

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Pellino2 [1 – 420]

Enzyme description:- Pellino2 [1 – 420]

Clone number:- DU 15004

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 73, 212.43 daltons

Average Mass 73, 259.50 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.46

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

Pellino2 [1 – 420]

<u>Protein</u>	Pellino2 [1 – 420]
<u>Clone number</u>	DU 15004
<u>Species</u>	Human
<u>Accession number</u>	AAH09476.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMFSPGQEEHCAPNK EPVKYGELVVLGYNGALPNGDRGRRKSRFALYKRPKANGVKPSTVHVIS TPQASKAISCKGQHSISYTL SRNQT VVVEYTHDKD TDMFQVGRSTESPI DFVVTDTISGSQNTDEAQITQSTISR FACRIVCDRNEPYTARI FAAGFD SSKNIFLGEKAAKWKNP DGHMDGLTTNGVLVMHPRGGFTEESQPGVWRE ISVCGDVYTLRETRSAQQRGKLVESETNVLQDGLIDL CGATLLWRTAD GLFHTPTQKHIEALRQEI NAARPOCPVGLNTLAFPSINRKEVVEEKQPW AYLSCGHVHG YHNWGHRS DTEANERECPM CRTVGPYVPLWLGCEAGFYV DAGPPTHAFTPCGHVCSEKSAKYWSQIPLPHGTHAFHAACPF CATQLVG EQNCIKLIFQGPID</p>
<u>Native sequence</u>	<p>Amino acids M1 – D420 (end) of human Pellino2. 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> 1 sites into pGEX6P-1

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Nucleotide Sequence of Insert:

ggatccATGTTTTCCCTGGCCAGGAGGAACACTGCGCCCCAATAAGGA
GCCAGTGAAATACGGGGAGCTGGTGGTGTCTCGGGTACAATGGTGTCTTAC
CCAATGGAGATAGAGGACGGAGGAAAAGTAGATTTGCCCTCTACAAGCGG
CCCAAGGCAAATGGTGTCAAACCCAGCACCGTCCATGTGATATCCACGCC
CCAGGCATCCAAGGCTATCAGCTGCAAAGGTCAACACAGTATATCCTACA
CTTTGTCAAGGAATCAGACTGTGGTGGTGGAGTACACACATGATAAGGAT
ACGGATATGTTTCAGGTGGGCAGATCAACAGAAAGCCCTATCGACTTCGT
TGTCACAGACACGATTTCTGGCAGCCAGAACACGGACGAAGCCCAGATCA
CACAGAGCACCATATCCAGGTTTCGCCTGCAGGATCGTGTGCGACAGGAAT
GAACCTTACACAGCACGGATATTCGCCGCCGGATTTGACTCTTCCAAAA
CATATTTCTTGAGAAAAGGCAGCAAAGTGGAAAAACCCCGACGGCCACA
TGGATGGGCTCACTACTAATGGCGTCTGGTGATGCATCCACGAGGGGGC
TTCACCGAGGAGTCCCAGCCCGGGGTCTGGCGCGAGATCTCTGTCTGTGG
AGATGTGTACACCTTGCGAGAAAACCAGGTCGGCCCAGCAACGAGGAAAGC
TGGTGGAAAGTGAGACCAACGTCCTGCAGGACGGCTCCCTCATTGACCTG
TGTGGGGCCACTCTCCTCTGGAGAACAGCAGATGGGCTTTTTTCATACTCC
AACTCAGAAGCACATAGAAGCCCTCCGGCAGGAGATTAACGCCGCCCGGC
CTCAGTGTCCCTGTGGGGCTCAACACCCCTGGCCTTCCCCAGCATCAACAGG
AAAGAGGTGGTGGAGGAGAAGCAGCCCTGGGCATATCTCAGTTGTGGCCA
CGTGCACGGGTACCACAACCTGGGGCCATCGGAGTGACACGGAGGCCAACG
AGAGGGAGTGTCCCATGTGCAGGACTGTGGGCCCTATGTGCCTCTCTGG
CTTGGCTGTGAGGCAGGATTTTATGTAGACGCAGGACCGCCAACCTCATGC
TTTCACTCCCTGTGGACACGTGTGCTCGGAGAAGTCTGAAAATACTGGT
CTCAGATCCCGTTGCCTCATGGAACCTCATGCATTTACGCTGCTTGCCCT
TTCTGTGCTACACAGCTGGTTGGGGAGCAAACCTGCATCAAATTAATTTT
CCAAGGTCCAATTGACtgagcggccgc

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