

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MINK1 [1 - 320]

<u>Enzyme description:-</u>	MINK1 [1 - 320]
<u>Clone number:-</u>	DU 19081
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 63, 174.33 daltons
Average Mass 63, 214.95 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.25

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate

Substrate:-

MBP Final concentration: 0.3 mg/ml

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Clone Data Sheet

MINK1 [1 - 320]

<u>Protein</u>	MINK1 [1 - 320]
<u>Clone number</u>	DU 19081
<u>Species</u>	Human
<u>Accession number</u>	NM_015716
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEELHYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMGDPAPARSLDDID LSALRDPAGIFELVEVVGNGTYGQVYKGRHVKTGQLAAIKVMDVTEDEE EEIKQEI NMLKKYSHHRNIATYYGAFIKKSPPGNDDQLWLVMEFCGAGS VTDLVKNTKGNALKEDCIAYICREILRGLAHLHAHKVIHRDIKQONVLL TENAEVKLVDFGVSAQLDRTVGRRTFIGTPYWMapeviACDENPDATY DYRSDIWSLGITAIEMAEGAPPLCDMHPMRALFLIPRNP PRLKSKKWS KKFIDFIDTCLIKTYLSRPPT EQLLKFPFIRDQPTERQVRIQLKDHIR SRKKRGEKEETE</p>
<u>Native sequence</u>	<p>Amino acids M1 – E320 (end W1295) of human MINK1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I site of pFastBac GST

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Nucleotide
Sequence of insert

ggatccATGGGCGACCCAGCCCCGCCCCGAGCCTGGACGACATCGACC
TGTCCGCCCTGCGGGACCCTGCTGGGATCTTTGAGCTTGTGGAGGTGGT
CGGCAATGGAACCTACGGACAGGTGTACAAGGGTCGGCATGTCAAGACG
GGCAGCTGGCTGCCATCAAGGTCATGGATGTCACGGAGGACGAGGAGG
AAGAGATCAAACAGGAGATCAACATGCTGAAAAAGTACTCTCACCACCG
CAACATCGCCACCTACTACGGAGCCTTCATCAAGAAGAGCCCCCGGGA
AACGATGACCAGCTCTGGCTGGTGATGGAGTTCTGTGGTGTCTGGTTTCAG
TGACTGACCTGGTAAAGAACACAAAAGGCAACGCCCTGAAGGAGGACTG
TATCGCCTATATCTGCAGGGAGATCCTCAGGGGTCTGGCCATCTCCAT
GCCACAAGGTGATCCATCGAGACATCAAGGGCAGAATGTGCTGCTGA
CAGAGAATGCTGAGGTCAAGCTAGTGGATTTTGGGGTGAGTGCTCAGCT
GGACCGCACCGTGGGCAGACGGAACACTTTTCATTGGGACTCCCTACTGG
ATGGCTCCAGAGGTCATCGCCTGTGATGAGAACCCTGATGCCACCTATG
ATTACAGGAGTGATATTTGGTCTCTAGGAATCACAGCCATCGAGATGGC
AGAGGGAGCCCCCCTCTGTGTGACATGCACCCCATGCGAGCCCTCTTC
CTCATTCCTCGGAACCCTCCGCCAGGCTCAAGTCCAAGAAGTGGTCTA
AGAAGTTCATTGACTTCATTGACACATGTCTCATCAAGACTTACCTGAG
CCGCCACCCACGGAGCAGCTACTGAAGTTTCCCTTCATCCGGGACCAG
CCCACGGAGCGGCAGGTCCGCATCCAGCTTAAGGACCACATTGACCGAT
CCCGGAAGAAGCGGGGTGAGAAAGAGGAGACAGAAtgagcggccgc