eurofins | Discovery Services

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.

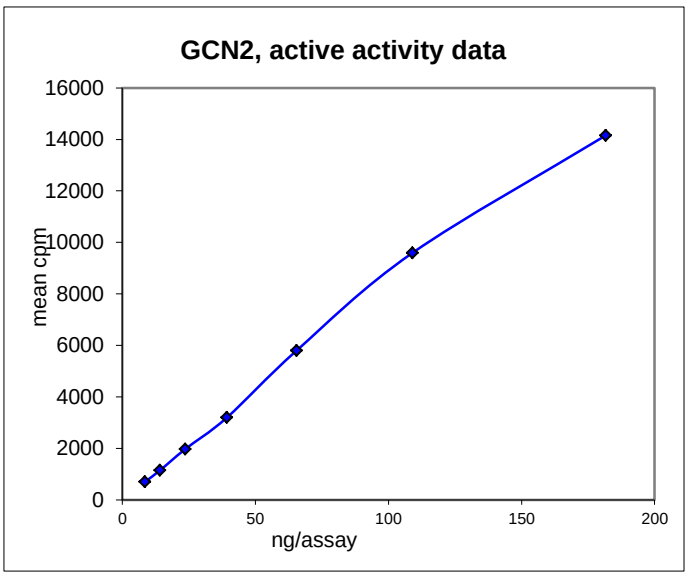
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

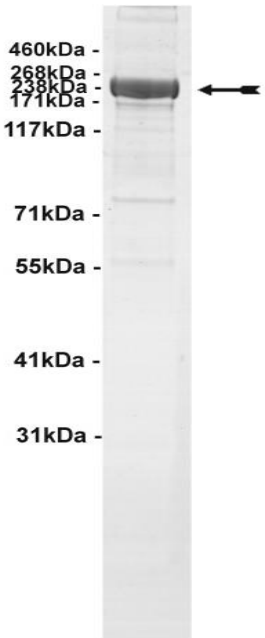
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

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 300µM. Prepare a 3mM stock and add 2.5µl of stock per assay point.
3. **10% Tween 20(w/v)**: Use at a final assay concentration of 0.1% (w/v). Use 0.25µl of a 10% stock solution per assay point.
4. **1M NaCl:** Use at a final assay concentration of 100mM. Use 2.5µl 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-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]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 1M NaCl.
3. Add 0.25µl 10% Tween 20.
4. Add 2.5µl of **(RSRSRSRSRSRSRSR)**.
5. Add 2.25µl of dH_2O.
6. Add **2.5µl (8–182ng) GCN2, active.**
7. Add 10µl of diluted [γ-^{33}P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl 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 1µl of 30% phosphoric acid.

eurofins | Discovery Services

Certificate of Analysis

GCN2 Sequence Information

Protein human GCN2

Tags *N*-terminal GST

Native sequence M237 of the recombinant protein is equivalent to M1 of human GCN2

