

Certificate of Analysis

NDR2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-028, 16-028-K, 16-028M

Parent Lot # 215688

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 NDR2 amino acids 83-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 72 kDa.

Specific Activity (Parent lot# 215688): 18 U/mg, where one unit of NDR2 activity is defined as 1 nmol phosphate incorporated into 300 μ M RSRSRSRSRSRSR per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 3.12 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 180 mM NaCl, 260 mM Imidazole, 0.09 mM EGTA, 270 mM sucrose, 0.9 mM benzamidine, 0.2 mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

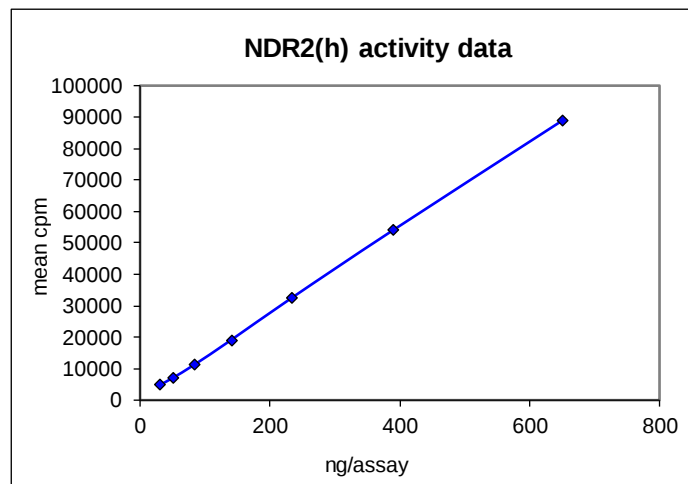
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

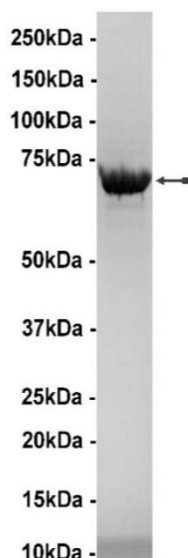
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 30.3–650 ng of this lot of enzyme phosphorylated 300 μ M 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 NDR2 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 NDR2, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA, other components.
- 2. Manganese Chloride (MnCl₂):** Use at a final assay concentration of 10 mM. Prepare a 100 mM stock and add 2.5 µl of stock per assay point.
- 3. RSRSRSRSRSRSR:** Use at a final assay concentration of 300 µM. Prepare a 3 mM stock and add 2.5 µl of stock per assay point.
- 4. Enzyme: NDR2, active** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 30.3–650 ng per assay point.
- 5. [γ-³³P]ATP:** 2.5 x MgAc/[γ-³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 µl of 5 x reaction buffer per assay to wells.
2. Add 2.5 µl of **RSRSRSRSRSRSR**.
3. Add 2.5 µl of manganese chloride.
4. Add **2.5 µl (30.3–650 ng) NDR2, active**.
5. Add 2.5 µl of dH₂O.
6. Add 10 µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 µl of 3% phosphoric acid.
9. Transfer a 10 µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the dried filtermat to a sealable plastic bag and add 4 ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 µl of 30% phosphoric acid.

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NDR2, active Sequence Information

<u>Protein</u>	Human NDR2
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	T231 of the recombinant protein is equivalent to T83 of human NDR2
<u>Accession number</u>	GenBank NM_015000.4

Recombinant NDR2 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 LEVLFQGPEF TRGLDDFES
241 LKVIGRGAFG EVRLVQKQDT GHIYAMKILR KSDMLEKEQV AHIRAERDIL VEADGAWVVK
301 MFYSFQDKRN LYLIMEFLPG GDMMTLLMKK DTLTEEETQF YISETVLAIK AIHQLGFIHR
361 DIKPDNLLLD AKGHVKLSDF GLCTGLKKAH RTEFYRNLTH NPPSDFSQON MNSKRKAETW
421 KKNRRQLAYS TVGTPDYIAP EVFMQTGYNK LCDWWSLGI MYEMLIGYPP FCSETPQETY
481 RKVMNWKETL VFPPEVPISE KAKDLILRFC IDSENRIKNS GVEEIKGHPF FEGVDWEHIR
541 ERPAAPIEIE KSIDDTSNFD DFPESDILQP VPNTTEPDYK SKDWVFLNYT YKRFEGLTQR
601 GSIPTYMKAG KLHHHHHHH
  
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Recombinant NDR2 nucleotide sequence:

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1 atggcaatga cggcagggac tacaacaacc tttcctatga gcaaccatac cccgggaaaga
61 gtgactgtag ccaagctcac attggagaat ttttatagca acctaatttt acagcatgaa
121 gagagagaaa ccaggcagaa gaaattagaa gtggccatgg aagaagaagg atttagcagat
181 gaagagaaaa agttacgtcg atcacaacac gctcgcaaaag aaacagagtt cttacggctc
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1381 gctgggaagt tatga
  
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Reviewed and approved by site quality representative.

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