

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

Calculated molecular mass:-

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

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, 0.2 mM PMSF, 1 mM Benzamidine.

<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
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<u>Assay:-</u>	Standard filter binding assay
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Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

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]

Protein GCK [2 – 812]

Clone Number DU 1760

Species Human

Accession number BC047865

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGMGIRNSKAYVDALLRDVSLQDPRD
RFELLQRVGAGTYGDVYKARDTVTSELAAVKIVKLDPGDDISSLQOEIT
ILRECRHPNVVAYIGSYLRNDRLWICMEFCGGGSLQEIYHATGPLEERQ
IAYVCREALKGLHHLHSQGKIHRDIKGANLLLTLOGDVKLADFGVSGEL
TASVAKRRSFIGTPYWMAPEVA AVERKGGYNELCDVWALGITAIELGEL
QPPLFHLHPMRALMLMSKSSFQPPKLRDKTRWTQNFHHFLKLALTKNPK
KRPTAEKLLQHPFTTQQLPRALLTQLLDKASDPHLGTPSPEDCELETYD
MFPDTIHSRGQHGPAERTPSEIQFHQVKFGAPRRKETDPLNEPWEEEW
LLGKEELSGSLLQSVQEALEERSLTIRSASEFQELDSPDDTMGTIKRAP
FLGPLPTDPPAEEPLSSPPGPNSSPLLPTAWATMKQREDPERSSCHGLP
PTPKVHMGACFSKVFNGCPLRIHAAVTWIHPVTRDQFLVVGAEEGIYTL
NLHELHEDTLEKLI SHRC SWLYCVNNVLLSLSGKSTHIWAHDLPGLFEQ
RRLQOQVPLSIPTNRLTQRIIPRRFALSTKIPDTKGCLOCRVVRNPYTG
ATFLLAALPTSLLLLQWYEPLOKFLLLKNFSSPLPSPAGMLEPLVLDGK
ELPQVCVGAEGPEGPGCRVLFHVLPLEAGLTPDILIPPEGIPGSAQQVI
QVDRDTILVSFERCVRIVNMQGEPTATLAPELTFDFPIETVVCLQDSVL
AFWSHGMQGRSLDTNEVTQEITDETRIFRVLGAHRDIILES IPTDNPEA
HSNLYILTGHOSTY

Native sequence 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.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Sal*1 and *Not*1 site of pFastBAC HTc

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**Nucleotide
sequence of
insert**