Accession number 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 KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRRMAGG
 241 RGAPGRGRDE PPESYPQRQD HELQALEAIY GADFQDLRPD ACGPVKEPPE INLVLYPQGL
 301 TGEEVYVKVD LRVKCPPTYP DVVPEIELKN AKGLSNESVN LLKSRLEELA KKHCGEVMIF
 361 ELAYHVQSFL SEHNKPPPKS FHEEMLERRA QEEQQRLLEA KRKEEQEQRE ILHEIQRRKE
 421 EIKEEKKRKE MAKQERLEIA SLSNQDHTSK KDPGGHRTAA ILHGGSPDFV GNGKHRANSS
 481 GRSRRERQYS VCNSEDSPGS CEILYFNMGS PDQLMVHKGK CIGSDEQLGK LVYNALETAT
 541 GGFVLLYEWV LQWQKKMGPF LTSQEKEKID KCKKQIQGTE TEFNSLVKLS HPNVVRYLAM
 601 NLKEQDDSIV VDILVEHISG VSLAAHLSHS GPIPVHQLRR YTAQLLSGLD YLHSNSVVHK
 661 VLSASNVLVD AEGTVKITDY SISKRLADIC KEDVFEQTRV RFSDNALPYK TGKKGDVWRL
 721 GLLLLSLSQG QECGEYPVTI PSDLPADFQD FLKKCVCLDD KERWSPQQLL KHSFINPQPK
 781 MPLVEQSPED SGGQDYVETV IPSNRLPSAA FFSETQRQFS RYFIEFEELQ LLGKGAFGAV
 841 IKVQNKLDGC CYAVKRIPIN PASRQFRRIK GEVTLLSRLH HENIVRYYNA WIERHERPAG
 901 PGTPPPDSGP LAKDDRAARG QPASDTDGLD SVEAAAPPPI LSSSVEWSTS GERSASARFP
 961 ATGPGSSDDE DDDEDEHGGV FSQSFLPASD SESDIIFDNE DENSKSQNQD EDCNEKNGCH
1021 ESEPSVTTEA VHYLYIQMEY CEKSTLRDTI DQGLYRDTVR LWRLFREILD GLAYIHEKGM
1081 IHRDLKPVNI FLDSDDHVKI GDFGLATDHL AFSADSKQDD QTGDLIKSDP SGHLTGMVGT
1141 ALYVSPEVQG STKSAYNQKV DLFSLGIIFF EMSYHPMVTA SERIFVLNQL RDPTSPKFPE
1201 DFDDGEHAKQ KSVISWLLNH DPAKRPTATE LLKSELLPPP QMEESELHEV LHHTLTNVDG
1261 KAYRTMMAQI FSQRISPAID YTYDSDILKG NFSIRTAKMQ QHVCETIIRI FKRHGAVQLC
1321 TPLLLPRNRQ IYEHNEAALF MDHSGMLVML PFDLRIPFAR YVARNNILNL KRYCIERVFR
1381 PRKLDRFHPK ELLECAFDIV TSTTNSFLPT AEIIYTIYEI IQEFPALQER NYSIYLNHTM
1441 LLKAILLHCG IPEDKLSQVY IILYDAVTEK LTRREVEAKF CNLSLSSNSL CRLYKFIEQK
1501 GDLQDLMPTI NSLIKQKTGI AQLVKYGLKD LEEVVGLLKK LGIKLQVLIN LGLVYKVQQH
1561 NGIIFQFVAF IKRRQRAVPE ILAAGGRYDL LIPQFRGPQA LGPVPTAIGV SIAIDKISAA
1621 VLNMEESVTI SSCDLLVVSV GQMSMSRAIN LTQKLWTAGI TAEIMYDWSQ SQEELQEYCR
1681 HHEITYVALV SDKEGSHVKV KSFEKERQTE KRVLETELVD HVLQKLRTKV TDERNGREAS
1741 DNLAVQNLKG SFSNASGLFE IHGATVVPIV SVLAPEKLSA STRRRYETQV QTRLQTSLAN
1801 LHQKSSEIEI LAVDLPKETI LQFLSLEWDA DEQAFNTTVK QLLSRLPKQR YLKLVCDEIY
1861 NIKVEKKVSV LFLYSYRDDY YRILF
```

Recombinant GCN2 nucleotide sequence:

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
```

