Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ NDR1

serine/threonine kinase 38

Recombinant Human Active Protein Kinase

HGNC Symbol: STK38

Synonyms: NDR

Product No.: 1775-0000-1

Lot: 001

Description: Human NDR1, full length, amino acids M_1-K_{465} (as in NCBI/Protein entry NP_009202.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: NDR1 Lot001, was confirmed as NDK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 82,280 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: By co-expression of MOB1B

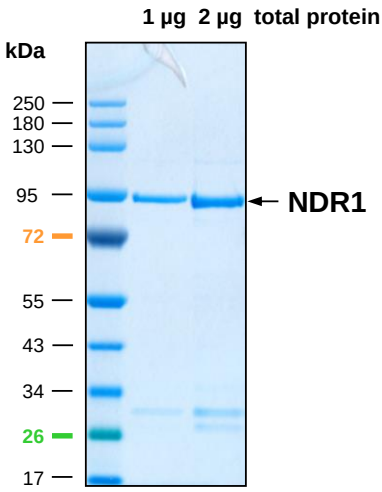
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

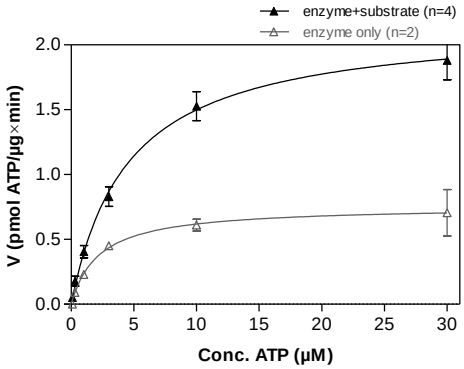
Protein concentration: 0.266 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 2.2 pmol/µg × min
ATP-K_M: 4.5 µM

NDR1 Lot001:
Coomassie stain



NDR1 Lot001:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: RBER-GSK3 80 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
NDR1 Lot 001 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase™ NDR1

Product No.: 1775-0000-1

GST-NDR1 Recombinant Fusion Protein Amino Acid Sequence

```
  1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241  PMAMTGSTPC SSMSNHTKER VTMTKVTLEN FYSNLIAQHE EREMRQKKLE KVMEEEGLKD 300
301  EEKRLRRSAH ARKETEFLRL KRTRLGLEDF ESLKVIGRGA FGEVRLVQKK DTGHVYAMKI 360
361  LRKADMLEKE QVGHIRAERD ILVEADSLWV VKMFYSFQDK LNLYLIMEFL PGGDMMTLLM 420
421  KKDTLTEEET QFYIAETVLA IDSIHQLGFI HRDIKPDNLL LDSKGHVKLS DFGLCTGLKK 480
481  AHRTEFYRNL NHSLPSDFTF QNMNSKRKAE TWKRNRRQLA FSTVGTPDYI APEVFMQTGY 540
541  NKLCDWWSLG VIMYEMLIGY PPFCSETPQE TYKKVMNWKE TLTFPPEVPI SEKAKDLILR 600
601  FCCEWEHRIG APGVEEIKSN SFFEGVDWEH IRERPAAISI EIKSIDDTSN FDEFPESDIL 660
661  KPTVATSNHP ETDYKNKDWV FINYTYKRFE GLTARGAIPS YMKAAK                720
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: NDR1

NDR1 wt[1] Amino Acid Sequence

```
1    MAMTGSTPCS SMSNHTKERV TMTKVTLENF YSNLIAQHEE REMRQKKLEK VMEEEGLKDE  60
61   EKRLRRSAHA RKETEFLRLK RTRLGLEDFE SLKVIGRGAF GEVRLVQKKD TGHVYAMKIL 120
121  RKADMLEKEQ VGHIRAERDI LVEADSLWVV KMFYSFQDKL NLYLIMEFLP GGDMMTLLMK 180
181  KDTLTEEETQ FYIAETVLAI DSIHQLGFIH RDIKPDNLLL DSKGHVKLSD FGLCTGLKKA 240
241  HRTEFYRNLN HSLPSDFTFQ NMNSKRKAET WKRNRRQLAF STVGTPDYIA PEVFMQTGYN 300
301  KLCDWWSLGV IMYEMLIGYP PFCSETPQET YKKVMNWKET LTFPPEVPIS EKAKDLILRF 360
361  CCEWEHRIGA PGVEEIKSNS FFEGVDWEHI RERPAAISIE IKSIDDTSNF DEFPESDILK 420
421  PTVATSNHPE TDYKNKDWVF INYTYKRFEG LTARGAIPSY MKAAK                 480
```

blue: NDR1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_009202.1

Recombinant Proteins