Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active WNK3 [1 - 579]

Enzyme description:- WNK3 [1 - 579]

Clone number:- DU 4631

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 92, 572.56 daltons
Average Mass 92, 631.85 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.79

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

WNK3 [1 - 579]

Protein	WNK3 [1 - 579]
Clone number	DU 4631
Species	Human
Accession number	NM_020922
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MATDSGDPAST** **EDSEKPDGISFENRVPQVAATLTVEARLKEKNSTFSASGETVERKRFFR** **KSVEMTEDDKVAESSPKDERIKAAMNIPRVDKLPSNVLRGGQEVKYEQC** **SKSTSEISKDCFKEKNEKEMEEEAEMKAVATSPSGRFLKFDIELGRGAF** **KTVYKGLDTETWVEVAWCELQDRKLTKAEQQRFKEEAEMLKGLQHPNIV** **RFYDSWESILKGKKCIVLVTELMTSGTLKTYLKRFKVMKPKVLRSWCRQ** **ILKGLQFLHTRTPPIIHRDLKCDNIFITGPTGSVKIGDLGLATLMRTSF** **AKSVIGTPEFMAPEMYEEHYDESVDVYAFGMCMLEMATSEYPYSECQNA** **AQIYRKVTSGIKPASFNKVTDPEVKEIIEGCIRQNKSERLSIRDLLNHA** **FFAEDTGLRVELAEEDDCSNSSLALRLWVEDPKKLKGKHKDNEAIEFSF** **NLETDTPEEVAYEMVKSGFFHESDSKAVAKSIRDRVTPIKKTREKKPAG** **CLEERRDSQCKSMGNVFPQPQNTTLPLAPAQQTGAECEETEVDQHVRQQ** **LLQRKPQQHCSSVTGDNLSEAGAASVIHS**
Native sequence	Amino acids M1 – S579 of human WNK3 [end residue is K1800]. Residue M235 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 *Not*1 sites of pGEX6P-1

Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC
TCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC
AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCCCGGAAT
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CCTGATGGAATTTCATTTGAAAACAGAGTTCCCCAGGTCGCTGCAACTTT
GACAGTAGAAGCTAGACTAAAGGAGAAAAACAGTACCTTCTCTGCTTCTG
GGGAAACTGTAGAAAGGAAGAGATTTTTCCGAAAGAGTGTTGAAATGACG
GAAGATGACAAAGTTGCCGAATCATCCCCCAAAGATGAGAGAATTAAGGC
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AACCAAAGCTGAGCAGCAAAGATTCAAGGAAGAAGCAGAGATGTTGAAGG
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GACCTTAAAGACGTACTTAAAACGATTTAAAGTCATGAAACCAAAGGTCT
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CACGGGACCCACTGGATCTGTGAAGATTGGTGATCTAGGATTAGCCACCT
TAATGCGTACCTCATTTGCTAAGAGTGTCATTGGAACTCCTGAGTTTATG
GCTCCAGAGATGTATGAAGAACACTATGATGAATCCGTAGATGTTTATGC
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AGGATGTATTCGTCAAAACAAATCTGAAAGGTTGTCTATCAGGGACCTAT
TAAACCATGCATTTTTTGCTGAGGATACAGGACTGAGGGTGGAGTTAGCA
GAAGAAGATGATTGCTCAAATTCATCCCTTGCTTTAAGACTCTGGGTTGA
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GTATTCCCTCAGCCCCAGAATACAACTTTACCCCTTGCTCCCGCTCAGCA
AACTGGGGCTGAATGTGAAGAAACTGAAGTTGATCAACATGTTAGACAAC
AGCTTCTACAAAGAAAACCACAGCAGCACTGCTCCTCTGTTACAGGTGAC
AATTTGTCTGAGGCAGGAGCTGCATCAGTTATACATTCAtgagcggccgc

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