Certificate of Analysis

ProQinase
TARGETING CANCER

CDC42BPB

CDC42-binding protein kinase beta

Recombinant Human Active Protein Kinase

HGNC Symbol: CDC42BPB

Synonyms: MRCKB, DMPK-like

Product No.: 0382-0000-1

Lot: 001

Description: Human CDC42BPB, N-terminal fragment, amino acids M_1-H_{472} (as in NCBI/Protein entry NP_006026.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CDC42BPB Lot 001, was confirmed as CDC42BPB by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 84,444 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

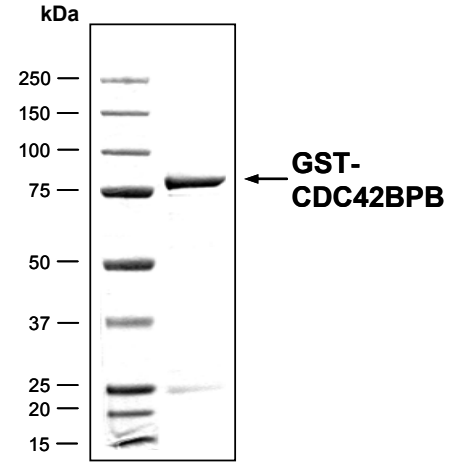
Storage buffer: 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

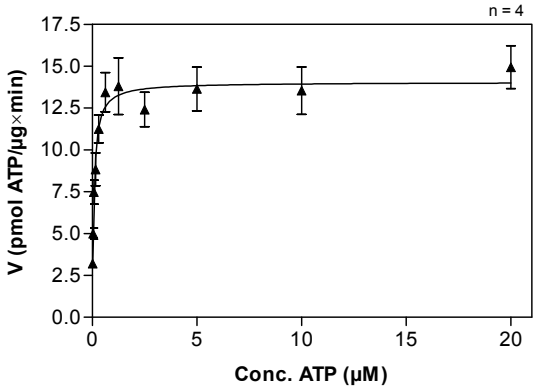
Biochemical Parameters:
Specific kinase activity (P_i transfer): 14 pmol/µg×min
ATP-K_M: 0.1 µM

CDC42BPB Lot 001:
Coomassie stain



2.0 µg GST-CDC42BPB

CDC42BPB 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: INSRR-derived peptide 20 µg/ml
  CDC42BPB: 1.0 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

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CDC42BPB 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 RRRASVAAGI  240
241  LVPRGSPGLD GIYARGIQAS MSAKVRLKKL EQLLLDGPWR NESALSVETL LDVLVCLYTE  300
301  CSHSALRRDK YVAEFLEWAK PFTQLVKEMQ LHREDFEIIK VIGRGAFGEV AVVKMKNTER  360
361  IYAMKILNKW EMLKRAETAC FREERDVLVN GDCQWITALH YAFQDENHLY LVMDYYVGGD  420
421  LLTLLSKFED KLPEDMARFY IGEMVLAIDS IHQLHYVHRD IKPDNVLLDV NGHIRLADFG  480
481  SCLKMNDDGT VQSSVAVGTP DYISPEILQA MEDGMGKYGP ECDWWSLGVC MYEMLYGETP  540
541  FYAESLVETY GKIMNHEERF QFPSHVTDVS EEAKDLIQRL ICSRERRLGQ NGIEDFKKHA  600
601  FFEGLNWENI RNLEAPYIPD VSSPSDTSNF DVDDDVLRNT EILPPGSHTG FSGLHLPFIG  660
661  FTFTTESCFS DRGSLKSIMQ SNTLTKDEDV QRDLEHSLQM EAYERRIRRL EQEKLELSRK  720
721  LQESTQTVQS LH                                                      780
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1-218: GST **Red:** HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDC42BPB fragment

