Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MLK3 [96 - 386]

Enzyme description:- MLK3 [96 - 386]

Clone number:- DU 8313

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 60, 759.92 daltons
Average Mass 60, 799.30 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.74

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

MLK3 [96 - 386]

Protein	MLK3 [96 - 386]
Clone number	DU 8313
Species	Human
Accession number	NM_002419
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWR NKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGG CPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKL PEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPM CLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATF GGGDHPPKSDLEVLFQGPLGSPGIGGGGGI**PSNYVSRGGGPP** **PCEVASFQELRLEEVIGIGGFGKVYRGSWRGELVAVKAARQD** **PDEDISVTAESVRQEARLFAMLAHPNIIALKAVCLEEPNLCL** **VMEYAAGGPLSRALAGRRVPPHVLVNWAVQIARGMHYLHCEA** **LVPVIHRDLKSNNILLLQPIESDDMEHKTLKITDFGLAREWH** **KTTQMSAAGTYAWMAPEVIKASTFSKGSDVWSFGVLLWELLT** **GEVPYRGIDCLAVAYGVAVNKLTLPIPSTCPEPFAQLMADCW** **AQDPHRRPDFASILQQLEALEAQVLREMPRDSFHSMQ**
Native sequence	Amino acids P96 – Q386 (end P847) of human MLK3. Residue P of the fusion protein is equivalent to P96 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

ggatcccccggtattggaggaggggcggtattCCGTCCAACTATGTGT
CTCGGGGTGGCGGCCCGCCCCCCTGCGAGGTGGCCAGCTTCCAGGAGCT
GCGGCTGGAGGAGGTGATCGGCATTGGAGGCTTTGGCAAGGTGTACAGG
GGCAGCTGGCGAGGTGAGCTGGTGGCTGTGAAGGCAGCTCGCCAGGACC
CCGATGAGGACATCAGTGTGACAGCCGAGAGCGTTCGCCAGGAGGCCCG
GCTCTTCGCCATGCTGGCACACCCCAACATCATTGCCCTCAAGGCTGTG
TGCCTGGAGGAGCCCAACCTGTGCCTGGTGATGGAGTATGCAGCCGGTG
GGCCCCTCAGCCGAGCTCTGGCCGGGCGGCGCGTGCCTCCCCATGTGCT
GGTCAACTGGGCTGTGCAGATTGCCCGTGGGATGCACTACCTGCACTGC
GAGGCCCTGGTGCCCGTCATCCACCGTGATCTCAAGTCCAACAACATTT
TGCTGCTGCAGCCCATTGAGAGTGACGACATGGAGCACAAGACCCTGAA
GATCACCGACTTTGGCCTGGCCCGAGAGTGGCACAAAACCACACAAATG
AGTGCCGCGGGCACCTACGCCTGGATGGCTCCTGAGGTTATCAAGGCCT
CCACCTTCTCTAAGGGCAGTGACGTCTGGAGTTTTGGGGTGCTGCTGTG
GGAACTGCTGACCGGGGAGGTGCCATACCGTGGCATTGACTGCCTTGCT
GTGGCCTATGGCGTAGCTGTTAACAAGCTCACACTGCCCATCCCATCCA
CCTGCCCCGAGCCCTTCGCACAGCTTATGGCCGACTGCTGGGCGCAGGA
CCCCCACCGCAGGCCCGACTTCGCCTCCATCCTGCAGCAGTTGGAGGCG
CTGGAGGCACAGGTCCTACGGGAAATGCCGCGGGACTCCTTCCATTCCA
TGCAGtaagcggccgc