Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of SPAK T233E [1 - 547]

Enzyme description:- SPAK T233E [1 – 547]

Clone number:- DU 6245

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 86, 439.21 daltons
Average Mass 86, 494.39 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.80

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, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

SPAK T233E [1 - 547]

Protein	SPAK T233E [1 - 547]
Clone number	DU 6245
Species	Human
Accession number	AF099989.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAEPSGSPV** **HVQLPQQAAPVTAAAAAAPAAATAAPAPAAPAAPAPAPAPAAQAVG** **WPICRDAYELQEVIGSGATAVVQAALCKPRQERVAIKRINLEKCQTSM** **DELLKEIQAMSQCSHPNVVTYYTSFVVKDELWLVMKLLSGGSMLDIIK** **YIVNRGEHKNGVLEEAIIATILKEVLEGLDYLHRNGQIHRDLKAGNIL** **LGEDGSVQIADFGVSAFLATGGDVTRNKVRKEFVGTPCWMAPEVMEQV** **RGYDFKADMWSFGITAIELATGAAPYHKYPPMKVLMLTLQNDPPTLET** **GVEDKEMMKKYGKSFRKLLSLCLQKDPSKRPTAAELLKCKFFQKAKNR** **EYLIEKLLTRTPDIAQRAKKVRRVPGSSGHLHKTEDGDWEWSDDEMDE** **KSEEGKAAFSQEKSRRVKEENPEIAVSASTIPEQIQSLSVHDSQGPPN** **ANEDYREASSCAVNLVLRLRNSRKELNDIRFEFTPGRDTADGVSQELF** **SAGLVDGHDVVIVAANLQKIVDDPKALKTLTFKLASGCDGSEIPDEVK** **LIGFAQLSVS**
Native sequence	Amino acids M1 – S547 (end) of human SPAK. Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a T233E mutation in order to mimic phosphorylation of the enzyme. Residue T185 is equivalent to E464 of the fusion protein
Protease cleavage	Prescission site (LEVLFQGP) at residues 221 – 228
Cloning sites	*Bam*H1 sites of pGex6P-1

Nucleotide sequence of insert

ggatccATGGCGGAGCCGAGCGGCTCGCCCGTGCACGTCCAGCTTCCC
CAGCAGGCGGCCCCGGTGACAGCGGCGGCGGCGGCGGCCCCGGCGGCC
GCGACAGCAGCGCCGGCCCCGGCAGCTCCCGCGGCCCCGGCCCCGGCC
CCGGCCCCGGCCCCGGCGGCACAGGCTGTCGGCTGGCCCATCTGCAGG
GACGCGTACGAGCTGCAGGAGGTTATCGGCAGTGGAGCTACTGCTGTG
GTTCAGGCAGCCCTATGCAAACCCAGGCAAGAACGTGTAGCAATAAAA
CGGATCAACTTGGAAAAATGCCAGACCAGTATGGATGAACTATTAAAA
GAAATTCAAGCCATGAGTCAGTGCAGCCATCCCAACGTAGTGACCTAT
TACACCTCTTTTGTGGTCAAAGATGAACTTTGGCTGGTCATGAAATTA
CTAAGTGGAGGTTCAATGTTGGATATCATAAAATACATTGTCAACCGA
GGAGAACACAAGAATGGAGTTCTGGAAGAGGCAATAATAGCAACAATT
CTTAAAGAGGTTTTGGAAGGCTTAGACTATCTACACAGAAACGGTCAG
ATTCACAGGGATTTGAAAGCTGGTAATATTCTTCTGGGTGAGGATGGT
TCAGTACAAATAGCAGATTTTGGGGTAAGTGCGTTCCTAGCAACAGGG
GGTGATGTTACCCGAAATAAAGTAAGAAAAGAATTCGTTGGCACCCCA
TGTTGGATGGCTCCTGAAGTCATGGAACAGGTGAGAGGCTATGACTTC
AAGGCTGACATGTGGAGTTTTGGAATAACTGCCATTGAATTAGCAACA
GGAGCAGCGCCTTATCACAAATATCCTCCCATGAAAGTGTTAATGTTG
ACTTTGCAAAATGATCCACCCACTTTGGAAACAGGGGTAGAGGATAAA
GAAATGATGAAAAAGTACGGCAAGTCCTTTAGAAAATTACTTTCACTG
TGTCTTCAGAAAGATCCTTCCAAAAGGCCCACAGCAGCAGAACTTTTA
AAATGCAAATTCTTCCAGAAAGCCAAGAACAGAGAGTACCTGATTGAG
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GACTGGGAGTGGAGTGACGACGAGATGGATGAGAAGAGCGAAGAAGGG
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CCAGAGATTGCAGTGAGTGCCAGCACCATCCCCGAACAAATACAGTCC
CTCTCTGTGCACGACTCTCAGGGCCCACCCAATGCTAATGAAGACTAC
AGAGAAGCTTCTTCTTGTGCCGTGAACCTCGTTTTGAGATTAAGAAAC
TCCAGAAAGGAACTTAATGACATACGATTTGAGTTTACTCCAGGAAGA
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TGTGATGGGTCGGAGATTCCTGATGAAGTGAAGCTGATTGGGTTTGCT
CAGTTGAGTGTCAGCTGAGCTGAGGTACCAAGGGCGAATTCCAGCACA
CTGGCGGCCGTTACtagtggatcc