CDC42BPB wt[1] Amino Acid Sequence

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  1  MSAKVRLKKL EQLLLDGPWR NESALSVETL LDVLVCLYTE CSHSALRRDK YVAEFLEWAK  60
 61  PFTQLVKEMQ LHREDFEIIK VIGRGAFGEV AVVKMKNTER IYAMKILNKW EMLKRAETAC  120
121  FREERDVLVN GDCQWITALH YAFQDENHLY LVMDYYVGGD LLTLLSKFED KLPEDMARFY  180
181  IGEMVLAIDS IHQLHYVHRD IKPDNVLLDV NGHIRLADFG SCLKMNDDGT VQSSVAVGTP  240
241  DYISPEILQA MEDGMGKYGP ECDWWSLGVC MYEMLYGETP FYAESLVETY GKIMNHEERF  300
301  QFPSHVTDVS EEAKDLIQRL ICSRERRLGQ NGIEDFKKHA FFEGLNWENI RNLEAPYIPD  360
361  VSSPSDTSNF DVDDDVLRNT EILPPGSHTG FSGLHLPFIG FTFTTESCFS DRGSLKSIMQ  420
421  SNTLTKDEDV QRDLEHSLQM EAYERRIRRL EQEKLELSRK LQESTQTVQS LHGSSRALSN  480
481  SNRDKEIKKL NEEIERLKNK IADSNRLERQ LEDTVALRQE REDSTQRLRG LEKQHRVVRQ  540
541  EKEELHKQLV EASERLKSQA KELKDAHQQR KLALQEFSEL NERMAELRAQ KQKVSRQLRD  600
601  KEEEMEVATQ KVDAMRQEMR RAEKLRKELE AQLDDAVAEA SKERKLREHS ENFCKQMESE  660
661  LEALKVKQGG RGAGATLEHQ QEISKIKSEL EKKVLFYEEE LVRREASHVL EVKNVKKEVH  720
721  DSESHQLALQ KEILMLKDKL EKSKRERHNE MEEAVGTIKD KYERERAMLF DENKKLTAEN  780
781  EKLCSFVDKL TAQNRQLEDE LQDLAAKKES VAHWEAQIAE IIQWVSDEKD ARGYLQALAS  840
841  KMTEELEALR SSSLGSRTLD PLWKVRRSQK LDMSARLELQ SALEAEIRAK QLVQEELRKV  900
901  KDANLTLESK LKDSEAKNRE LLEEMEILKK KMEEKFRADT GLKLPDFQDS IFEYFNTAPL  960
961  AHDLTFRTSS ASEQETQAPK PEASPSMSVA ASEQQEDMAR PPQRPSAVPL PTTQALALAG  1020
1021 PKPKAHQFSI KSFSSPTQCS HCTSLMVGLI RQGYACEVCS FACHVSCKDG APQVCPIPPE  1080
1081 QSKRPLGVDV QRGIGTAYKG HVKVPKPTGV KKGWQRAYAV VCECKLFLYD LPEGKSTQPG  1140
1141 VIASQVLDLR DDEFSVSSVL ASDVIHATRR DIPCIFRVTA SLLGAPSKTS SLLILTENEN  1200
1201 EKRKWVGILE GLQSILHKNR LRNQVVHVPL EAYDSSLPLI KAILTAAIVD ADRIAVGLEE  1260
1261 GLYVIEVTRD VIVRAADCKK VHQIELAPRE KIVILLCGRN HHVHLYPWSS LDGAEGSFDI  1320
1321 KLPETKGCQL MATATLKRNS GTCLFVAVKR LILCYEIQRT KPFHRKFNEI VAPGSVQCLA  1380
1381 VLRDRLCVGY PSGFCLLSIQ GDGQPLNLVN PNDPSLAFLS QQSFDALCAV ELESEEYLLC  1440
1441 FSHMGLYVDP QGRRARAQEL MWPAAPVACS CSPTHVTVYS EYGVDVFDVR TMEWVQTIGL  1500
1501 RRIRPLNSEG TLNLLNCEPP RLIYFKSKFS GAVLNVPDTS DNSKKQMLRT RSKRRFVFKV  1560
1561 PEEERLQQRR EMLRDPELRS KMISNPTNFN HVAHMGPGDG MQVLMDLPLS AVPPSQEERP  1620
1621 GPAPTNLARQ PPSRNKPYIS WPSSGGSEPS VTVPLRSMSD PDQDFDKEPD SDSTKHSTPS  1680
1681 NSSNPSGPPS PNSPHRSQLP LEGLEQPACD T                                  1740
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blue: CDC42BPB sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_006026.2