

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active WNK1 [1 - 667]

<u>Enzyme description:-</u>	WNK1 [1 – 667]
<u>Clone number:-</u>	DU 34823
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose
<u>Calculated molecular mass:-</u>	
Monoisotopic	100, 374.72 daltons
Average Mass	100, 437.92 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.67
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Auto-activation
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF	
<u>Storage temperature:-</u>	-70 °C
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 5 mM MnCl ₂	
<u>Substrate:-</u>	
GST-OSR1 [DU 6099]	Final concentration: 0.5 mg/ml

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Clone Data Sheet

WNK1 [1 - 667]

Protein WNK1 [1 - 667]

Clone number DU 34823

Species Human

Accession number NM_018979.3

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSTMSGGAAE
KQSSTPGSLFLSPPAPAPKNGSSSDSSVGEKLGAAAADAVTGRTEEYR
RRRHTMDKDSRGAAATTTTTEHRFFRRSVICDSNATALELPGLPLSLP
QPSIPAAPVQSAPEPHREETVTATATSQVAQPPAAAAAPGEQAVAGP
APSTVPSSTSKDRPVQPSLVGSKEEPPPARSGSGGSAKEPQEERSQ
QDDIEELETKAVGMSNDGRFLKFDIEIGRGSFKTVYKGLDTETTVEV
AWCELQDRKLTCSRQRFKEEAEMKGLQHPNIVRFYDSWESTVKGKK
CIVLVTELMTSGTLKTYLKRFKVMKIKVLRSWCRQILKGLQFLHTRTP
PIIHRDLKCDNIFITGPTGSVKIGDLGLATLKRASFASVIGTPEFMA
PEMYEEKYDESVDVYAFGCMCMLEMATSEYPYSECQNAAQIYRRVTSGV
KPASFDKVAIPEVKEIIIEGCIRQNKDERYSIKDLLNHAFFQEETGVRV
ELAEEDDGEKIAIKLWLRIEDIKKLKGKYKDNEAIEFSFDLERDVPED
VAQEMVESGYVCEGDHKTMAKAIKDRVSLIKRKREQRQLVREEQEKKK
QEESLQQVEQSSASQTGIKQLPSASTGIPTASTTSASVSTQVEPEE
PEADQHQQQLQYQQPSISVLSDGTVDSGQGSSVFTE

Native sequence Amino acids M1 – S667 of human WNK1 [end residue is T2382].

Residue M234 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission site (LEVLFQGP) residues 221 – 228

Cloning sites *Bam*H1 *Kpn*1 and *Not*1 sites of pFB GST 6P

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Nucleotide sequence of insert

ggatccactagtATGTCTGGCGGCGCCGAGAGAAGCAGAGCAGCACT
CCCGGTTCCCTGTTCTCTCGCCGCGGCTCCTGCCCCAAGAATGGC
TCCAGCTCCGATTCTCTCGTGGGGGAGAACTGGGAGCCGCGGCCGCC
GACGCTGTGACCGGCAGGACCGAGGAGTACAGGCGCCGCCGCCACACT
ATGGACAAGGACAGCCGTGGGGCGGCCGCGACCACTACCACCACTGAG
CACCGCTTCTTCCGCCGGAGCGTCATCTGCGACTCCAATGCCACTGCA
CTGGAGCTTCCCGGCCTTCTCTTCCCTGCCCCAGCCCAGCATCCCC
GCGGCTGTCCCGCAGAGTGCTCCACCGGAGCCCCACCGGGAAGAGACC
GTGACCGCCACCGCCACTTCCCAGGTAGCCAGCAGCCTCCAGCCGCT
GCCGCCCTTGGGGAACAGGCCGTCGCGGGCCCTGCCCCCTCGACTGTC
CCCAGCAGTACCAGCAAAGACCGCCAGTGTCCAGCCTAGCCTTGTG
GGGAGCAAAGAGGAGCCGCCGCCGGCGAGAAGTGGCAGCGGCGGCGGC
AGCGCCAAGGAGCCACAGGAGGAACGGAGCCAGCAGCAGGATGATATC
GAAGAGCTGGAGACCAAGGCCGTGGGAATGTCTAACGATGGCCGCTTT
CTCAAGTTTGACATCGAAATCGGCAGAGGCTCCTTTAAGACGGTCTAC
AAAGGTCTGGACACTGAAACCACCGTGGAAGTCGCCTGGTGTGAACTG
CAGGATCGAAAATTAACAAAGTCTGAGAGGCAGAGATTTAAAGAAGAA
GCTGAAATGTTAAAAGGTCTTCAGCATCCCAATATTGTTAGATTTTAT
GATTCCTGGGAATCCACAGTAAAAGGAAAGAAGTGCATTGTTTTGGTG
ACTGAACCTTATGACGTCTGGAACACTTAAAACGTATCTGAAAAGGTTT
AAAGTGATGAAGATCAAAGTTCTAAGAAGCTGGTGCCGTCAGATCCTT
AAAGGTCTTCAGTTTCTTCATACTCGAACTCCACCTATCATTCACCGC
GATCTTAAATGTGACAACATCTTTATCACCGGCCCTACTGGCTCAGTC
AAGATTGGAGACCTCGGTCTGGCAACCCTGAAGCGGGCTTCTTTTGCC
AAGAGTGTGATAGGTACCCAGAGTTCATGGCCCCTGAGATGTATGAG
GAGAAATATGATGAATCCGTTGACGTTTATGCTTTTGGGATGTGCATG
CTTGAGATGGCTACATCTGAATATCCTTACTCGGAGTGCCAAAATGCT
GCGCAGATCTACCGTCGCGTGACCAGTGGGGTGAAGCCAGCCAGTTTTT
GACAAAGTAGCAATTCTGAAGTGAAGGAAATTATTGAAGGATGCATA
CGACAAAACAAAGATGAAAGATATTCCATCAAAGACCTTTTGAACCAT
GCCTTCTTCCAAGAGGAAACAGGAGTACGGGTAGAATTAGCAGAAGAA
GATGATGGAGAAAAAATAGCCATAAAATTATGGCTACGTATTGAAGAT
ATTAAGAAATTAAAGGGAAAATACAAAGATAATGAAGCTATTGAGTTT
TCTTTTGGATTTAGAGAGAGATGTCCAGAAAGATGTTGCACAAGAAATG
GTAGAGTCTGGGTATGTCTGTGAAGGTGATCACAAGACCATGGCTAAA
GCTATCAAAGACAGAGTATCATTAATTAAGAGGAAACGAGAGCAGCGG
CAGTTGGTACGGGAGGAGCAAGAAAAAAAAAAGCAGGAAGAGAGCAGT
CTCAAACAGCAGGTAGAACAATCCAGTGCTTCCCAGACAGGAATCAAG
CAGCTCCCTTCTGCTAGCACCGGCATACCTACTGCTTCTACCACTTCA
GCTTCAGTTTCTACACAAGTAGAACCTGAAGAACCTGAGGCAGATCAA
CATCAGCAACTACAGTACCAGCAACCCAGTATATCTGTGTTATCTGAT
GGGACGGTTGACAGTGGTCAGGGATCCTCTGTCTTCACAGAATCT