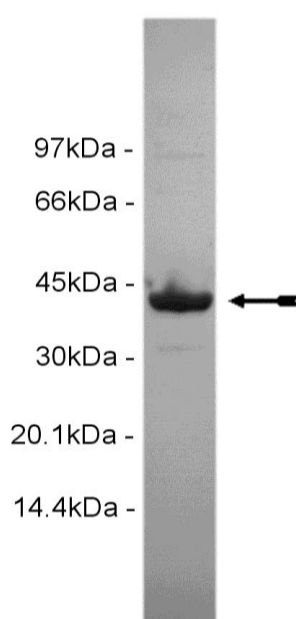
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 0.13–0.95ng of this lot of enzyme phosphorylated 200 $\mu$ 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 $\gamma$ 3 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 3 $\mu$ g of Casein Kinase 1 $\gamma$ 3, 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 $\mu$ M. Make up a 2mM stock. Add 2.5 $\mu$ l of stock per assay point.
3. **Casein Kinase 1 $\gamma$ 3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.13–0.95ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **(KRRRALS(p)VASLPGL)**.
3. Add **2.5 $\mu$ l (0.13–0.95ng) Casein Kinase 1 $\gamma$ 3, active.**
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ 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 $\mu$ l of 30% phosphoric acid.

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### Casein Kinase 1 $\gamma$ 3 Sequence Information

<b><u>Protein</u></b>	Human Casein Kinase 1 $\gamma$ 3
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	M31 of the recombinant protein is equivalent to M1 of human Casein Kinase 1 $\gamma$ 3
<b><u>Accession number</u></b>	GenBank NM_004384. The recombinant protein contains the amino acid substitutions K91E, R174G and E302D with reference to NM_004384. These mutations are reported in GenBank BC047567.

#### ***Recombinant Casein Kinase 1 $\gamma$ 3 amino acid sequence:***

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MENKKKDKDK SDDRMARPSG RSGHNTRGTG
61 SSSSGVLMVG PNFVRGKKIG CGNFGELRLG KNLYTNEYVA IKLEPMKSRA PQLHLEYRFY
121 EQLGSGDGIP QVYFPGCGK YNAMVLELLG PSLEDLFDLC DRTFSLKTVL MIAIQLISRM
181 EYVHSKNLIY RDVKPENFLI GRPGNKTQQV IHIIDFGLAK EYIDPETKKH IPYREHKS LT
241 GTARYMSINT HLGKEQSRRD DLEALGHMFM YFLRGSLPWQ GLKADTLKER YQKIGDTKRA
301 TPIEVLCEFN PEMATYLRVY RRLDFFEKPD YDYLRKLF TD LFDRKGYMFD YEYDWIGKQL
  
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#### ***Recombinant Casein Kinase 1 $\gamma$ 3 nucleotide sequence:***

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcccatgga tccggaattc atggaaaata aaaagaaaga caaggacaaa
121 tcagatgata gaatggcacg acctagtggc cgatcgggac acaacactcg aggaactggg
181 tcttcatcgt ctggagtttt aatggttgga cctaacttta gagttggaaa aaaaattgga
241 tgtggcaatt ttggagaatt gcgattaggg aaaaatttat acacaaatga atatgtggca
301 attaagttgg agcccatgaa atcaagagca ccacagctac atttggaata cagattctat
361 gagcagttag gatctggaga tgggtatacct caagtttact atttcggccc ttgtggtaaa
421 tacaatgcta tgggtctgga actgctggga cctagtttgg aagacttggt tgacttgtgt
481 gacagaacat tttctcttaa aacagttctc atgatagcta tacaactgat ttctcgcgatg
541 gaatatgtcc attcaaagaa cttgatatac agagatgtaa aacctgagaa cttcttaata
601 ggacgaccag gaaacaaaac ccagcaagtt attcacatta tagattttgg tttggcaaag
661 gaatatattg atccggagac aaagaaacac ataccataca gagaacacaa gaggccttaca
721 ggaacagcta gatatatgag cataaacaca catttaggaa aagaacaaag tagaagagac
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961 agaaggctag atttttttga aaaaccagac tatgactact taagaaagct ttttactgac
1021 ttgtttgatc gaaaaggata tatgtttgat tatgaatatg actggattgg taaacagttg
1081 taa
  
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Reviewed and approved by site quality representative.

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