Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MINK1 [1 - 320]

Enzyme description:- MINK1 [1 - 320]

Clone number:- DU 19081

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 3 mg/L

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:- Standard filter binding assay

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

Specific activity range:- To be determined

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Clone Data Sheet

MINK1 [1 - 320]

Protein	MINK1 [1 - 320]
Clone number	DU 19081
Species	Human
Accession number	NM_015716
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MGDPAPARSLDDID LSALRDPAGIFELVEVVGNGTYGQVYKGRHVKTGQLAAIKVMDVTEDEE EEIKQEINMLKKYSHHRNIATYYGAFIKKSPPGNDDQLWLVMEFCGAGS VTDLVKNTKGNALKEDCIAYICREILRGLAHLHAHKVIHRDIKGQNVLL TENAEVKLVDFGVSAQLDRTVGRRNTFIGTPYWMAPEVIACDENPDATY DYRSDIWSLGITAIEMAEGAPPLCDMHPMRALFLIPRNPPPRLKSKKWS KKFIDFIDTCLIKTYLSRPPTEQLLKFPFIRDQPTERQVRIQLKDHIDR SRKKRGEKEETE**
Native sequence	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.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1site of pFastBac GST

Nucleotide Sequence of insert

ggatccATGGGCGACCCAGCCCCCGCCCGCAGCCTGGACGACATCGACC
TGTCCGCCCTGCGGGACCCTGCTGGGATCTTTGAGCTTGTGGAGGTGGT
CGGCAATGGAACCTACGGACAGGTGTACAAGGGTCGGCATGTCAAGACG
GGGCAGCTGGCTGCCATCAAGGTCATGGATGTCACGGAGGACGAGGAGG
AAGAGATCAAACAGGAGATCAACATGCTGAAAAAGTACTCTCACCACCG
CAACATCGCCACCTACTACGGAGCCTTCATCAAGAAGAGCCCCCCGGGA
AACGATGACCAGCTCTGGCTGGTGATGGAGTTCTGTGGTGCTGGTTCAG
TGACTGACCTGGTAAAGAACACAAAAGGCAACGCCCTGAAGGAGGACTG
TATCGCCTATATCTGCAGGGAGATCCTCAGGGGTCTGGCCCATCTCCAT
GCCCACAAGGTGATCCATCGAGACATCAAGGGGCAGAATGTGCTGCTGA
CAGAGAATGCTGAGGTCAAGCTAGTGGATTTTGGGGTGAGTGCTCAGCT
GGACCGCACCGTGGGCAGACGGAACACTTTCATTGGGACTCCCTACTGG
ATGGCTCCAGAGGTCATCGCCTGTGATGAGAACCCTGATGCCACCTATG
ATTACAGGAGTGATATTTGGTCTCTAGGAATCACAGCCATCGAGATGGC
AGAGGGAGCCCCCCCTCTGTGTGACATGCACCCCATGCGAGCCCTCTTC
CTCATTCCTCGGAACCCTCCGCCCAGGCTCAAGTCCAAGAAGTGGTCTA
AGAAGTTCATTGACTTCATTGACACATGTCTCATCAAGACTTACCTGAG
CCGCCCACCCACGGAGCAGCTACTGAAGTTTCCCTTCATCCGGGACCAG
CCCACGGAGCGGCAGGTCCGCATCCAGCTTAAGGACCACATTGACCGAT
CCCGGAAGAAGCGGGGTGAGAAAGAGGAGACAGAAtgagcggccgc