

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active WNK1 [1 - 661]

Enzyme description:- WNK1 [1 - 661]

Clone number:- DU 43603

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 99, 536.35 daltons

Average Mass 99, 599.05 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.71

Purity:- >80 %

Activation protocol:- Constitutively active

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

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 10 mM DTT, 2 mM manganese chloride

Substrate:-

Myelin Basic Protein (MBP)

Final concentration: 0.3 mg/ml

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Clone Data Sheet

WNK1 [1 - 661]

<u>Protein</u>	WNK1 [1 - 661]
<u>Clone number</u>	DU 43603
<u>Species</u>	Human
<u>Accession number</u>	Q9H4A3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMSGGAAEKQSSTPG SLFLSPPAPAPKNGSSSDSSVGEKLGAAAADAVTGRTEEYRRRRHTMDK DSRGAAATTTTTEHRFFRRSVICDSNATALELPGLPLSLPQPSIPAAVP QSAPPEPHREETVTATATSQVAQQPPAAAAPGEQAVAGPAPSTVPSSTS KDRPVSQPSLVGSKEEPPPARSGSGGSAKEPQEERSQQQDDIEELETG AVGMSNDGRFLKFDIEIGRGSFKTVYKGLDTETTVEVAWCELQDRKLTG SERQRFKEEAEMLKGLQHPNIVRFYDSWESTVKGKKCIVLVTLM TSGT LKTYLKRFKVMKIKVLRSWCRQILKGLQFLHTRTPPIIHRDLKCDNIFI TGPTGSVKIGDLGLATLKRASF AKSVIGTPEFMAPEMYEEKYDESVDVY AFGMCMLEMATSEYPYSECQNAAQIYRRVTSGVKPASFDKVAIPEVKEI IEGCIRQNKDERYSIKDLLNHAF FQEETGVRVELAEEDDGEKIAIKLWL RIEDIKKLKGKYKDNEAIEFSFDLERDVPEDVAQEMVESGYVCEGDHKT MAKAIKDRVSLIKRKREQRQLVREEQEKKKQEESLKKQOVEQSSASQTG IKQLPSASTGIPTASTTSASVSTQVEPEEPEADQHQQQLQYQQPSISVLS DGTVDSGQGS
<u>Native sequence</u>	Amino acids M1 – S661 of human WNK3 [end residue is T2382]. Residue M233 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Xho</i> 1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGTCTGGCGGCGCCGCAGAGAAGCAGAGCAGCACTCCCGGTT
CCCTGTTTCCTCTCGCCGCGGCTCCTGCCCCCAAGAATGGCTCCAGCTC
CGATTCTCCGTGGGGGAGAACTGGGAGCCGCGGCCGCCGACGCTGTG
ACCGGCAGGACCGAGGAGTACAGGCGCCGCGGCCACACTATGGACAAGG
ACAGCCGTGGGGCGGCCGCGACCACTACCACCACTGAGCACCGCTTCTT
CCGCCGGAGCGTCATCTGCGACTCCAATGCCACTGCACTGGAGCTTCCC
GGCCTTCCTCTTTCCCTGCCCCAGCCCAGCATCCCCGCGGCTGTCCCGC
AGAGTGCTCCACCGGAGCCCCACCGGGAAGAGACCGTGACCGCCACCGC
CACTTCCCAGGTAGCCCAGCAGCCTCCAGCCGCTGCCGCCCTGGGGAA
CAGGCCGTCGCGGGCCCTGCCCCCTCGACTGTCCCCAGCAGTACCAGCA
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CGGCAGAGGCTCCTTTAAGACGGTCTACAAAGGTCTGGACACTGAAACC
ACCGTGGAAGTCGCCTGGTGTGAACTGCAGGATCGAAAATTAACAAAGT
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