

Certificate of Analysis

GCN2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-934, 14-934-K, 14-934M

Parent Lot # WAD0197

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 GCN2 full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Purity 91% by SDS-PAGE and Coomassie blue staining. MW = 215kDa.

Specific Activity (Parent lot# WAD0197): 83U/mg, where one unit of GCN2, active activity is defined as 1nmol phosphate incorporated into 300µM (RSRSRSRSRSRSRSR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.09mg/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.

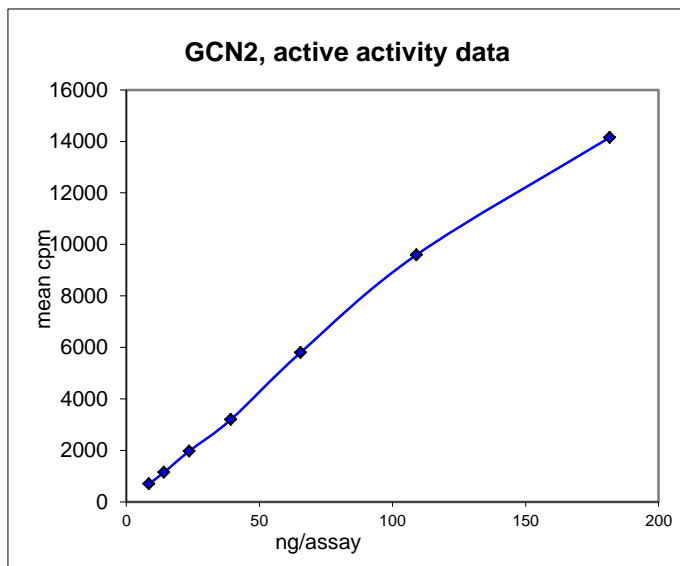
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

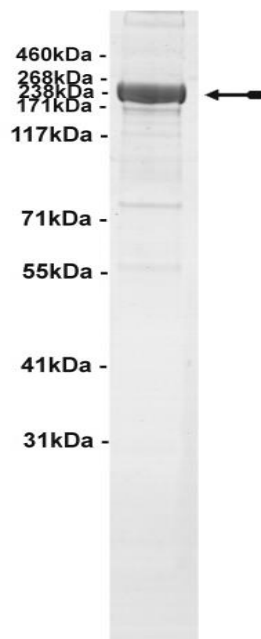
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 8–182ng of this lot of enzyme phosphorylated 300µM (RSRSRSRSRSRSRSR) 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 GCN2 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of GCN2, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 100mM Tris/HCl pH8.5, 1mM EDTA.
2. **(RSRSRSRSRSRSRSR):** Use at a final assay concentration of 300 μ M. Prepare a 3mM stock and add 2.5 μ l of stock per assay point.
3. **10% Tween 20(w/v):** Use at a final assay concentration of 0.1% (w/v). Use 0.25 μ l of a 10% stock solution per assay point.
4. **1M NaCl:** Use at a final assay concentration of 100mM. Use 2.5 μ l of a 1M stock solution per assay point.
5. **GCN2, active:** Dilute with 20mM Tris/HCl pH8.5, 0.2mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 8–182ng per assay point.
6. **[γ -³³P]ATP:** 2.5 x MgAc/[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 1M NaCl.
3. Add 0.25 μ l 10% Tween 20.
4. Add 2.5 μ l of **(RSRSRSRSRSRSRSR)**.
5. Add 2.25 μ l of dH₂O.
6. Add **2.5 μ l (8–182ng) GCN2, active**.
7. Add 10 μ l of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
10. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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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GCN2 Sequence Information

<u>Protein</u>	human GCN2
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M237 of the recombinant protein is equivalent to M1 of human GCN2
<u>Accession number</u>	GenPept NP_001013725 The recombinant protein contains the amino acid substitution E556G with reference to GenPept NP_001013725. E556G is reported in GenPept CAH10626.

Recombinant GCN2 amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYE  HLYERDEGD  WRNKKFELG  EFPNLPYYI
61  GDVKLTQSM  IIRYIADKH  MLGGCPKER  EISMLEGAV  DIRYGVSRI  YSKDFETLK
121 DFLSKLPEML  KMFEDRLCH  TYLNGDHV  PDFMLYDAL  VVLYMDPM  DAFPKLVCF
181 KRIEAIPQID  KYLKSSKYI  WPLQGWQAT  GGDHPPKSD  LEVLFQGPE  KGLRRRMAG
241 RGAPGRGRDE  PPESYPQRQ  HELQALEAI  GADFQDLRP  ACPVKEPPE  INLVLYPQGL
301 TGEEVYVKVD  LRVKCPPTY  DVVPEIELK  AKGLSNESV  LLKSRLEEL  KKHCGEVMIF
361 ELAYHVQSFL  SEHNKPPPK  FHEEMLERR  QEEQRLLEA  KRKEEQEQR  ILHEIQRRKE
421 EIKEEKRRKE  MAKQERLEI  SLSNQDHTS  KDPGGHRTA  ILHGGSPDF  GNGKHRANSS
481 GRSRRERQYS  VCNSDSPGS  CEILYFNM  PDQLMVHKG  CIGSDEQLG  LVSNALETAT
541 GGFVLLYEW  LQWQKKMGP  LTSQEKEKI  KCKKQIQGT  TEFNSLVKL  HPNVVRYLAM
601 NLKEQDDSI  VDILVEHIS  VSLAAHLS  GPIPVHQLR  YTAQLLSGL  YLHSNSVVHK
661 VLSASNVLVD  AEGTVKITD  SISKRLADI  KEDVFEQTR  RFSNALPYK  TGKKGDVWRL
721 GLLLLSLSQ  QECGEYPVI  PSDLPADFQ  FLKCKVCLD  KERWSPQQL  KHSFINPQPK
781 MPLVEQSPED  SGGQDYVET  IPSNRLPSA  FFSETQRQF  RYFIEFEEL  LLGKGAFGAV
841 IKVQNKLDGC  CYAVKRIPIN  PASRQFRR  GEVTLLSRL  HENIVRYNA  WIERHERPAG
901 PGTPPPDSGP  LAKDDRAAR  QPASDTDGL  SVEAAAPPI  LSSSVEWST  GERSASARFP
961 ATGPGSSDDE  DDEDEHGGV  FSQSFLPAS  SESDIIFDN  DENSKSQNQ  EDCNEKNGCH
1021 ESEPSVTTEA  VHLYIQMEY  CEKSTLRDI  DQGLYRDTV  LWRLFREIL  GLAYIHEKGM
1081 IHRDLKPVNI  FLDSDDHVK  GDFGLATDH  AFSADSKQD  QTGDLIKSD  SGHLTGMVGT
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1201 DFDDGEHAK  KSVISWLLN  DPAKRPTAT  LLKSELLPP  QMEESELHE  LHHTLTNVDG
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1381 PRKLDRFHP  ELLECAFDI  TSTTNSFL  AEIITYIYE  IQEFPALQE  NYSIYLNHTM
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1741 DNLAVQNKG  SFSNASGLF  IHGATVPIV  SVLAPEKLS  STRRRYETQ  QTRLQTSLAN
1801 LHQKSSEIE  LAVDLPKET  LQFLSLEWD  DEQAFNTTV  QLLSRLPKQ  YLKLVCDEIY
1861 NIKVEKKVS  LFLYSYRDD  YRILF
    
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Recombinant GCN2 nucleotide sequence:

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421	acataattaa	atggtgatca	tgtaacccat	cctgacttca	tgttgatga	cgctcttgat
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