Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Mixed Lineage Kinase 1 [132 - 413]

Enzyme description:- MLK1 [132 - 413]

Clone number:- DU 15482

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 58, 295.96 daltons
Average Mass 58, 333.68 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.53

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

MLK1 [132 - 413]

Protein	MLK1 [132 - 413]
Clone number	DU 15482
Species	Human
Accession number	NM_033141
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PPIQLLEIDFAELT** **LEEIIGIGGFGKVYRAFWIGDEVAVKAARHDPDEDISQTIENVRQEAKL** **FAMLKHPNIIALRGVCLKEPNLCLVMEFARGGPLNRVLSGKRIPPDILV** **NWAVQIARGMNYLHDEAIVPIIHRDLKSSNILILQKVENGDLSNKILKI** **TDFGLAREWHRTTKMSAAGTYAWMAPEVIRASMFSKGSDVWSYGVLLWE** **LLTGEVPFRGIDGLAVAYGVAMNKLALPIPSTCPEPFAKLMEDCWNPDP** **HSRPSFTNILDQLTTIEESGFFE**
Native sequence	Amino acids P132 – E413 (end S1118) of human MLK1. Residue P232 of the fusion protein is equivalent to P132 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1site of pFastBac GST

Nucleotide Sequence of insert

ggatccCCGCCCATTCAGTTGTTAGAAATTGATTTTGCGGAGCTCACCT
TGGAAGAGATTATTGGCATCGGGGGCTTTGGGAAGGTCTATCGTGCTTT
CTGGATAGGGGATGAGGTTGCTGTGAAAGCAGCTCGCCACGACCCTGAT
GAGGACATCAGCCAGACCATAGAGAATGTTCGCCAAGAGGCCAAGCTCT
TCGCCATGCTGAAGCACCCCAACATCATTGCCCTAAGAGGGGTATGTCT
GAAGGAGCCCAACCTCTGCTTGGTCATGGAGTTTGCTCGTGGAGGACCT
TTGAATAGAGTGTTATCTGGGAAAAGGATTCCCCCAGACATCCTGGTGA
ATTGGGCTGTGCAGATTGCCAGAGGGATGAACTACTTACATGATGAGGC
AATTGTTCCCATCATCCACCGCGACCTTAAGTCCAGCAACATATTGATC
CTCCAGAAGGTGGAGAATGGAGACCTGAGCAACAAGATTCTGAAGATCA
CTGATTTTGGCCTGGCTCGGGAATGGCACCGAACCACCAAGATGAGTGC
GGCAGGGACGTATGCTTGGATGGCACCCGAAGTCATCCGGGCCTCCATG
TTTTCCAAAGGCAGTGATGTGTGGAGCTATGGGGTGCTACTTTGGGAGT
TGCTGACTGGTGAGGTGCCCTTTCGAGGCATTGATGGCTTAGCAGTCGC
TTATGGAGTGGCCATGAACAAACTCGCCCTTCCTATTCCTTCTACGTGC
CCAGAACCTTTTGCCAAACTCATGGAAGACTGCTGGAATCCTGATCCCC
ACTCACGACCATCTTTCACGAATATCCTGGACCAGCTAACCACCATAGA
GGAGTCTGGTTTCTTTGAAtaagcggccgc