Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ DDR2 N456S

discoidin domain receptor family, member 2

Recombinant Human Active Protein Kinase

HGNC Symbol: DDR2

Synonyms: MIG20a, NTRKR3, TKT, TYRO10

Product No.: 1190-0000-1

Lot: 001

Description: Human DDR2, C-terminal fragment, amino acids R_{422}-E_{855} (as in NCBI/Protein entry NP_006173.2), with a N456S mutation, activated, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: DDR2 N456S Lot 001, was confirmed as DDR2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 77,774 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

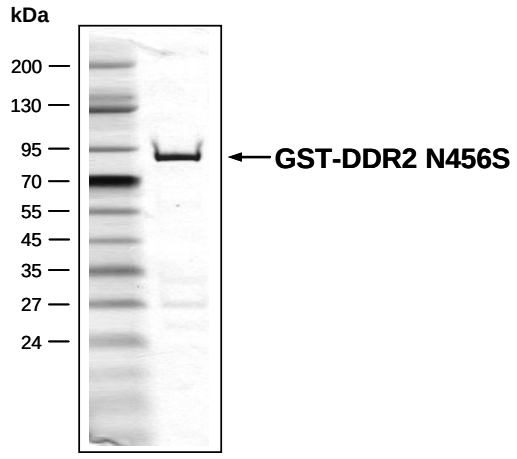
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

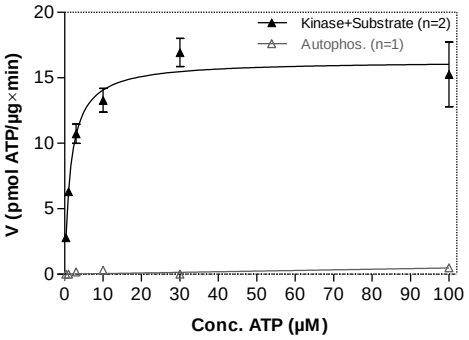
Biochemical Parameters:
Specific kinase activity (P_i transfer): 16 pmol/µg × min
ATP-K_M: 1.6 µM

DDR2 N456S Lot 001:
Coomassie stain



2.0 µg GST-DDR2 N456S

DDR2 N456S Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- 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: TRK-C-derived peptide 80 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
DDR2 N456S 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™ DDR2 N456S

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GST-DDR2 N456S Recombinant Fusion Protein Amino Acid Sequence

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241 PLAMLRQFWQ KMLEKASRRM LDDEMTVSLS LPSDSSMFNS NRSSSPSEQG SNSTYDRIFP 300
301 LRPDYQEPSR LIRKLPEFAP GEEESGCSGV VKPVQPSGPE GVPHYAEADI VNLQGVTGGN 360
361 TYSVPAVTMD LLSGKDVAVE EFPRKLLTFK EKLGEGQFGE VHLCEVEGME KFKDKDFALD 420
421 VSANQPVLVA VKMLRADANK NARNDFLKEI KIMSRLKDPN IIHLLAVCIT DDPLCMITEY 480
481 MENGDLNQFL SRHEPPNSSS SDVRTVSYTN LKFMATQIAS GMKYLSSLNF VHRDLATRNC 540
541 LVGKNYTIKI ADFGMSRNLY SGDYYRIQGR AVLPIRWMSW ESILLGKFTT ASDVWAFGVT 600
601 LWETFTFCQE QPYSQLSDEQ VIENTGEFFR DQGRQTYLPQ PAICPDSVYK LMLSCWRRDT 660
661 KNRPSFQEIH LLLLQQGDE                                            720
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: DDR2 fragment **boxed**: N456S

DDR2 wt[1] Amino Acid Sequence

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  1 MILIPRMLLV LFLLLPILSS AKAQVNPAIC RYPLGMSGGQ IPDEDITASS QWSESTAAKY  60
 61 GRLDSEEGDG AWCPEIPVEP DDLKEFLQID LHTLHFITLV GTQGRHAGGH GIEFAPMYKI 120
121 NYSRDGTRWI SWRNRHGKQV LDGNSNPYDI FLKDLEPPIV ARFVRFIPVT DHSMNVCMRV 180
181 ELYGCVWLDG LVSYNAPAGQ QFVLPGGSII YLNDSVYDGA VGYSMTEGLG QLTDGVSGLD 240
241 DFTQTHEYHV WPGYDYVGWR NESATNGYIE IMFEFDRIRN FTTMKVHCNN MFAKGVKIFK 300
301 EVQCYFRSEA SEWEPNAISF PLVLDDVNPS ARFVTVPLHH RMASAIKCQY HFADTWMMFS 360
361 EITFQSDAAM YNNSEALPTS PMAPTTYDPM LKVDDSNTRI LIGCLVAIIF ILLAIIVIIL 420
421 WRQFWQKMLE KASRRMLDDE MTVSLSLPSD SSMFNNNRSS SPSEQGSNST YDRIFPLRPD 480
481 YQEPSRLIRK LPEFAPGEEE SGCSGVVKPV QPSGPEGVPH YAEADIVNLQ GVTGGNTYSV 540
541 PAVTMDLLSG KDVAVEEFPR KLLTFKEKLG EGQFGEVHLC EVEGMEKFKD KDFALDVSAN 600
601 QPVLVAVKML RADANKNARN DFLKEIKIMS RLKDPNIIHL LAVCITDDPL CMITEYMENG 660
661 DLNQFLSRHE PPNSSSSDVR TVSYTNLKFM ATQIASGMKY LSSLNFVHRD LATRNCLVGK 720
721 NYTIKIADFG MSRNLYSGDY YRIQGRAVLP IRWMSWESIL LGKFTTASDV WAFGVTLWET 780
781 FTFCQEQPYS QLSDEQVIEN TGEFFRDQGR QTYLPQPAIC PDSVYKLMLS CWRRDTKNRP 840
841 SFQEIHLLLL QQGDE                                                900
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blue: DDR2 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_006173.2

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