Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TcPINK1 [1 – 570]

Enzyme description:- TcPINK1 [1 – 570]

Clone number:- DU 34701

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal Maltose Binding Protein (MBP)

Purification method:- Amylose agarose

Calculated molecular mass:-
Monoisotopic 108, 129.50 daltons
Average Mass 108, 197.55 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.04

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
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

Storage temperature:- –70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
GST-PARK2 (1 – 108) [DU 37370]

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Clone Data Sheet

TcPINK1 [1 – 570]

Protein	TcPINK1 [1 - 570]
Clone number	DU 34701
Species	*Tribolium castaneum*
Accession number	XM_963274
Tags	N-terminal MBP
Bacterially expressed protein	MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLE EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIK DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVKD PRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDE ALKDAQTNSSSNNNNNNNNNLGDDDDKVPEFLEVLFQGPGS**MSV** **RAVGSRLFKHGRSLIQQFCKRDLNTTIGDKINAVSQATAAPSSLP** **KTQIPKNFALRNVGVQLGLQARRILIDNVLNRVTNSLSAELRKKA** **TRRILFGDSAPFFALVGVSIASGTGILTKEEELEGVCWEIREAIS** **KIKWQYYDIDESRFESNPITLNDLSLGKPIAKGTNGVVYSAKVKD** **DETDDNKYPFALKMMFNYDIQSNSMEILKAMYRETVPARMYYSNH** **DLNNWEIELANRRKHLPPHPNIVAIFSVFTDLIQELEGSKDLYPA** **ALPPRLHPEGEGRNMSLFLLMKRYDCNLQSFLSTAPSTRTSLLLL** **AQLLEGVAHMTAHGIAHRDLKSDNLLLDTSEPESPILVISDFGCC** **LADKTNGLSLPYTSYEMDKGGNTALMAPEIICQKPGTFSVLNYSK** **ADLWAVGAIAYEIFNCHNPFYGPSRLKNFNYKEGDLPKLPDEVPT** **VIQALVANLLKRNPNKRLDPEVAANVCQLFLWAPSTWLKPGLKVP** **TSGEILQWLLSLTTKVLCEGKINNKSFGEKFTRNWRRTYPEYLLI** **SSFLCRAKLANVRNALHWIQENLPELD**
Native sequence	Amino acids M1 – D570 (end) of *Tribolium castaneum* PINK1. Residue M403 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 - 392.
Protease cleavage	PreScission (LEVLFQGP) residues 392 - 400
Cloning sites	*Bam*H1 and *Not*I sites of pMal (modified)

Nucleotide sequence of insert

ggatccATGAGCGTCCGTGCAGTTGGCAGCCGCCTGTTCAAACAT
GGCCGTAGCCTGATCCAGCAGTTCTGTAAACGTGACCTGAATACC
ACCATTGGCGATAAAATCAATGCCGTGAGCCAGGCAACCGCCGCA
CCGAGCTCTCTGCCGAAAACCCAAATTCCGAAAAACTTTGCCCTG
CGTAATGTCGGCGTGCAGCTGGGTCTGCAAGCACGTCGCATTCTG
ATCGATAACGTCCTGAATCGTGTGACCAATTCACTGTCGGCCGAA
CTGCGCAAAAAAGCAACGCGTCGCATCCTGTTCGGCGACAGCGCA
CCGTTTTTCGCTCTGGTTGGTGTCAGTATTGCCTCCGGCACCGGT
ATCCTGACGAAAGAAGAAGAACTGGAAGGCGTTTGCTGGGAAATC
CGTGAAGCGATCTCTAAAATCAAATGGCAGTACTACGATATCGAC
GAAAGCCGCTTTGAATCTAACCCGATCACCCTGAATGATCTGAGT
CTGGGTAAACCGATCGCCAAAGGCACGAACGGTGTGGTTTACTCC
GCAAAAGTCAAAGATGACGAAACCGATGACAATAAATATCCGTTT
GCCCTGAAAATGATGTTCAACTACGATATTCAGAGCAATTCTATG
GAAATCCTGAAAGCTATGTATCGTGAAACCGTTCCGGCGCGCATG
TATTACTCAAACCACGACCTGAACAATTGGGAAATTGAACTGGCT
AACCGTCGCAAACATCTGCCGCCGCACCCGAATATTGTGGCAATC
TTTAGCGTTTTCACCGATCTGATCCAAGAACTGGAAGGTTCTAAA
GACCTGTATCCGGCAGCGCTGCCGCCGCGTCTGCATCCGGAAGGT
GAAGGTCGCAACATGAGCCTGTTTCTGCTGATGAAACGTTATGAT
TGTAATCTGCAGTCATTCCTGAGCACCGCTCCGAGTACCCGCACC
TCCCTGCTGCTGCTGGCCCAACTGCTGGAAGGCGTGGCGCATATG
ACGGCCCACGGTATTGCACATCGTGATCTGAAATCAGACAACCTG
CTGCTGGATACCTCAGAACCGGAATCGCCGATTCTGGTTATCTCG
GATTTTGGCTGCTGTCTGGCGGACAAAACCAACGGTCTGAGCCTG
CCGTATACGTCTTACGAAATGGATAAAGGCGGTAATACGGCGCTG
ATGGCCCCGGAAATTATCTGCCAGAAACCGGGCACCTTTAGTGTC
CTGAATTATTCCAAAGCTGACCTGTGGGCGGTGGGTGCAATCGCT
TACGAAATCTTCAACTGTCACAATCCGTTCTACGGCCCGTCTCGC
CTGAAAAACTTCAACTACAAAGAAGGTGATCTGCCGAAACTGCCG
GACGAAGTGCCGACCGTTATTCAGGCACTGGTGGCTAACCTGCTG
AAACGTAACCCGAATAAACGCCTGGACCCGGAAGTTGCGGCCAAT
GTCTGCCAGCTGTTTCTGTGGGCACCGAGTACCTGGCTGAAACCG
GGTCTGAAAGTTCCGACGTCAGGTGAAATTCTGCAATGGCTGCTG
TCGCTGACCACGAAAGTCCTGTGCGAAGGCAAAATCAACAACAAA
TCCTTCGGTGAAAAATTCACCCGTAATTGGCGTCGCACGTATCCG
GAATACCTGCTGATTAGTTCCTTCCTGTGTCGCGCGAAACTGGCT
AATGTTCGTAATGCTCTGCACTGGATTCAAGAAAACCTGCCGGAA
CTGGATtaagcggccgc