Certificate of Analysis

```
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggaagttc tgttccaggg gcccgaattc aaaggcctac gtcgacgaat ggcaggtgga
 721 agaggagccc caggtagagg aagagatgag cctcctgaat cctaccccca aaggcaagac
 781 catgaattgc aggcactcga ggccatttac ggagccgact tccaggacct gcgccccgat
 841 gcatgtggcc ccgtgaaaga gccccctgag atcaacctgg ttctgtatcc ccaaggactc
 901 actggagaag aggtctacgt caaagtggat ttgagagtga aatgcccacc tacatatcca
 961 gacgttgtgc cagagatcga gttgaaaaat gccaagggac tgtctaatga aagcgtgaac
1021 ttgttgaagt cacgcctcga agaattggcc aagaaacatt gcggcgaggt catgatcttt
1081 gaactcgcat accacgtgca gagcttcctg agcgagcaca acaaacctcc acccaagtca
1141 tttcacgaag aaatgctgga gcgcagagca caagaggagc agcagaggct gctggaggct
1201 aagcgcaaag aggaacaaga acaacgcgag atcctccacg agattcagcg cagaaaagag
1261 gaaatcaaag aagagaagaa gcgcaaggaa atggctaaac aagagcgcct ggaaattgct
1321 tcactcagca accaggacca tacctccaaa aaggaccctg gaggccatcg cacagctgcc
1381 atcctgcacg gtggctcacc cgacttcgtt ggaaatggta agcacagagc caatagctca
1441 ggccgctcaa ggagagaacg ccaatacagc gtgtgtaact ccgaggattc accaggtagc
1501 tgcgagatct tgtacttcaa tatgggttcc cccgatcagc tgatggtgca caaaggaaaa
1561 tgtatcggct ccgacgaaca attgggtaaa ttggtctata atgcattgga aaccgcaacc
1621 ggcggtttcg ttctcctgta tgaatgggtc ctgcagtggc agaaaaagat gggtcccttt
1681 ctcacctcac aagagaagga gaagattgat aagtgcaaga aacagattca gggaaccgag
1741 acagagttta actctctcgt taagttgtcc cacccaaatg tggtcagata cctggcaatg
1801 aatctcaagg agcaagacga ctccattgtt gtggatatcc tcgtggagca catctccgga
1861 gtcagcctcg ctgctcacct ctcacacagc ggcccaattc ctgtccacca actcagaagg
1921 tacacagctc aattgttgtc cggtctggac tatttgcact caaatagcgt ggtccacaag
1981 gtcctgtctg cctccaacgt gctcgttgat gccgagggca ccgtcaaaat taccgattat
2041 agcatctcta aacgcctggc tgatatctgc aaggaggacg tgttcgaaca gactagggtc
2101 aggttttctg ataacgcatt gccatacaaa actggcaaaa agggtgatgt ttggagactg
2161 ggcctgttgc tgttgtccct ctcacaaggt caagaatgcg gagaatatcc tgtgacaatt
2221 ccttcagacc tccccgctga tttccaggac tttctcaaaa agtgtgtctg cctggacgac
2281 aaggagaggt ggtcacccca acagctgctg aagcactcct tcatcaaccc ccagcccaaa
2341 atgcccctgg tggaacagtc ccctgaagat tctggcggcc aggactatgt tgaaacagtg
2401 atcccttcta atagactgcc ttctgctgca ttctttttctg aaacccaaag gcagttctct
2461 cgctacttta tcgagttcga agaactgcaa ctgctgggaa aaggtgcctt tggcgctgtc
2521 atcaaagtgc agaataagct cgacggttgc tgttacgctg tgaagagaat ccctattaac
2581 ccagctagca gacaattcag gagaatcaaa ggtgaggtca ccttgctctc tagattgcat
2641 cacgagaaca tcgtgagata ttacaacgca tggatcgaga ggcatgagag gcccgcaggt
2701 ccaggcacac ctcccccaga tagcggccca ctggctaagg atgaccgcgc tgcacgcggc
2761 cagccagcct ccgataccga tggcttggat tccgttgaag ctgctgcacc acccccctatc
2821 ctctcctctt cagtggaatg gtccactagc ggcgagcgct cagcttccgc tcgcttccca
2881 gccactggac ctggatcttc agacgacgaa gacgacgatg aggatgagca cggcggagtg
2941 tttagccagt ccttttttgcc agccagcgac tctgagtccg atattatctt cgataatgag
3001 gacgagaata gcaaaagcca gaatcaggac gaggattgca acgaaaagaa cggatgtcat
3061 gagtcagagc catcagtgac cactgaagca gtgcactact tgtacatcca gatggagtat
3121 tgtgagaagt ctaccctcag agatactatt gatcaaggcc tgtataggga tactgtccgc
3181 ctgtggcgcc tcttcaggga gatcctggat ggactggctt acatccacga gaagggcatg
3241 atccatcgcg atctcaagcc cgtcaacatt tttctggatt cagacgatca cgtgaaaatc
3301 ggagatttcg gcctggccac cgaccatctc gccttttccg ctgatagcaa acaagacgac
3361 cagaccggcg acctgattaa gtccgatcct agcggtcatt tgactggcat ggtcggcaca
3421 gccctgtacg tgagccctga agttcaaggt agcactaaat ctgcatacaa tcagaaagtg
3481 gacttgttct ccctcggcat tattttcttt gaaatgagct accatcctat ggtgacagcc
3541 agcgaacgca ttttcgtgct gaatcagctc agggacccta catcacccaa gttccctgaa
```

