

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of KPNA1 [1 – 538]

Enzyme description:- KPNA1 [1 - 538]

Clone number:- DU 5344

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 87, 017.20 daltons

Average Mass 87, 072.96 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.13

Purity:- >80 %

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

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Clone Data Sheet

KPNA1 [1 - 538]

<u>Protein</u>	KPNA1 [1 - 538]
<u>Clone number</u>	DU 5344
<u>Species</u>	Human
<u>Accession number</u>	BT006959.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFFQGPLGSMTTPGKENFRLKSY KNKSLNPDEMRRRREEEGLQLRKQKREEQLFKRRNVATAEEETEEVMS DGGFHEAQINMEMAPGGVITSDMIEMIFSKSPEQQLSATQKFRKLLSK EPNPPIDEVISTPGVVARFVEFLKRKENCTLQFESAWVLTNIASGNSLQ TRIVIQAGAVPIFIELLSSEFEDVQEQAVWALGNIAGDSTMCRDYVLDC NILPPLLQLFSKQNRMTMTRNAVWALSNLGRGKSPPEFAKVSPCLNVL SWLLFVSDTDVLADACWALS YLS DGPNDKIQAVIDAGVCRRLVELLMHN DYKVVSPALRAVGNI VTGDDIQ TQVILNCSALQSLHLLSSPKESIKKE ACWTISNITAGNRAQIQ TVIDANIFPALISILQTAEFRTRKEAAWAITN ATSGGSAEQIKYLVELGC IKPLCDLLTVMDSKIVQVALNGLENILRLGE QEA KRNGTGINPYCALIEEAYGLDKIEFLQSHENQEIYQKAFDLIEHYF GTEDEDSSIAPQVDLNQQQYIFQQCEAPMEGFQL
<u>Native sequence</u>	Amino acids M1 – L538 (end) of human KPNA1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatccATGACCACCCCAGGAAAAGAGAACTTTCGCCTGAAAAGTTACAAGAACAAATCTCTGAAT
CCCGATGAGATGCGCAGGAGGAGGGAGGAAGAAGGACTGCAGTTACGAAAGCAGAAAAGAGAAGAG
CAGTTATTCAAGCGGAGAAATGTTGCTACAGCAGAAGAAGAAACAGAAGAAGAAGTTATGTCAGAT
GGAGGCTTTCATGAGGCTCAGATTAATAACATGGAGATGGCACCAGGTGGTGTTCATCACTTCTGAC
ATGATTGAAATGATATTTTCCAAAAGCCCAGAGCAACAGCTTTCAGCAACACAGAAATTCAGGAAG
CTGCTTTCAAAGAACCTAACCCTCCTATTGATGAAGTTATCAGCACACCAGGAGTAGTGGCCAGG
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AATATTGCTTCAGGAAATTCCTTCAGACCCGAATTGTGATTTCAGGCAGGAGCTGTGCCCATCTTC
ATAGAGTTGCTCAGCTCAGAGTTTGAAGATGTCCAGGAACAGGCAGTCTGGGCTCTTGCCAACATT
GCTGGAGATAGTACCATGTGCAGGGACTATGTCTTAGACTGCAATATCCTTCCCCCTCTTTTGCAG
TTATTTTCAAAGCAAAACCGCCTGACCATGACCCGGAATGCAGTATGGGCTTTGTCTAATCTCTGT
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CAACAGTGTGAGGCTCCTATGGAAGGTTTCAGCTTtgagcgccgc

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