

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active GCK [2 - 812]

<u>Enzyme description:-</u>	GCK [2 – 812]
<u>Clone number:-</u>	DU 1760
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	2 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	94, 949.59 daltons
Average Mass	95, 009.63 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.96
<u>Purity:-</u>	75 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
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, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay Buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc	
<u>Substrate:-</u>	
MBP	Final concentration: 0.3 mg/ml
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

GCK [2 - 812]

<u>Protein</u>	GCK [2 – 812]
<u>Clone Number</u>	DU 1760
<u>Species</u>	Human
<u>Accession number</u>	BC047865
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	<p>MSYYHHHHHDYDIPTTENLYFQAMGIRNSKAYVDALLRDVSLQDPRD RFELLQRVGAGTYGDVYKARDTVTSELAAVKIVKLDPGDDISSLQOEIT ILRECRHPNVVAYIGSYLRNDRLWICMEFCGGGSLQEIYHATGPLEERQ IAYVCREALKGLHHLHSQGKIHRDIKGANLLLTLOQDVKLADFGVSGEL TASVAKRRSFIGTPYWMAPEVA AVERKGGYNELCDVWALGITAIELGEL QPPLFHLHPMRALMLMSKSSFQPPKLRDKTRWTQNFHHFLKLALTKNPK KRPTAEKLLQHPFTTQQLPRALLTQLLDKASDPHLGTPSPEDCELETYD MFPDTIHSRGQHGPAERTPSEIQFHQVKFGAPRRKETDPLNEPWEEEW LLGKEELSGSLLQSVQEALEERSLTIRSASEFQELDSPDDTMGTIKRAP FLGPLPTDPPAEPLSSPPGPNSSPLLPTAWATMKQREDPERSSCHGLP PTPKVHMGACFSKVFNGCPLRIHAAVTWIHPVTRDQFLVVGAEEGIYTL NLHELHEDTLEKLI SHRC SWLYCVNNVLLSLSGKSTHIWAHDLPLGLFEQ RRLQOQVPLSIPTNRLTQRIIPRRFALSTKIPDTKGCLOCRVVRNPYTG ATFLLAALPTSLLLQWYEPLOKFLLLKNFSSPLPSPAGMLEPLVLDGK ELPQVCVGAEGPEGPGCRVLFHVLPLEAGLTPDILIPPEGIPGSAQQVI QVDRDTILVSFERCVRIVNMQGEPTATLAPELTFDFPIETVVCLQDSVL AFWSHGMQGRSLDTNEVTQEITDETRIFRVLGAHRDI ILES IPTDNPEA HSNLYILTGHOSTY</p>
<u>Native sequence</u>	<p>Amino acids A2 – Y812 (end) of human GCK. Residue A37 of fusion protein is equivalent to A2 of the native enzyme. The His(6) tag is located at residues 5 – 10.</p>
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Sal</i> 1 and <i>Not</i> 1 site of pFastBAC HTc

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Nucleotide sequence of insert

