Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of SAPK2A D168A [1 - 360]

Enzyme description:- SAPK2A D168A [1 – 360]

Clone number:- DU 12428

Source:- Recombinant

Expression system:- *E.coli* expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 68, 029.66 daltons
Average Mass 68, 073.44 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.63

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM Sucrose, 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 MgAc

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Clone Data Sheet

SAPK2A D168A [1 - 360]

Protein SAPK2A D168A [1 - 360]

Clone number DU 12428

Species Human

Accession number L35264

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSQERPTFY**
RQELNKTIWEVPERYQNLSPVGSGAYGSVCAAFDTKTGLRVAVKKLSR
PFQSIIHAKRTYRELRLLKHMKHENVIGLLDVFTPARSLEEFNDVYLV
THLMGADLNNIVKCQKLTDDHVQFLIYQILRGLKYIHSADIIHRDLKP
SNLAVNEDCELKILA**FGLARHTDDEMTGYVATRWYRAPEIMLNWMHYN**
QTVDIWSVGCIMAELLTGRTLFPGTDHIDQLKLILRLVGTPGAELLKK
ISSESARNYIQSLTQMPKMNFANVFIGANPLAVDLLEKMLVLDSDKRI
TAAQALAHAYFAQYHDPDDEPVADPYDQSFESRDLLIDEWKSLTYDEV
ISFVPPPLDQEEMES

Native sequence Amino acids M1 – S360 (end) of human SAPK2A.

Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

The enzyme has a D168A mutation, which produces a kinase dead enzyme. Residue D168A is equivalent to A399 of the fusion protein.

Protease cleavage PreScission site (LEVLFQGP) residues 221 – 228

Cloning sites *Bam*H1 and *Not*1 sites of pGex6P1

Nucleotide sequence of insert

ggatccATGTCTCAGGAGAGGCCCACGTTCTACCGGCAGGAGCTGAAC
AAGACAATCTGGGAGGTGCCCGAGCGTTACCAGAACCTGTCTCCAGTG
GGCTCTGGCGCCTATGGCTCTGTGTGTGCTGCTTTTGACACAAAGACG
GGGTTACGTGTGGCAGTGAAGAAGCTCTCCAGACCATTTCAGTCCATC
ATTCATGCGAAAAGAACCTACAGAGAACTGCGGTTACTTAAACATATG
AAACATGAAAATGTGATTGGTCTGTTGGACGTTTTTACACCTGCAAGG
TCTCTGGAGGAATTCAATGATGTGTATCTGGTGACCCATCTCATGGGG
GCAGATCTGAACAACATTGTGAAATGTCAGAAGCTTACAGATGACCAT
GTTCAGTTCCTTATCTACCAAATTCTCCGAGGTCTAAAGTATATACAT
TCAGCTGACATAATTCACAGGGACCTAAAACCTAGTAATCTAGCTGTG
AATGAAGACTGTGAGCTGAAGATTCTGGCTTTTGGACTGGCTCGGCAC
ACAGATGATGAAATGACAGGCTACGTGGCCACTAGGTGGTACAGGGCT
CCTGAGATCATGCTGAACTGGATGCATTACAACCAGACAGTTGATATT
TGGTCAGTGGGATGCATAATGGCCGAGCTGTTGACTGGAAGAACATTG
TTTCCTGGTACAGACCATATTGATCAGTTGAAGCTCATTTTAAGACTC
GTTGGAACCCCAGGGGCTGAGCTTTTGAAGAAAATCTCCTCAGAGTCT
GCAAGAAACTATATTCAGTCTTTGACTCAGATGCCGAAGATGAACTTT
GCGAATGTATTTATTGGTGCCAATCCCCTGGCTGTCGACTTGCTGGAG
AAGATGCTTGTATTGGACTCAGATAAGAGAATTACAGCGGCCCAAGCC
CTTGCACATGCCTACTTTGCTCAGTACCACGATCCTGATGATGAACCA
GTGGCCGATCCTTATGATCAGTCCTTTGAAAGCAGGGACCTCCTTATA
GATGAGTGGAAAAGCCTGACCTATGATGAAGTCATCAGCTTTGTGCCA
CCACCCCTTGACCAAGAAGAGATGGAGTCCtgagcggccgc