

## Certificate of Analysis

### NUAK2, active

#### (Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-005, 15-005-K, 15-005M

Parent Lot # WAE0132

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 NUAK2 full length expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Purity 54% by SDS-PAGE and Coomassie blue staining. MW = 96kDa.

**Specific Activity (Parent lot# WAE0132):** 54U/mg, where one unit of NUAK2 activity is defined as 1nmol phosphate incorporated into KKKVSRSGLYRSPSPENLNRPR per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 0.21mg/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.

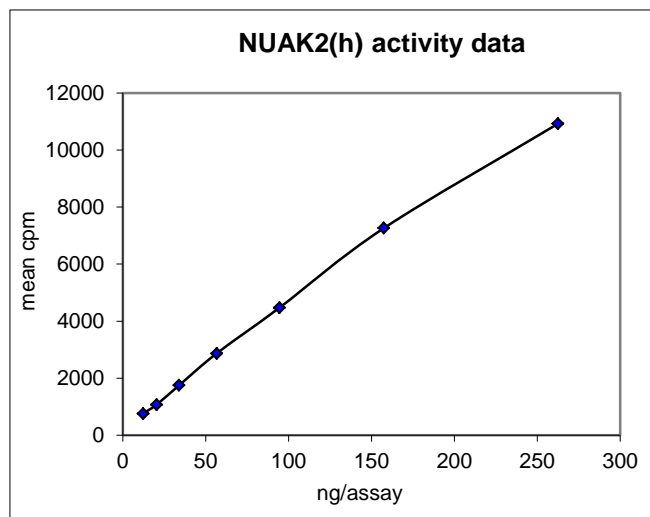
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

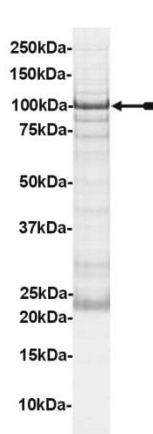
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 12.25–262.50ng of this lot of enzyme phosphorylated KKKVSRSGLYRSPSPENLNRPR 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 NUAK2 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 NUAK2, active.

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### Kinase Assay Protocol

#### Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. KKKVSRSGLYRSPSPENLNRPR:** Use at a final assay concentration of 300 $\mu$ M. Prepare a 3mM stock and add 2.5 $\mu$ l of stock per assay point.
- 3. NUAK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 12.25–262.50ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<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 KKKVSRSGLYRSPSPENLNRPR.
3. Add **2.5 $\mu$ l (12.25–262.50ng) NUAK2, 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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### NUAK2, active Sequence Information

<b><u>Protein</u></b>	Human NUAK2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	M230 of the recombinant protein is equivalent to M1 of human NUAK2
<b><u>Accession number</u></b>	GenBank BC017306.2

### **Recombinant NUAK2 amino acid sequence:**

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFSLKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFM ESLVFARRSG
241 PTPSAAELAR PLAEGLIKSP KPLMKKQAVK RHHHKHNL RH RYEFLET LGK GTYGKVKKAR
301 ESSGRLVAIK SIRKDKIKDE QDLMHIRREI EIMSSLNHPH IIAIHEVFEN SSKIVIVMEY
361 ASRGDLYDYI SERQQLSERE ARHFFRQIVS AVHYCHQNRV VHRDLKLENI LLDANGNIKI
421 ADFGLSNLYH QGKFLQTF CG SPLYASPEIV NGKPYTGPEV DSWSLGVLLY ILVHGTMPFD
481 GHDHKILVKQ ISNGAYREPP KPSDACGLIR WLLMVNPTRR ATLEDVASHW WVNWGYATRV
541 GEQEAPHEGG HPGSDSARAS MADWLRRSSR PLENGAKVC SFFKQHAPGG GSTTPGLERQ
601 HSLKKS RKEN DMAQSLHSDT ADDTAHRPGK SNLKL PKGIL KKKVSASAEG VQEDPPELSP
661 IPASPGQAAP LLPKKGILKK PRQRESGYYS SPEPSESGEL LDAGDVFVSG DPKEQKPPQA
721 SGLLLHRKGI LKLNKFSQT ALELAAP TTF GSLDELAPPR PLARASRPSG AVSEDSILSS
781 ESFDQLDLPE RLPEPPLRGC VSVDNLTGLE EPPSEGGPGSC LRRWRQDPLG DSCFSLTDCQ
841 EVTATYRQAL RVCSKLT

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### **Recombinant NUAK2 nucleotide sequence:**

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1 atgtccccta tactaggtta ttgaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatc ttgaagaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggttg gagtttcca atcttcctta ttatattgat
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1381 gacagctggt ccctgggtgt tctcctctac atcctggtgc atggcaccat gccctttgat

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1861 gctgatgaca ctgcccacg ccctggcaag agcaacctca agctgcaaaa gggcattctc
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1981 atccctgcga gccagggca ggctgcccc ctgctcccca agaagggcat tctcaagaag
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