

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SAPK2a [1 - 360] T106M

Enzyme description:- SAPK2a [1 - 360] T106M

Clone number:- DU 3315

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 67, 572.38 daltons

Average Mass 67, 615.93 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.69

Purity:- >85 %

Activation protocol:-

SAPK2a (3.5 μ M) is activated in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM MgAc, 0.1 mM ATP with 200 nM MBP-MKK6 DD [DU 1662] at 30 °C for 30 min. Following activation, SAPK2a is re-purified by GSH Sepharose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 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:- Standard filter binding

Assay buffer:-

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

Substrate:-

Myelin Basic Protein

Final concentration: 0.3 mg/ml

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Clone Data Sheet

SAPK2a [1 - 360] T106M

<u>Protein</u>	SAPK2a [1 – 360] T106M
<u>Clone number</u>	DU 3315
<u>Species</u>	Human
<u>Accession number</u>	L35264
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSMSQERPTFYRQELN KTIWEVPERYQNLSPVSGAYGSVCAAFDTKTGLRVAVKKLSRPFQSI IHAKRTYRELRLKHKHENVIGLLDVFTPARSLEEFNDVYLVMHLMG ADLNNIVKCQKLTDDHVQFLIYQILRGLKYIHSADIIHRDLKPSNLAV NEDCELKILDFGLARHTDDEMTGYVATRWYRAPEIMLNWMHYNQTVDI WSVGCIMAELLTGRTLFPGTDHIDQLKLILRLVGTPGAELLKKISSES ARNYIQSLTQMPKMNFAVFIGANPLAVDLLEKMLVLDSDKRITAAQA LAHAYFAQYHDPDDEPVADPYDQSFESRDLLIDEWKSLTYDEVISFVP PPLDQEEMES
<u>Native sequence</u>	Amino acids M1 – S360 (end) of human SAPK2a. Residue M227 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has the gatekeeper mutation T106M mutation. This mutation increases resistance to SB203580 as compared to the wildtype enzyme. Residue T106 is equivalent to M332 of the fusion protein.
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226
<u>Cloning sites</u>	<i>Bam</i> HI site of pGEX-4T1

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Nucleotide sequence of insert

ggatccATGTCTCAGGAGAGGCCACGTTCTACCGGCAGGAGCTGAAC
AAGACAATCTGGGAGGTGCCCCGAGCGTTACCAGAACCTGTCTCCAGTG
GGCTCTGGCGCCTATGGCTCTGTGTGTGCTGCTTTTGACACAAAAACG
GGGTACGTGTGGCAGTGAAGAAGCTCTCCAGACCATTTTCAGTCCATC
ATTCATGCGAAAAGAACCTACAGAGAAGCTGCGGTACTTAAACATATG
AAACATGAAAATGTGATTGGTCTGTTGGACGTTTTTACACCTGCAAGG
TCTCTGGAGGAATTCAATGATGTGTATCTGGTGATGCATCTCATGGGG
GCAGATCTGAACAACATTGTGAAATGTCAGAAGCTTACAGATGACCAT
G TTCAGTTCCTTATCTACCAAATTCTCCGAGGTCTAAAGTATATACAT
TCAGCTGACATAATTCACAGGGACCTAAAACCTAGTAATCTAGCTGTG
AATGAAGACTGTGAGCTGAAGATTCTGGATTTTGGACTGGCTCGGCAC
ACAGATGATGAAATGACAGGCTACGTGGCCACTAGGTGGTACAGGGCT
CCTGAGATCATGCTGAACTGGATGCATTACAACCAGACAGTTGATATT
TGGTCAGTGGGATGCATAATGGCCGAGCTGTTGACTGGAAGAACATTG
TTTCCTGGTACAGACCATATTGATCAGTTGAAGCTCATTTTAAGACTC
GTTGGAACCCCAGGGGCTGAGCTTTTGAAGAAAATCTCCTCAGAGTCT
GCAAGAAACTATATTCAGTCTTTGACTCAGATGCCGAAGATGAACTTT
GCGAATGTATTTATTGGTGCCAATCCCCCTGGCTGTCGACTTGCTGGAG
AAGATGCTTGTATTGGACTCAGATAAGAGAATTACAGCGGCCCAAGCC
CTTGACATGCCTACTTTGCTCAGTACCACGATCCTGATGATGAACCA
GTGGCCGATCCTTATGATCAGTCCTTTGAAAGCAGGGACCTCCTTATA
GATGAGTGGAAGCCTGACCTATGATGAAGTCATCAGCTTTGTGCCA
CCACCCCTTGACCAAGAAGAGATGGAGTCCTgaggatcc