Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active WNK1 [1 - 491]

Enzyme description:- WNK1 [1 - 491]

Clone number:- DU 41859

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 80, 599.83 daltons
Average Mass 80, 651.08 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.21

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

Clone Data Sheet

WNK1 [1 - 491]

Protein WNK1 [1 - 491]

Clone number DU 41859

Species Human

Accession number Q9H4A3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSA**MSGGAAEKQSSTP**
GSLFLSPPAPAPKNGSSSDSSVGEKLGAAAADAVTGRTEEYRRRRHTMD
KDSRGAAATTTTTEHRFFRRSVICDSNATALELPGLPLSLPQPSIPAAV
PQSAPPEPHREETVTATATSQVAQQPPAAAAPGEQAVAGPAPSTVPSST
SKDRPVSQPSLVGSKEEPPPARSGSGGGSAKEPQEERSQQQDDIEELET
KAVGMSNDGRFLKFDIEIGRGSFKTVYKGLDTETTVEVAWCELQDRKLT
KSERQRFKEEAEMLKGLQHPNIVRFYDSWESTVKGKKCIVLVTELMTSG
TLKTYLKRFKVMKIKVLRSWCRQILKGLQFLHTRTPPIIHRDLKCDNIF
ITGPTGSVKIGDLGLATLKRASFAKSVIGTPEFMAPEMYEEKYDESVDV
YAFGMCMLEMATSEYPYSECQNAAQIYRRVTSGVKPASFDKVAIPEVKE
IIEGCIRQNKDERYSIKDLLNHAFFQEETGVRVELAE

Native sequence Amino acids M1 – E491 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.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Xho*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

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ggatccgccATGTCTGGCGGCGCCGCAGAGAAGCAGAGCAGCACTCCCG
GTTCCCTGTTCCTCTCGCCGCCGGCTCCTGCCCCCAAGAATGGCTCCAG
CTCCGATTCCTCCGTGGGGGAGAAACTGGGAGCCGCGGCCGCCGACGCT
GTGACCGGCAGGACCGAGGAGTACAGGCGCCGCCGCCACACTATGGACA
AGGACAGCCGTGGGGCGGCCGCGACCACTACCACCACTGAGCACCGCTT
CTTCCGCCGGAGCGTCATCTGCGACTCCAATGCCACTGCACTGGAGCTT
CCCGGCCTTCCTCTTTCCCTGCCCCAGCCCAGCATCCCCGCGGCTGTCC
CGCAGAGTGCTCCACCGGAGCCCCACCGGGAAGAGACCGTGACCGCCAC
CGCCACTTCCCAGGTAGCCCAGCAGCCTCCAGCCGCTGCCGCCCCTGGG
GAACAGGCCGTCGCGGGCCCTGCCCCCTCGACTGTCCCCAGCAGTACCA
GCAAAGACCGCCCAGTGTCCCAGCCTAGCCTTGTGGGGAGCAAAGAGGA
GCCGCCGCCGGCGAGAAGTGGCAGCGGCGGCGGCAGCGCCAAGGAGCCA
CAGGAGGAACGGAGCCAGCAGCAGGATGATATCGAAGAGCTGGAGACCA
AGGCCGTGGGAATGTCTAACGATGGCCGCTTTCTCAAGTTTGACATCGA
AATCGGCAGAGGCTCCTTTAAGACGGTCTACAAAGGTCTGGACACTGAA
ACCACCGTGGAAGTCGCCTGGTGTGAACTGCAGGATCGAAAATTAACAA
AGTCTGAGAGGCAGAGATTTAAAGAAGAAGCTGAAATGTTAAAAGGTCT
TCAGCATCCCAATATTGTTAGATTTTATGATTCCTGGGAATCCACAGTA
AAAGGAAAGAAGTGCATTGTTTTGGTGACTGAACTTATGACGTCTGGAA
CACTTAAAACGTATCTGAAAAGGTTTAAAGTGATGAAGATCAAAGTTCT
AAGAAGCTGGTGCCGTCAGATCCTTAAAGGTCTTCAGTTTCTTCATACT
CGAACTCCACCTATCATTCACCGCGATCTTAAATGTGACAACATCTTTA
TCACCGGCCCTACTGGCTCAGTCAAGATTGGAGACCTCGGTCTGGCAAC
CCTGAAGCGGGCTTCTTTTGCCAAGAGTGTGATAGGTACCCCAGAGTTC
ATGGCCCCTGAGATGTATGAGGAGAAATATGATGAATCCGTTGACGTTT
ATGCTTTTGGGATGTGCATGCTTGAGATGGCTACATCTGAATATCCTTA
CTCGGAGTGCCAAAATGCTGCGCAGATCTACCGTCGCGTGACCAGTGGG
GTGAAGCCAGCCAGTTTTGACAAAGTAGCAATTCCTGAAGTGAAGGAAA
TTATTGAAGGATGCATACGACAAAACAAAGATGAAAGATATTCCATCAA
AGACCTTTTGAACCATGCCTTCTTCCAAGAGGAAACAGGAGTACGGGTA
GAATTAGCAGAAtgactcgag
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