

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of KPNB1 [1 – 876]

Enzyme description:- KPNB1 [1 - 876]

Clone number:- DU 5345

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 123, 911.65 daltons

Average Mass 123, 994.35 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.83

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

KPNB1 [1 - 876]

<u>Protein</u>	KPNB1 [1 - 876]
<u>Clone number</u>	DU 5345
<u>Species</u>	Human
<u>Accession number</u>	L3895.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEELHYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGGPLGSMELITILEKTVSPD RLELEAAQKFLERA AVENLPTFLVELSRVLANPGNSQVARVAAGLOIKN SLTSKDPDIKAQYQQRWLAI DANARREVKNYVLQTLGTETYPSSASQC VAGIACAEIPVNQWPELIPQLVANVTNPNSTEHMKESTLEAIGYICQDI DPEQLQDKSNEILTAI IQGMRKEEPSNNVKLAATNALLNSLEFTKANFD KESERHFIMQVVCEATQCPDTRVRVAALQNLVKIMSLYQYMETYMGPA LFAITIEAMKSDIDEVALQGIEFWSNVCDEEMDLAIEASEAAEQGRPPE HTSKFYAKGALQYLVPILTQTTLTKQDENDDDDWNPCKAAGVCLMLLAT CCEDDIVPHVLPFIKEHIKNPDWRYRDAAVMAFGCILEGPEPSQLKPLV IQAMPTLIELMKDPSVVVRDTAAWTVGRICELLPEAAINDVYLAPLLQC LIEGLSAEPRVASNVCWAFSSLAEEA YEADVADDQEEPATYCLSSSFE LIVQKLLETDRPDGHQNNLRSSAYESLMEIVKNSAKDCYPVQKTTLV IMERLQQVLQ MESHIOQSTSDRIQFNDLQSLLCATLQNVLRKVQHQDALQ ISDVVMASLLRMFQSTAGSGGVQEDALMAVSTLVEVLGGEFLKYMEAFK PFLGIGLKNYAEYQVCLA AVGLVGDLCRALQSNIPFCDEVMQLLLENL GNENVHRSVKPQILSVFGDIALAIGGEFKKYLEVVLNTLQOASQAQVQDK SDYDMVDYLNELRESCLEAYTGIVQGLKGDQENVHPDVMLVQPRVEFIL SFIDHIAGDEDHTDGVVACAAGLIGDLCTAFGKDVLKLVEARPMIHELL TEGRRSKTNKAKTLATWATKELRKLKNQA
<u>Native sequence</u>	Amino acids M1 – A876 (end) of human KPNB1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

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Protease cleavage PreScission (LEVLFGQP) residues 221 - 228

Cloning sites *Bam*H1 sites of pGEX6P-1

Nucleotide Sequence of insert

ggatccATGGAGCTGATCACCATTCTCGAGAAGACCGTGTCTCCCGATCGGCTGGAGCTGGAAGCG
GCGCAGAAGTTCTTGGAGCGTGCGGCCGTGGAGAACCTGCCCACTTTCCTTGTGGAACGTGCCAGA
GTGCTGGCAAATCCAGGAAACAGTCAGGTTGCCAGAGTTGCAGCTGGTCTACAAATCAAGAACTCT
TTGACATCTAAAGATCCAGATATCAAGGCACAATATCAGCAGAGGTGGCTTGCTATTGATGCTAAT
GCTCGACGAGAAGTCAAGAACTATGTTTTGCAGACATTGGGTACAGAACTTACCGGCCTAGTTCT
GCCTCACAGTGTGTGGCTGGTATTGCTTGTGCAGAGATCCAGTAAACCAGTGGCCAGAACTCATT
CCTCAGCTGGTGGCCAAATGTCACAAACCCCAACAGCACAGAGCACATGAAGGAGTCGACATTGGAA
GCCATCGGTTATATTTGCCAAGATATAGACCCAGAGCAGCTACAAGATAAATCCAATGAGATTCTG
ACTGCCATAATCCAGGGGATGAGGAAAGAAGAGCCTAGTAATAATGTGAAGCTAGCTGCTACGAAT
GCACTCCTGAACTCATTGGAGTTCACCAAAGCAAACCTTTGATAAAGAGTCTGAAAGGCACTTTATT
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AAAGCAAAAACCCCTTGCTACATGGGCAACAAAAGAACTGAGGAACTGAAGAACCAAGCTtgagga
tcc

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