eurofins | Discovery Services

Certificate of Analysis

```
3601 gacttcgacg atggagaaca cgctaagcaa aagtctgtga tctcctggct gttgaatcat
3661 gatcccgcca agcgcccaac tgctactgaa ttgctgaagt ctgaattgct cccaccacct
3721 cagatggaag aatccgaact ccacgaggtg ctccatcaca ctttgacaaa cgtcgatggt
3781 aaggcttacc gcactatgat ggcacaaatc ttttcccaga ggatctcccc cgctattgac
3841 tacacctacg actcagatat tctcaagggt aatttctcca tcagaaccgc aaagatgcag
3901 cagcatgtgt gtgagacaat tattcgcatc tttaagcgcc acggcgctgt gcaactctgt
3961 actcctctct tgctgcctag aaaccgccag atctatgagc acaatgaggc agccctgttc
4021 atggaccatt ctggcatgct ggttatgctg ccatttgacc tccgcattcc tttcgcccgc
4081 tacgtcgccc gcaataacat cctgaatctg aaaaggtact gcatcgagcg cgtgttccgc
4141 cccagaaaac tggataggtt ccacccaaag gagttgctgg aatgtgcatt tgacatcgtt
4201 acatccacta caaacagctt tctccctacc gctgagatca tttataccat ttacgagatc
4261 atccaggagt tccccgccct gcaggagaga aattactcta tttatctcaa tcacactatg
4321 ttgttgaagg ccatcctgct gcattgcggc attcctgagg acaaactgtc ccaagtctac
4381 atcatcttgt acgacgctgt gacagaaaag ctgaccagaa gggaggttga ggctaagttc
4441 tgtaacctgt ccctgtcttc taattctttg tgtaggctgt ataagtttat cgagcagaag
4501 ggcgacctcc aggatctcat gcccaccatc aactctctga tcaagcagaa aaccggtatt
4561 gctcaactgg tcaagtacgg cctgaaggat ctggaggagg tggtcggact gctgaagaaa
4621 ctgggcatca agttgcaggt tttgatcaat ttgggcctgg tttataaggt gcagcagcat
4681 aacggcatta ttttccaatt cgtggctttt atcaagagac gccaacgcgc cgtgcctgag
4741 attctcgctg ctggcggtag atatgatctg ctgatcccac agtttcgcgg tccccaggca
4801 ctgggtcccg tgcccaccgc tatcggagtc agcatcgcca tcgataagat ctcagctgct
4861 gtgctgaaca tggaagagag cgttaccatc agctcctgtg acctgctggt ggtttccgtg
4921 ggacaaatgt caatgtcaag agccatcaac ctcactcaga agctgtggac cgcaggcatc
4981 acagctgaga tcatgtacga ttggtcacag agccaggagg aactccagga atactgtcgc
5041 caccacgaga ttacatatgt tgctctggtg tccgacaagg agggctccca cgtgaaagtg
5101 aaatccttcg aaaaggaaag acaaactgag aagcgcgtgc tggagactga actcgtggac
5161 cacgtcctgc agaaactccg cacaaaggtc accgatgaaa gaaacggtag agaggcttct
5221 gacaatctcg ctgttcagaa tctcaaaggc tcatttagca acgctagcgg tttgtttgag
5281 attcatggcg ccactgttgt gcctattgtc tcagtgctgg ctcctgaaaa gctgtcagct
5341 agcaccagac gcagatacga aacacaggtg caaaccaggc tccagactag cctggctaac
5401 ctgcatcaaa agtcttctga aatcgagatt ctggccgtcg atctgcccaa agagacaatc
5461 ctgcaatttc tgtcactcga gtgggacgcc gacgagcagg cctttaacac taccgttaaa
5521 caattgctgt cccgcctccc caaacagcgc tatctgaagc tggtgtgcga tgaaatctac
5581 aacattaagg tggaaaagaa agtctctgtg ttgttcttgt actcatacag agatgactac
5641 tacagaatct tgttttag
```

Reviewed and approved by site quality representative.