

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of WNK3 [1 - 579] D294A

Enzyme description:- WNK3 [1 - 579] D294A

Clone number:- DU 27094

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 92, 528.57 daltons

Average Mass 92, 587.84 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.85

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] D294A

<u>Protein</u>	WNK3 [1 - 579] D294A
<u>Clone number</u>	DU 27094
<u>Species</u>	Human
<u>Accession number</u>	NM_020922
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSPEFMATDSGDPAST EDSEKPDGISFENRVPQVAATLTVEARLKEKNSTFSASGETVERKRFFR KSVEMTEDDKVAESSPKDERIKAAMNIPRVDKLP SNVLRGGQEVKYEQC SKSTSEISKDCFK EKNEKEMEEEEAE MKAVATSPSGRFLKFDIELGRGAF KTVYKGLDTETWVEVAWCELODRKLTKAEQQRFKEEAEMLKGLQHPNIV RFYDSWESILKGKKCIVLVTELM TSGTLKTYLKRFKVMKPKVLRSWCRQ ILKGLQFLHTRTPPIIHRDLKCDNIFITGPTGSVKIGALGLATLMRTSF AKSVIGTPEFMAPEMYEEHYDESVDVYAFGMCMLEMATSEYPYSECQNA AQIYRKVTSGIKPASFNKVTDP EVKEIIIEGCIRQNKSERLSIRDLLNHA FFAEDTGLRVELAEEDDCSNSSLALRLWVEDPKKLKGKHKDNEAIEFSF NLETDTP EEVAYEMVKSGFFHESDSKAVAKSIRDRVTP IKKTREKKPAG CLEERRDSQCKSMGNVFPQPQNTTLPLAPAQQTGAECETEVDQHVRQQ LLQRKPQQHCSSVTGDNLSEAGAASVIHS
<u>Native sequence</u>	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. The enzyme has a D294A mutation. Residue D294 is equivalent to A528 of the fusion protein.
<u>Protease cleavage</u>	PreScission (LEVLFQGP) residues 221 - 228
<u>Cloning sites</u>	<i>Eco</i> R1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide Sequence of Insert

gaattcATGGCCACTGATTcAGGGGATCCAGCCAGCACAGAAGATTCTGAGAAACCT
GATGGAATTTcATTTGAAAACAGAGTTCcCCcAGGTCGCTGCAACTTTGACAGTAGAA
GCTAGACTAAAGGAGAAAAACAGTACCTTCTCTGCTTCTGGGGAAACTGTAGAAAG
GAAGAGATTTTTCCGAAAGAGTGTTGAAATGACGGAAGATGACAAAGTTGCCGAAT
CATCCCCCAAAGATGAGAGAAATTAAGGCCGCAATGAATATTCCAAGAGTAGATAAG
CTTCCTTCAAATGTGTTGAGAGGTGGACAAGAAGTTAAATATGAACAGTGTTCAAAG
TCAACCTCAGAAATCTCAAAAGATTGTTTCAAGGAGAAAAATGAAAAGGAAATGGAA
GAAGAAGCAGAAATGAAGGCTGTAGCTACTTCTCCTAGTGGCAGATTCTGAAATTT
GACATAGAACTAGGAAGAGGAGCATTTAAACAGTATATAAAGGACTGGACACTGA
AACATGGGTTGAGGTTGCTTGGTGTGAGCTGCAGGACCGAAAGTTAACCAAAGCTG
AGCAGCAAAGATTCAAGGAAGAAGCAGAGATGTTGAAGGCTCTCCAGCACCCCAAT
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TAGTGACTGAACAAATGACATCTGGGACCTTAAAGACGTACTTAAACGATTTAAAG
TCATGAAACCAAAGGTCTTAAGGAGCTGGTGCAGGCAAATTTTAAAGGGGTTGCAG
TTCTTGCACTAGGACTCCTCCTATTATTCACCGGGACCTGAAGTGTGACAATATT
TTCATCACGGGACCCACTGGATCTGTGAAGATTGGTGTCTAGGATTAGCCACCTTA
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TCTTCATGAAAGTGATTCCAAAGCTGTTGCTAAATCCATTAGAGACCGGGTGACGC
CAATAAAGAAGACAAGAGAGAAGAAGCCTGCTGGCTGTTTGAAGAACGCAGGGA
TTCTCAGTGCAAGTCTATGGGGAATGTATTCCCTCAGCCCCAGAATACAACCTTTACC
CCTTGCTCCCGCTCAGCAAACCTGGGGCTGAATGTGAAGAAACTGAAGTTGATCAAC
ATGTTAGACAACAGCTTCTACAAAGAAAACACAGCAGCACTGCTCCTCTGTTACAG
GTGACAATTTGTCTGAGGCAGGAGCTGCATCAGTTATACATTCAtgagcgccgc

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