

Certificate of Analysis

NDR1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-989, 14-989-K, 14-989M

Parent Lot # D18EP021N

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 and C-terminal 6His-tagged, recombinant, human NDR1 full length and N-terminal 6His-tagged, recombinant, human MOBKL1A full length co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Complex activated *in vivo* with recombinant MST2. Purity 97% (combined) by SDS-PAGE and Coomassie blue staining. NDR1 MW = 83kDa, MOBKL1A MW = 30kDa.

Specific Activity (Parent lot# D18EP021N):

1405U/mg, where one unit of NDR1 activity is defined as 1nmol phosphate incorporated into 150µM KKLRRTLSVA per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.45mg/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.

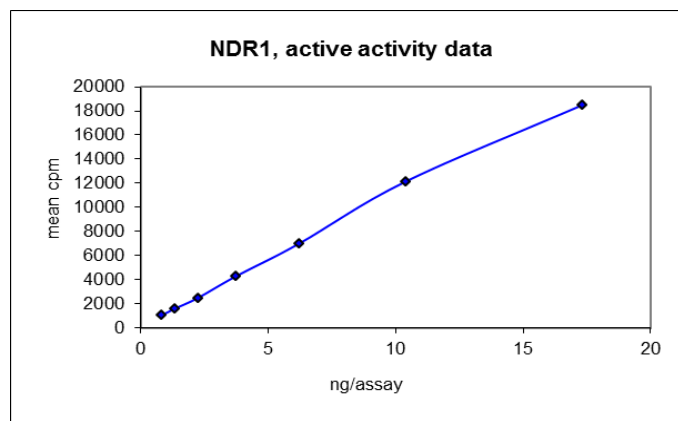
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

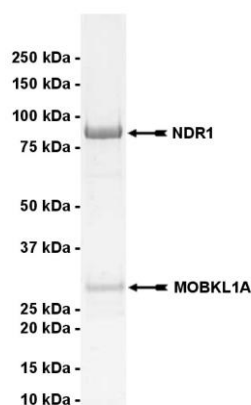
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.81–17.31ng of this lot of enzyme phosphorylated 150µM KKLRRTLSVA 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 NDR1 and MOBKL1A with the translated sequences listed on pages three and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of NDR1, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. KKLRRTLSVA:** Use at a final assay concentration of 150µM. Prepare a 1.5mM stock and add 2.5µl of stock per assay point.
- 3. NDR1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.81–17.31ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of KKLRRTLSVA.
3. Add **2.5µl (0.81–17.31ng) NDR1, active.**
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [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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NDR1, active Sequence Information

<u>Protein</u>	Human NDR1
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	M236 of the recombinant protein is equivalent to M1 of human NDR1
<u>Accession number</u>	GenBank NM_007271.2

Recombinant NDR1 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKEFK  GLRRQMAMTG
241  STPCSSMSNH  TKERVMTMKV  TLENFYSNLI  AQHEEREMRQ  KKLEKVMEEE  GLKDEEKRLR
301  RSAHARKETE  FLRLKRTRLG  LEDFESLKVI  GRGAFGEVRL  VQKKDTGHVY  AMKILRKADM
361  LEKEQVGHIR  AERDILVEAD  SLWVVKMFYS  FQDKLNLYLI  MEFLPGGDMM  TLLMKKDTLT
421  EEETQFYIAE  TVLAIDSIHQ  LGFIHRDIKP  DNLLLDSKGH  VKLSDFGLCT  GLKKAHRTEF
481  YRNLNHSPLS  DFTFQNMNSK  RKAETWKRNR  RQLAFSTVGT  PDYIAPEVFM  QTGYNKLCDW
541  WSLGVIMYEM  LIGYPPFCSE  TPQETYKKVM  NWKETLTFPP  EVPISEKAKD  LILRFCCWE
601  HRIGAPGVEE  IKSNSFFEGV  DWEHIRERPA  AISIEIKSID  DTSNFDEFPE  SDILKPTVAT
661  SNHPETDYKN  KDWVFINYTY  KRFEGLTARG  AIPSYMKAAC  HHHHHH

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Recombinant NDR1 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
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241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatattaa  atggtgatca  tgtaacccat  cctgacttca  tgttgtatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
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601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggttccgc  gtggatccaa  ggaattcaaa  ggcctacgtc  gacaaatggc  aatgacaggc
721  tcaacacctt  gctcatccat  gagtaaccac  acaaaggaaa  gggtgacaat  gaccaaagtg
781  aactgggaga  atttttatag  caaccttatc  gctcaacatg  aagaacgaga  aatgagacaa
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1441  tataggaatc  tgaaccacag  cctccccagt  gatttcactt  tccagaacat  gaattccaaa
1501  aggaaagcag  aaacctggaa  aagaaataga  cgtcagctag  cttctctcac  agtaggcact

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1561 cctgactaca ttgctcctga ggtgttcatg cagaccgggt acaacaagct ctgtgattgg
1621 tggtcgcttg gggatgatcat gtatgagatg ctcatcggct acccaccttt ctgttctgag
1681 acccctcaag agacatataa gaaggtgatg aactggaaag aaactttgac ttttcctcca
1741 gaagttccca tctctgagaa agccaaggat ctaattttga ggttctgctg tgaatgggaa
1801 catagaattg gagctcctgg agttgaggaa ataaaaagta actctttttt tgaaggcggt
1861 gactgggaac atatcagaga gagacctgct gcaatatcta ttgaaatcaa aagcattgat
1921 gataacctcaa acttcgatga gtttccagaa tctgatattc ttaagccaac agtggccaca
1981 agtaatcatc ctgagactga ctacaagaac aaagactggg tcttcatcaa ctacacgtac
2041 aagcgctttg agggcctgac tgcaaggggg gcaatacctt cctacatgaa agcagcaaaa
2101 catcaccatc accatcacta a
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MOBKL1A Sequence Information

<u>Protein</u>	Human MOBKL1A
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M37 of the recombinant protein is equivalent to M1 of human MOBKL1A
<u>Accession number</u>	GenBank BC038112.1

Recombinant MOBKL1A amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMSFL FGSRSSKTFK PKKNIPEGSH
61 QYELLKHAEA TLGSGNLRMA VMLPEGEDLN EWVAVNTVDF FNQINMLYGT ITDFCTEESC
121 PVMSAGPKYE YHWADGTNIK KPIKCSAPKY IDYLMTWVQD QLDDETLFPS KIGVPFPKNF
181 MSVAKTILKR LFRVYAHYH QHFDPVIQLQ EEHLNLSFK HFIFVQEFN LIDRRELAPL
241 QELIEKLTSK DR

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Recombinant MOBKL1A nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gagcttcttg
121 ttggtagtc gctcttctaa aactttttaa ccaaagaaga acattccaga gggttctcac
181 cagtatgagc tcttaaaaca cgcagaagcc acacttggca gtggcaacct tcggatggct
241 gtcattgctt ctgaagggga agatctcaat gaatgggttg cagttaacac tgtggatttc
301 ttcaatcaga tcaacatgct ttatggaact atcacagact tctgtacaga agagagttgt
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421 aaacctatta agtgctctgc accaaagtat attgattact tgatgacttg ggttcaggac
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601 cagcattttg accctgtgat ccagcttcag gaggaagcac atctaaatac atctttcaag
661 cactttattt tttttgtcca ggaattcaac cttattgata gaagagaact tgcaccactc
721 caagaactga ttgaaaaact cacctcaaaa gacagataa

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