

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.

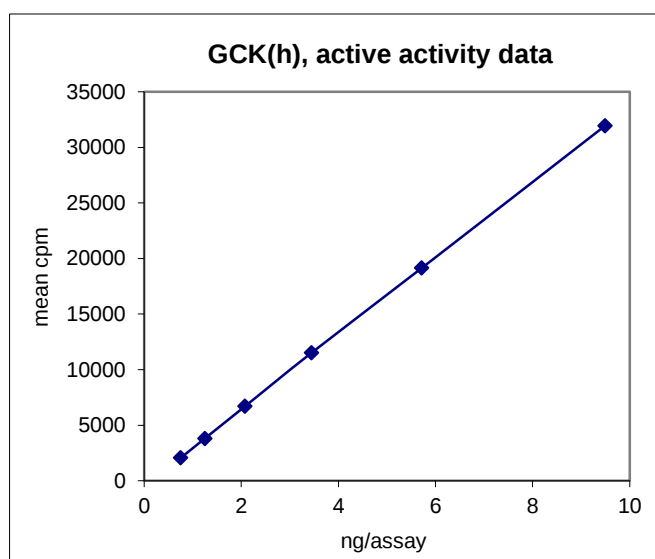
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

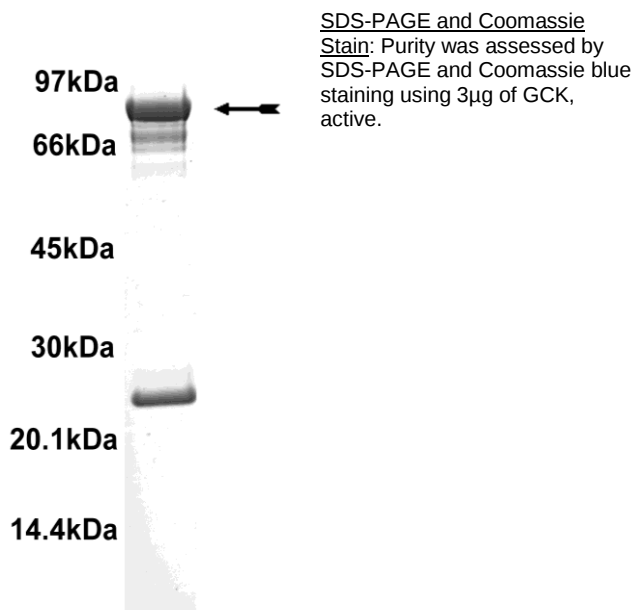
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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.



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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.

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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:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSM A IIRYIADKH N MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRQMALL
241 RDVSLQDPRD RFELLQRVGA GTYGDVYKAR DTVTSELA AV KIVKLDPGDD ISSLQQEITI
301 LRECRHPNVV AYIGSYLRND RLWICMEFCG GGS LQEIYHA TGPLEERQIA YVCREALKGL
361 HHLHSQGKIH RDIKGANLLL TLQGDVKLAD FGVSGELTAS VAKRRSFIGT PYWMAPEVAA
421 VERKGGYNEL CDVWALGITA IELGELQPPL FHLHPMRALM LMSKSSFQPP KLRDKTRWTQ
481 NFHHFLKLAL TKNPKKRPTA EKLLQHPFTT QQLPRALLTQ LLDKASDPHL GTPSPEDCEL
541 ETYDMFPDTI HSRGQHGP AE RTPSEIQFHQ VKFGAPRRKE TDPLNEPWEE EWTLLGKEEL
601 SGSL LQSVQE ALEERSLTIR SASEFQELDS PDDTMGTIKR APFLGLPLTD PPAAEPLSSP
661 PGPNSSPLLP TAWATMKQRE DPERSSCHGL PPTPKVHMGA CFSKVFNGC
  
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Recombinant GCK nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121  tggcgaaaca aaaagtttga atttgggtttg gaggtttcca atcttcctta ttatatgtat
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1621 gagacctatg acatgtttcc agacaccatt cactcccggg ggcagcacgg cccagccgag
  
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1801 agtgggagcc tgctgcagtc ggtccaggag gccctggagg aaaggagtct gactattcgg
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1921 gccccgttcc tagggccact cccactgac cctccagcag aggagcctct gtccagtccc
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2101 tgcttctcca aggtcttcaa tggctgctaa
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