gtcgacGCGCTGCTGCGGGATGTGTGCTGCGCTGCAGGACCCGCGGGACCGCT
TCGAGCTGCTGCAGCGCGTGGGGGCCGGGACCTATGGCGACGTCTACAA
GGCCCGGACACGGTACGTCCTCGAAGTGGCCGCCGTGAAGATAGTCAAG
CTAGGCCAGGGGACGACATCAGCTCCCTCCAGCAGGAAATCACCATCC
TGCGTGAGTGCCGCCACCCCAATGTGGTGGCCTACATTGGCAGCTACCT
CAGGAATGACCGCTTGTGGATCTGCATGGAGTTCTGCGGAGGGGGCTCC
CTGCAGGAGATTTACCATGCCACTGGGCCCTGGAGGAGCGGCAGATTG
CCTACGTCTGCCGAGAGGCACTGAAGGGGCTCCACCACCTGCATTCTCA
GGGAAGATCCACAGAGACATCAAGGGAGCCAACCTTCTCCTCACTCTC
CAGGGAGATGTCAAAGTGGCTGACTTTGGGGTGTGAGGCGAGCTGACAG
CGTCTGTGGCCAAGAGGAGGTCTTTCATTGGGACTCCCTACTGGATGGC
TCCCGAGGTGGCTGCTGTGGAGCGCAAAGGTGGCTACAATGAGCTATGT
GACGTCTGGGCCCTGGGCATCACTGCCATTGAGCTGGGCGAGCTGCAGC
CCCCTCTGTTCCACCTGCACCCCATGAGGGCCCTGATGCTCATGTCGAA
GAGCAGCTTCCAGCCGCCCAAAGTGGAGATAAGACTCGCTGGACCCAG
AATTTCCACCACTTTCTCAAAGTGGCCCTGACCAAGAATCCTAAGAAGA
GGCCGACAGCAGAGAAGCTCCTGCAGCACCCGTTACGACTCAGCAGCT
CCCTCGGGCCCTCCTCACACAGCTGCTGGACAAAGCCAGTGACCCTCAT
CTGGGGACCCCTCCCTGAGGACTGTGAGCTGGAGACCTATGACATGT
TTCCAGACACCATTCACTCCCGGGGGCAGCACGGCCAGCCGAGAGGAC
CCCCTCGGAGATCCAGTTTACCAGGTGAAATTTGGCGCCCCACGCAGG
AAGGAAACTGACCCACTGAATGAGCCGTGGGAGGAAGAGTGGACACTAC
TGGGAAAGGAAGAGTTGAGTGGGAGCCTGCTGCAGTCGGTCCAGGAGGC
CCTGGAGGAAGGAGTCTGACTATTCGGTCAGCCTCAGAATTCAGGAG
CTGGACTCCCCAGACGATACCATGGGAACCATCAAGCGGGCCCCGTTCC
TAGGGCCACTCCCCACTGACCCTCCAGCAGAGGAGCCTCTGTCCAGTCC
CCCAGGCCCAACAGCTCCCCACTGCTGCCACGGCCTGGGCCACCATG
AAGCAGCGGGAGGATCCTGAGAGGTCATCCTGCCACGGGCTCCCCCAA
CTCCCAAGGTGCATATGGGCGCCTGCTTCTCCAAGGTCTTCAATGGCTG
CCCCCTGCGGATCCACGCTGCTGTACCTGGATTACCCCTGTTACTCGG
GACCAGTTCCTGGTGGTAGGGGCCGAGGAAGGCATCTACACACTCAACC
TGCATGAACTGCATGAGGATACGCTGGAGAAGCTGATTTACATCGCTG
CTCCTGGCTCTACTGCGTGAACAACGTGCTGCTGCTCACTCTCAGGGAAA
TCCACGCACATCTGGGCCATGACCTCCAGGCCTGTTTGAGCAGCGGA
GGCTACAGCAACAGGTTCCCCTCTCCATCCCCACCAACCGCTCACCCA
GCGCATCATCCCCAGGCGTTTGCTCTGTCCACCAAGATTCCTGACACC
AAAGGCTGCTTGCAGTGTCTGTGGTGCAGGAAACCCCTACACGGGTGCCA
CCTTCCTGCTGGCCGCCCTGCCACAGCCTGCTCCTGCTGCAGTGGTA
TGAGCCGCTGCAGAAGTTTCTGCTGCTGAAGAACTTCTCCAGCCCTCTG
CCCAGCCAGCTGGGATGCTGGAGCCGCTGGTGTGCTGGATGGGAAGGAGC
TGCCGCAGGTGTGTGTTGGGGCCGAGGGGCTGAGGGGCCCGGCTGCCG
CGTCTGTTCATGTCTGCCCCCTGGAGGCTGGCCTGACGCCCGACATC
CTCATCCCACCTGAGGGGATCCCAGGCTCGGCCAGCAGGTGATCCAGG
TGGACAGGGACACAATCCTAGTCAGCTTTGAACGCTGTGTGAGGATTGT
CAACATGCAGGGCGAGCCCACGGCCACACTGGCACCTGAGCTGACCTTT
GATTTCCCATCGAGACTGTGGTGTGCTGCTGCAGGACAGTGTGCTGGCCT
TCTGGAGCCATGGGATGCAAGGCCGAAGCCTGGATAACCAATGAGGTGAC

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CCAGGAGATCACAGATGAAACAAGGATCTTCCGAGTGCTTGGGGCCCAC
AGAGACATCATCCTGGAGAGCATTCCCCTGACAACCCAGAGGCGCACA
GCAACCTCTACATCCTCACGGGCCACCAGAGCACCTACTaagcgccgc