

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TcPINK1 [1 – 570]

<u>Enzyme description:-</u>	TcPINK1 [1 – 570]
<u>Clone number:-</u>	DU 34701
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal Maltose Binding Protein (MBP)
<u>Purification method:-</u>	Amylose agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	108, 129.50 daltons
Average Mass	108, 197.55 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.04
<u>Purity:-</u>	85 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine	
<u>Storage temperature:-</u>	–70 °C
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
GST-PARK2 (1 – 108) [DU 37370]	

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Clone Data Sheet

TcPINK1 [1 – 570]

<u>Protein</u>	TcPINK1 [1 - 570]
<u>Clone number</u>	DU 34701
<u>Species</u>	<i>Tribolium castaneum</i>
<u>Accession number</u>	XM_963274
<u>Tags</u>	N-terminal MBP
<u>Bacterially expressed protein</u>	MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLE EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIK DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYEELVKD PRIAATMENAQKGEIMPNIPOMSAFWYAVRTAVINAASGRQTVDE ALKDAQTNSSSSNNNNNNNNNNLGDGDDDKVPEFLEVLFGQPGSMSV RAVGSRLFKHGRSLIQQFCRDLNTTIGDKINAVSQATAAPSSLP KTQIPKNFALRNVGVQLGLQARRILIDNVLRVTNSLSAELRKA TRRILFGDSAPFFALVGVSIASTGILTKEEELEGVCWEIREAIS KIKWQYYDIDESRFESNPITLNDLSLGKPIAKGTNGVVYSKVKD DETDDNKYPFALKMMFNVDIQNSMEILKAMYRETVPARMYYSNH DLNNWEIELANRRKHLPPHPNIVAIFSVFTDLIQELEGSKDLPA ALPPRLHPEGEGRNMSLFLMKRYDCNLQSFLSTAPSTRTSLLL AQLLEGVAHMTAHGIAHRDLKSDNLLLDLTSEPESPIVISDFGCC LADKTNGLSLPYTSYEMDKGGNTALMAPEIICQKPGTFSVLNYSK ADLWAVGAIAYEIFNCHNPFYGPSRLKNFNKYGEDLPKLPDEVPT VIQALVANLLKRNPKNRLDPEVAANVCQLFLWAPSTWLKPKLKP TSGEILQWLLSLTTKVLCEGKINNKSFGKFTRNWRRTYPEYLLI SSFLCRAKLANVRNALHWIQENLPELD
<u>Native sequence</u>	Amino acids M1 – D570 (end) of <i>Tribolium castaneum</i> PINK1. Residue M403 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 - 392.
<u>Protease cleavage</u>	PreScission (LEVLFQGP) residues 392 - 400
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pMal (modified)

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Nucleotide sequence of insert

ggatccATGAGCGTCCGTGCAGTTGGCAGCCGCCTGTTCAAACAT
GGCCGTAGCCTGATCCAGCAGTTCTGTAAACGTGACCTGAATACC
ACCATTGGCGATAAAATCAATGCCGTGAGCCAGGCAACCGCCGCA
CCGAGCTCTCTGCCGAAAACCCAAATTCCGAAAAACTTTGCCCTG
CGTAATGTCGGCGTGCAGCTGGGTCTGCAAGCACGTCGCATTCTG
ATCGATAACGTCCTGAATCGTGTGACCAATTCACTGTCTGGCCGAA
CTGCGCAAAAAAGCAACGCGTCGCATCCTGTTCTGGCGACAGCGCA
CCGTTTTTTCGCTCTGGTTGGTGTCTAGTATTGCCTCCGGCACCGGT
ATCCTGACGAAAGAAGAAGAACTGGAAGGCGTTTGCTGGGAAATC
CGTGAAGCGATCTCTAAATCAAATGGCAGTACTACGATATCGAC
GAAAGCCGCTTTGAATCTAACCCGATCACCTGAATGATCTGAGT
CTGGGTAAACCGATCGCCAAAGGCACGAACGGTGTGGTTTACTCC
GCAAAAGTCAAAGATGACGAAACCGATGACAATAAATATCCGTTT
GCCCTGAAAATGATGTTCAACTACGATATTCAGAGCAATTCTATG
GAAATCCTGAAAGCTATGTATCGTGAAACCGTTCCGGCGCGCATG
TATTACTCAAACCACGACCTGAACAATTGGGAAATTGAACTGGCT
AACCGTCGAAACATCTGCCGCCGCACCCGAATATTGTGGCAATC
TTTAGCGTTTTTCACCGATCTGATCCAAGAACTGGAAGGTTCTAAA
GACCTGTATCCGGCAGCGCTGCCGCCGCGTCTGCATCCGGAAGGT
GAAGGTCGCAACATGAGCCTGTTTCTGCTGATGAAACGTTATGAT
TGTAATCTGCAGTCATTCTGAGCACCGCTCCGAGTACCCGCACC
TCCCTGCTGCTGCTGGCCCAACTGCTGGAAGGCGTGGCGCATATG
ACGGCCCACGGTATTGCACATCGTGATCTGAAATCAGACAACCTG
CTGCTGGATACCTCAGAACCGGAATCGCCGATTCTGGTTATCTCG
GATTTTGGCTGCTGTCTGGCGGACAAAACCAACGGTCTGAGCCTG
CCGTATACGTCTTACGAAATGGATAAAGGCGGTAAATACGGCGCTG
ATGGCCCCGGAAATTATCTGCCAGAAACCGGGCACCTTTAGTGTC
CTGAATTATTCCAAAGCTGACCTGTGGGCGGTGGGTGCAATCGCT
TACGAAATCTTCAACTGTCACAATCCGTTCTACGGCCCGTCTCGC
CTGAAAAACTTCAACTACAAAGAAGGTGATCTGCCGAAACTGCCG
GACGAAGTGCCGACCGTTATTACGGCACTGGTGGCTAACCTGCTG
AAACGTAACCCGAATAAACGCCTGGACCCGGAAGTTGCGGCCAAT
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GGTCTGAAAGTTCCGACGTCAGGTGAAATCTGCAATGGCTGCTG
TCGCTGACCACGAAAGTCCTGTGCGAAGGCAAAATCAACAACAAA
TCCTTCGGTGAAAAATTACCCGTAATTGGCGTCGCACGTATCCG
GAATACCTGCTGATTAGTTCCTTCCTGTGTCGCGCGAAACTGGCT
AATGTTGTAATGCTCTGCACTGGATTCAAGAAAACCTGCCGGAA
CTGGATtaagcggccgc