

Certificate of Analysis

GCK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-743, 14-743-K, 14-743M

Parent Lot # D7HN049U

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 GCK amino acids 1–473, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 54.2% by SDS-PAGE and Coomassie blue staining. MW = 80.6kDa.

Specific Activity (Parent lot# D7HN049U): 2100U/mg, where one unit of GCK, active activity is defined as 1nmol phosphate incorporated into 0.8mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.759mg/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.

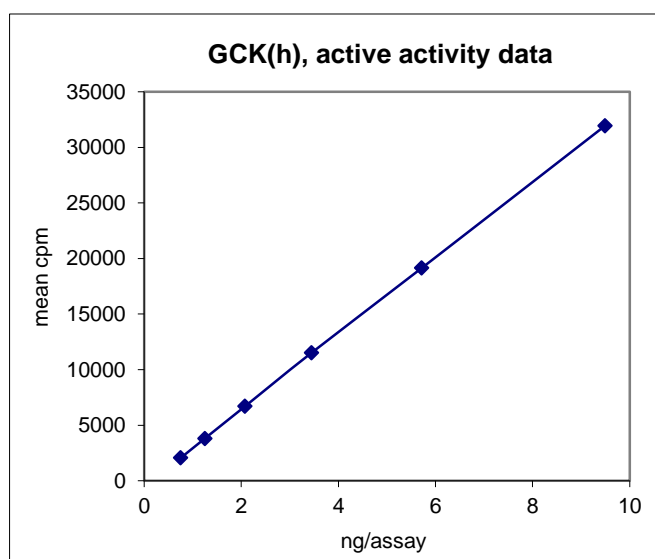
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

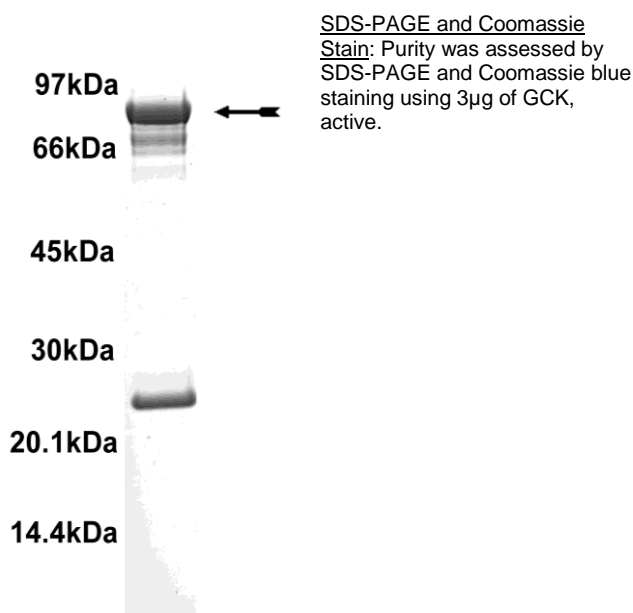
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–9ng of this lot of enzyme phosphorylated 0.8mg/ml myelin basic protein 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 GCK 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. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.8mg/ml. Make up an 8.0mg/ml stock. Use 2.5µl of stock per assay point.
- 3. GCK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–9ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein**.
3. Add **2.5µl (1–9ng) GCK, active**.
4. Add 5µl of dH₂O
5. Add 10µl of [γ -³³P] 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.

Certificate of Analysis

GCK Active Sequence Information

<u>Protein</u>	Human GCK
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M237 of recombinant sequence = M1 of native human GCK
<u>Accession number</u>	GenBank BC047865

Recombinant GCK amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRL L EYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFSLKPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGP EF KGLRRQMALL
241 RDVSLQDPRD RFELLQRVGA GTYGDVYKAR DTVTSELA AV KIVKLDPGDD ISSLQQEITI
301 LRECRHPNVV AYIGSYLRND RLWICMEFCG GGSLQEIYHA TGPLEERQIA YVCREALKGL
361 HHLHSQK I H RDIKANLLL TLQGDVKLAD FGVSGELTAS VAKRRSFIGT PYWMAPEVAA
421 VERKGGY NEL CDVWALGITA IELGELQPPL FHLHPMRALM LMSKSSFQPP KLRDKTRWTQ
481 NFHHFLK LAL TKNPKKRPTA EKLLQH PFTT QQLPRALLTQ LLDKASDPHL GTPSPEDCEL
541 ETYDMFP D TI HSRGQHGP AE RTPSEIQFHQ VKFGAPRRKE TDPLNEPWEE EWTLLGKEEL
601 SG SLLQSVQE ALEERSLTIR SASEFQELDS PDDTMGTIKR APFLGPLPTD PPAEEPLSSP
661 PGPNSSPLLP TAWATMKQRE DPERSSCHGL PPTPKVHMGA CFSKVFNGC

```

Recombinant GCK nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgctc aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtgggtggcg accatcctcc aaaatcggat
661 ctggaagtctc tgttcaggg gcccgaattc aaaggcctac gtcgacaaat ggcgctgctg
721 cgggatgtgt cgctgcagga cccgcgggac cgcttcgagc tgctgcagcg cgtggggggc
781 gggacctatg gcgacgtcta caaggccgc gacacggtca cgtccgaact ggccgccgtg
841 aagatagtca agctagacc aggggacgac atcagctccc tccagcagga aatcaccatc
901 ctgcgtgagt gccgccacc caatgtggtg gcctacattg gcagctacct caggaatgac
961 cgcttgtgga tctgcatgga gttctgcgga gggggctccc tgcaggagat ttaccatgcc
1021 actgggcccc tggaggagcg gcagattgcc tacgtctgcc gagaggcact gaaggggctc
1081 caccacctgc attctcaggg gaagatccac agagacatca agggagccaa ccttctctc
1141 actctccagg gagatgtcaa actggctgac tttgggggtgt caggcgagct gacagcgtct
1201 gtggccaaga ggaggtcttt cattgggact ccctactgga tggctcccga ggtggctgct
1261 gtggagcgca aaggtggcta caatgagcta tgtgacgtct gggccctggg catcactgcc
1321 attgagctgg gcgagctgca gccccctctg ttccacctgc acccatgag ggccctgatg
1381 ctcatgtcga agagcagctt ccagccgcc aaactgagag ataagactcg ctggaccag
1441 aatttccacc actttctcaa actggcctg accaagaatc ctaagaagag gccgacagca
1501 gagaagctcc tgcagcacc gttcacgact cagcagctcc ctgggccct cctcacacag
1561 ctgctggaca aagccagtga ccctcatctg gggacccccct cccctgagga ctgtgagctg
1621 gagacctatg acatgtttcc agacaccatt cactcccggg ggcagcacgg cccagccgag

```

Certificate of Analysis

```
1681 aggaccccct cggagatcca gtttcaccag gtgaaatttg gcgccccacg caggaaggaa
1741 actgacccac tgaatgagcc gtgggaggaa gagtggacac tactgggaaa ggaagagttg
1801 agtgggagcc tgctgcagtc ggtccaggag gccctggagg aaaggagtct gactattcgg
1861 tcagcctcag aattccagga gctggactcc ccagacgata ccatgggaac catcaagcgg
1921 gccccgttcc tagggcact ccccactgac cctccagcag aggagcctct gtccagtccc
1981 ccaggcccca acagctccc actgctgccc acggcctggg ccaccatgaa gcagcgggag
2041 gatcctgaga ggtcatcctg ccacgggctc cccccaactc ccaaggtgca tatgggcgcc
2101 tgcttctcca aggtcttcaa tggctgctaa
```

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