gtcgacGCGCTGCTGCGGGATGTGTGCTGCAGGACCCGCGGGACCGCT
TCGAGCTGCTGCAGCGCGTGGGGGCCGGGACCTATGGCGACGCTCTACAA
GGCCCGGACACGGTACGTCCTCGAAGTGGCCGCCGTGAAGATAGTCAAG
CTAGGCCAGGGGACGACATCAGCTCCCTCCAGCAGGAAATCACCATCC
TGCGTGAGTGCCGCCACCCCAATGTGGTGGCCTACATTGGCAGCTACCT
CAGGAATGACCGCTTGTGGATCTGCATGGAGTTCTGCGGAGGGGGCTCC
CTGCAGGAGATTTACCATGCCACTGGGCCCTGGAGGAGCGGCAGATTG
CCTACGTCTGCCGAGAGGCACTGAAGGGGCTCCACCACCTGCATTCTCA
GGGAAGATCCACAGAGACATCAAGGGAGCCAACCTTCTCCTCACTCTC
CAGGGAGATGTCAAAGTGGCTGACTTTGGGGTGTGAGGCGAGCTGACAG
CGTCTGTGGCCAAGAGGAGGTCTTTCATTGGGACTCCCTACTGGATGGC
TCCCGAGGTGGCTGCTGTGGAGCGCAAAGGTGGCTACAATGAGCTATGT
GACGTCTGGGCCCTGGGCATCACTGCCATTGAGCTGGGCGAGCTGCAGC
CCCCTCTGTTCCACCTGCACCCCATGAGGGCCCTGATGCTCATGTCGAA
GAGCAGCTTCCAGCCGCCCAAAGTGGAGATAAGACTCGCTGGACCCAG
AATTTCCACCACTTTCTCAAAGTGGCCCTGACCAAGAATCCTAAGAAGA
GGCCGACAGCAGAGAAGCTCCTGCAGCACCCGTTACGACTCAGCAGCT
CCCTCGGGCCCTCCTCACACAGCTGCTGGACAAAGCCAGTGACCCTCAT
CTGGGGACCCCTCCCTGAGGACTGTGAGCTGGAGACCTATGACATGT
TTCCAGACACCATTCACTCCCGGGGGCAGCACGGCCCGAGCCGAGAGGAC
CCCCTCGGAGATCCAGTTTACCAGGTGAAATTTGGCGCCCCACGCAGG
AAGGAAACTGACCCACTGAATGAGCCGTGGGAGGAAGAGTGGACACTAC
TGGGAAAGGAAGAGTTGAGTGGGAGCCTGCTGCAGTCGGTCCAGGAGGC
CCTGGAGGAAGGAGTCTGACTATTCGGTCAGCCTCAGAATTCAGGAG
CTGGACTCCCCAGACGATACCATGGGAACCATCAAGCGGGCCCCGTTCC
TAGGGCCACTCCCCACTGACCCTCCAGCAGAGGAGCCTCTGTCCAGTCC
CCCAGGCCCAACAGCTCCCCACTGCTGCCACGGCCTGGGCCACCATG
AAGCAGCGGGAGGATCCTGAGAGGTCATCCTGCCACGGGCTCCCCCAA
CTCCCAAGGTGCATATGGGCGCCTGCTTCTCCAAGGTCTTCAATGGCTG
CCCCCTGCGGATCCACGCTGCTGTCACCTGGATTACCCCTGTTACTCGG
GACCAGTTCCTGGTGGTAGGGGCCGAGGAAGGCATCTACACACTCAACC
TGCATGAACTGCATGAGGATACGCTGGAGAAGCTGATTTACATCGCTG
CTCCTGGCTCTACTGCGTGAACAACGTGCTGCTGTCACTCTCAGGGAAA
TCCACGCACATCTGGGCCATGACCTCCAGGCCTGTTTGAGCAGCGGA
GGCTACAGCAACAGGTTCCCCTCTCCATCCCCACCAACCGCTCACCCA
GCGCATCATCCCCAGGCGTTTGCTCTGTCCACCAAGATTCCTGACACC
AAAGGCTGCTTGCAGTGTCTGTGGTGCAGAACCCCTACACGGGTGCCA
CCTTCCTGCTGGCCGCCCTGCCACAGCCTGCTCCTGCTGCAGTGGTA
TGAGCCGCTGCAGAAGTTTCTGCTGCTGAAGAACTTCTCCAGCCCTCTG
CCCAGCCAGCTGGGATGCTGGAGCCGCTGGTGTGCTGGATGGGAAGGAGC
TGCCGCAGGTGTGTGTTGGGGCCGAGGGGCTGAGGGGCCCGGCTGCCG
CGTCTGTTCCATGTCTGCCCCCTGGAGGCTGGCCTGACGCCCGACATC
CTCATCCCACCTGAGGGGATCCCAGGCTCGGCCAGCAGGTGATCCAGG
TGGACAGGGACACAATCCTAGTCAGCTTTGAACGCTGTGTGAGGATTGT
CAACATGCAGGGCGAGCCCACGGCCACACTGGCACCTGAGCTGACCTTT
GATTTCCCATCGAGACTGTGGTGTGCCTGCAGGACAGTGTGCTGGCCT
TCTGGAGCCATGGGATGCAAGGCCGAAGCCTGGATAACCAATGAGGTGAC

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CCAGGAGATCACAGATGAAACAAGGATCTTCCGAGTGCTTGGGGCCCAC
AGAGACATCATCCTGGAGAGCATTCCCCTGACAACCCAGAGGCGCACA
GCAACCTCTACATCCTCACGGGCCACCAGAGCACCTACTaagcggccgc