

Certificate of Analysis

NIM1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-945, 14-945-K, 14-945M

Parent Lot # D14EP011N

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 NIM1 full length expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by gel filtration.

Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 77kDa.

Specific Activity (Parent lot# D14EP011N):

838U/mg, where one unit of NIM1, active activity is defined as 1nmol phosphate incorporated into 300 μ M (KKKVSRSGLYRSPSPENLNRRP) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.205mg/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.

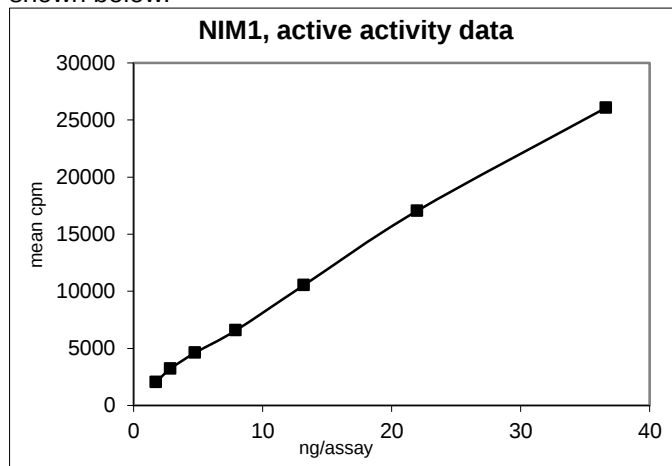
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

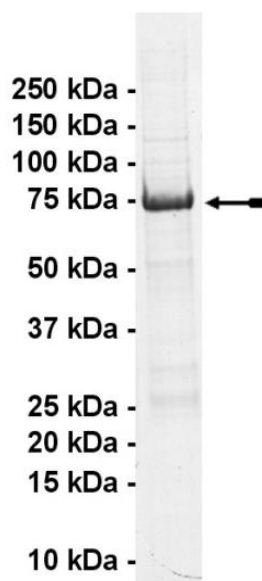
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–37ng of this lot of enzyme phosphorylated 300 μ M (KKKVSRSGLYRSPSPENLNRRP) 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 NIM1 with the translated sequence listed on page three



SDS-PAGE and Coomassie Stain:
Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of NIM1, 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 μ M. Prepare a 3mM stock and add 2.5 μ l of stock per assay point.
3. **NIM1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–37ng per assay point.
4. **[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 (KKKVSRSGLYRSPSPENLNRPR).
3. Add **2.5 μ l (2–37ng) NIM1, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ 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 μ l of 30% phosphoric acid.

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NIM1 Sequence Information

Protein	human NIM1
Tags	N-terminal GST
Native sequence	M237 of the recombinant protein is equivalent to M1 of human NIM1
Accession number	GenBank NM_153361

Recombinant NIM1 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPEF  KGLRRQMTAV
241 YMNGGGLVNP  HYARWDRRDS  VESGCQTESS  KEGEEGQPRQ  LTPFEKLTQD  MSQDEKVVRE
301 ITLGKRIGFY  RIRGEIGSGN  FSQVKLG IHS  LTKEKVAIKI  LDKTKLDQKT  QRLLSREISS
361 MEKLHHPNII  RLYEVVETLS  KLHLVMEYAG  GGELFGKIST  EGKLSEPESE  LIFSQIVSAV
421 KHMHENQIIH  RDLKAENVFY  TSNTCVKVG D  FGFSTVSKKG  EMLNTFCGSP  PYAAPELFRD
481 EHYIGIYVDI  WALGVLLYFM  VTGTMFPRAE  TVAKLKKSIL  EGTYSVPPHV  SEPCHRLIRG
541 VLQQIPTERY  GIDCIMNDEW  MQGVPYPTPL  EPFQLDPKHL  SETSTLKEEE  NEVKSTLEHL
601 GITEEHIRNN  QGRDARSSIT  GYRIILHRV  QRKKALESVP  VMMLPDPKER  DLKKGSRVYR
661 GIRHTSKFCS  IL

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Recombinant NIM1 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
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1501 gtgactggca  ccatgccatt  tcgggcagaa  accgtggcca  aactaaaaaa  gagcatcctc
1561 gagggcacat  acagtgtacc  gccgcacgtg  tcagagccct  gccaccgact  catccgagga
1621 gtccttcagc  agatccccac  ggagaggtac  ggaatcgact  gcatcatgaa  tgatgaatgg

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1801 ggcattacag aagagcatat tcgaaataac caaggagag atgctcgag ctcaatcaca
1861 ggggtctata gaattatttt acatagagtc caaaggaaga aggctttgga aagtgtccca
1921 gtcatgatgc taccagaccc taaagaaaga gacctcaaaa aagggtcccg tgtctacaga
1981 gggataagac acacatccaa attttgctcg attttataa
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