

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of KPNA4 [1 – 521]

Enzyme description:- KPNA4 [1 - 521]

Clone number:- DU 5343

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 84, 657.28 daltons

Average Mass 84, 710.92 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.02

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

KPNA4 [1 - 521]

<u>Protein</u>	KPNA4 [1 - 521]
<u>Clone number</u>	DU 5343
<u>Species</u>	Human
<u>Accession number</u>	NM_002268.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMADNEKLDNQRKLN FKNKGRDLETMRRQRNEVVVELRKNKRDEHLLKRRNVPHEDICEDSDID GDYRVQNTSLEAIVQNASSDNQGIQLSAVQAARKLLSSDRNPPIDDLIK SGILPILVHCLERDDNPSLQFEAAWALTNIASGTSEQTQAVVQSNVPL FLRLLHSPHQNVCEQAVWALGNIIGDGPQCRDYVISLGVVKPLLSFISP SIPITFLRNVTWVMVNLCRHKDPPPPMETIQEILPALCVLIHHTDVNIL VDTVWALSYLTDAGNEQIQMVIDSGIVPHLVPLLSHQEVKVQTAALRAV GNIVTGTDEQTQVVLNCDALSHFPALLTHPKEKINKEAVWFLSNITAGN QQQVQAVIDANLVPMIHLDDKGFDTQKEAAWAI SNLTISGRKDQVAY LIQQNVIPFCNLLTVKDAQVVQVVDGLSNILKMAEDEAETIGNLIEE CGGLEKIEQLQNHENEDIYKLAYEIIDQFFSSDDIDEDPSLVPEAIQGG TFGFNSSANVPTEGFQF</p>
<u>Native sequence</u>	<p>Amino acids M1 – F521 (end) of human KPNA4. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<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

ggatccATGGCGGACAACGAGAACTGGACAACCAACGGCTCAAGAATTTCAAGAACAAAGGCCGC
GACTTGAGACTATGAGAAGGCAACGAAATGAAGTTGTAGTTGAATTAAGGAAGAATAAAAGAGAT
GAACATCTCTTAAAGAGAAGGAATGTACCACATGAAGATATCTGTGAAGACTCTGATATAGATGGT
GATTATAGAGTGCAAAATACCTCTCTAGAAGCTATTGTTCAAAATGCTTCAAGTGATAACCAAGGA
ATTC AATTAAGTGCAGTTC AAGCTGCTAGGAAGCTTTTGTCCAGTGATCGAAATCCACCAATTGAT
GACTTAATAAAATCTGGAATATTGCCCATTTTAGTCCATTGTCTTGAAAGAGATGACAATCCTTCT
TTACAGTTTGAAGCTGCATGGGCTTTGACAAACATTGCATCTGGAAC TCTGAACAAACTCAAGCA
GTAGTTCAGTCCAATGCTGTGCCACTTTTCTGAGGCTTCTCCATTCACCCCATCAGAATGTCTGT
GAGCAAGCAGTGTGGGCATTGGGAAATATCATAGGTGATGGGCCCCAGTGTAGAGATTATGTCATA
AGTCTTGGAGTTGTGAAACCTTTACTTTCCTTCATAAGTCCATCTATTCCTATAACATTCTTAAGA
AATGTTACTTGGGTTATGGTCAACTTATGTCGCCACAAAGACCCACCACCACCAATGGAAACCATT
CAGGAGATTCTTCCAGCCCTTTGTGTTTTAATTCATCACACAGATGTAAATATACTGGTAGACACA
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