

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Pellino1 [1 - 418]

Protein description:- Pellino1 [1 - 418]

Clone number:- DU 8981

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 3 mg/L

Calculated molecular mass:-

Monoisotopic 73, 123.44 daltons

Average Mass 73, 170.49 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.06

Purity:- >75 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

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CLONE DATA SHEET

Pellino1 [1 - 418]

<u>Protein</u>	Pellino1 [1 - 418]
<u>Clone number</u>	DU 8981
<u>Species</u>	Human
<u>Accession no</u>	NM_020651.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGAVL DIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMFSPDQENHPFKAPVKYGE LIVLGYNGSLPNGDRGRRKSRFALFKRPKANGVKPSTVHIACTPQAAKAI SNKDQHSISYTL SRAQTVVVEYTHDSNTDMFQIGRSTESPIDFVVTDTVP GSQSNSDTQSVQSTISR FACRIICERNPPFTARIYAAGFDSSKNIFLGEK AAKWKTS DGQMDGLTTNGVLVMHPRNGFTEDSKPGIWREISVCGNVFSLR ETRSAQQRGKMVEIETNQLQDGLIDL CGATLLWRTAEGLSHTPTVKHLE ALRQEINAARPQCPVGFNTLAFPSMKRKDVVDEKQPWVYLNCGHVHGYHN WGNKEERD GKDRECPMCRSVGPYVPLWLGC EAGFYVDAGPPTHAFSPCGH VCSEKTTAYWSQIPLPHGTHTFHAACPFCAHQ LAGEQGYIRLIFQGPLD
<u>Native sequence</u>	Amino acids M1 – D418 (end) of human Pellino1. Residue M232 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I of pGEX 6P-1

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Nucleotide sequence of insert

ggatccATGTTTTCTCCTGATCAAGAAAATCATCCATTTAAAGCACCAGT
AAAATATGGTGAACTCATTGTCTTAGGGTATAATGGGTCTCTCCCAAATG
GCGATAGAGGAAGGAGGAAAAGTAGGTTTGCTTTGTTTAAAAGACCTAAG
GCAAATGGGGTGAAGCCCAGCACTGTGCATATTGCTTGTACTCCTCAGGC
TGCAAAGGCAATAAGCAACAAAGACCAGCATAGCATATCATATACTTTGT
CTCGGGCCCAGACTGTGGTGGTTGAATATACTCATGACAGCAACACAGAT
ATGTTTCAAATTGGCCGGTCGACTGAAAGCCCCATTGATTTTGTAGTAAC
TGACACGGTTCCTGGAAGTCAAAGTAATTCTGATACACAGTCAGTACAAA
GCACTATATCAAGATTTGCCTGCAGAATCATATGTGAACGGAATCCTCCC
TTTACAGCACGGATTTATGCTGCAGGATTTGACTCATCAAAAAACATCTT
TCTTGGGGAGAAGGCTGCCAAATGGAAGACATCAGATGGACAGATGGATG
GCTTGACCACTAATGGTGTTCCTTGTGATGCATCCACGCAATGGGTTTACA
GAAGACTCCAAGCCTGGAATATGGAGAGAAATATCGGTGTGTGGAAATGT
ATTTAGCCTACGTGAAACCAGATCGGCTCAGCAGAGAGGAAAAATGGTGG
AAATTGAAACCAATCAGTTACAAGATGGCTCGTTAATTGACCTCTGTGGT
GCAACATTGTTATGGCGTACTGCAGAAGGCCTTTCCACACTCCTACCGT
GAAGCATTTAGAAGCTTTAAGACAGGAAATCAATGCAGCACGACCTCAGT
GCCCTGTAGGGTTCAACACACTAGCATTTCCCTAGTATGAAGAGGAAAGAC
GTTGTAGATGAAAAACAACCATGGGTATATCTAACTGCGGCCATGTACA
TGGCTATCATAACTGGGGAAACAAAGAAGAACGTGATGGAAAAGATCGTG
AATGTCCTATGTGTAGGTCTGTTGGTCCCTATGTTCCCTCTGTGGCTTGGA
TGTGAAGCTGGATTTTATGTGGACGCCGGCCCTCCAACCCATGCGTTTAG
CCCGTGTGGGCATGTGTGTTTCAAAAAGACAACCTGCCTATTGGTCCCAGA
TCCCCTTCCTCATGGTACTCATACTTTTCATGCAGCCTGTCCCTTTTGT
GCACATCAGTTGGCTGGTGAACAAGGCTACATCAGACTTATTTTTCAAGG
ACCTCTAGACTaagcggccgc