

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Diacylglycerol Kinase beta isoform 1 [1 - 804]

<u>Enzyme description:-</u>	DGK beta [1 - 804]
<u>Clone number:-</u>	DU 9153
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	4 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	95, 295.55 daltons
Average Mass	95, 357.49 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.70
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	50 mM HEPES pH 7.0, 150 mM NaCl, 20 % glycerol, 5 mM DTT
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	ADP Glo
<u>Assay buffer:-</u>	20 mM Tris-HCl, 67 mM KCl, 1 mM DTT, 0.05 mg/ml BSA, 10 mM MgCl ₂
<u>Substrate:-</u>	Diacylglycerol Final concentration: 0.1 mM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

Diacylglycerol Kinase beta isoform 1 [1 – 804]

Protein DGK beta [1 - 804]

Clone number DU 9153

Species Human

Accession number NM_004080.2

Tags N-terminal His(6)

Baculovirus
Expressed protein

MSYYHHHHHDYDIPTTENLYFQ**G**AMGIRNSKAYVDELTSMT**NQ**EKWAH
LSPSEFSQ**L**QKYAEYST**KK**LKDVLEEFHGNGVLAKYNPEG**KQ**DIL**NT**I
DFEGFKLF**M**KTFLEAELPDD**F**TAHLFMSFS**N**KFP**H**SSPMVKS**K**PALLSG
GLRMNKGAI**T**PP**R**TTSPANTCSPEVIHLKD**I**VCYLS**L**LERGR**P**ED**K**LEF
MFRLYDTDGNGFLDSSELENIISQ**M**MHVAEYLEWDVTELNPILHE**M**EE
IDYDHDGTVSLEEWI**Q**GG**M**TTI**P**LLVLLGLENNV**K**DDG**Q**HVWRL**K**HFNK
PAYCNLCL**N**MLIGVG**K**QGLCCSFCKY**T**VERC**V**ARAPP**S**CI**K**TY**V**KSKR
NTD**V**MHHYWVEGNCPT**K**CDK**C**HKT**V**KCY**Q**GLTGLHC**V**WC**Q**ITL**H**NKCAS
HL**K**PECD**C**GPL**K**DHIL**P**PT**T**IC**P**VVL**Q**TL**P**TS**G**VS**V**PEER**Q**ST**V**K**K**E**S**
GS**Q**Q**P**N**K**VID**K**N**K**M**Q**RANS**V**TV**D**G**Q**GL**Q**VT**P**VP**G**TH**P**LL**V**F**V**NP**K**SG**G**K
Q**G**ER**I**Y**R**K**F**Q**Y**LL**N**PR**Q**V**S**LS**G**NG**P**MP**G**LN**F**FR**D**VP**D**FR**V**LAC**G**GD**G**T
V**G**W**V**LD**C**IE**K**AN**V**G**K**HP**P**V**A**IL**P**LG**T**GN**D**LAR**C**LR**W**GG**G**Y**E**GEN**L**M**K**IL
K**D**IEN**S**TE**I**ML**D**R**W**K**F**EV**I**P**N**D**K**DE**K**GD**P**VP**S**I**I**NN**Y**F**S**IG**V**D**A**S**I**A**H**
R**F**H**I**M**R**E**K**H**P**E**K**F**N**S**R**M**K**N**K**FW**Y**FE**F**GT**S**ET**F**S**A**T**C**CK**L**H**S**VE**I**EC**D**G
V**Q**ID**L**IN**I**S**L**E**G**I**A**IL**N**IP**S**M**H**GG**S**N**L**W**G**ES**K**RR**S**HR**R**IE**K**KG**S**D**K**R**T**
T**V**T**D**A**K**EL**K**F**A**S**Q**D**L**S**D**Q**L**LE**V**V**G**LE**G**A**M**EM**G**Q**I**Y**T**GL**K**S**A**G**R**RL**A**Q**C**S
C**V**V**I**R**T**SK**S**L**P**M**Q**ID**G**EP**W**M**Q**TP**C**TI**K**IT**H**KN**Q**AP**M**L**M**GP**P**PK**T**GL**F**CS
L**V**K**R**TR**N**RS**K**E

Native sequence Amino acids M1 – E804 (end) of human DGK beta isoform 1.
Residue M41 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (**ENLYFQG**) residues 18 – 24

Cloning sites *Spe*1 and *Kpn*1 of pFastBac HTc

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**Nucleotide
of sequence of
insert**

actagtATGACAAACCAGGAAAAATGGGCCACCTCAGCCCTTCGGAAT
TTTCCCAACTTCAGAAATATGCTGAGTATTCTACAAAGAAATTAAGGA
TGTTCTTGAAGAATTCATGGTAATGGTGTGCTTGCAAAGTATAATCCT
GAAGGAAACAAGACATTCCTAACCACCAATAGATTTTGAAGGTTTCA
AACTATTCATGAAGACATTCCTGGAAGCCGAGCTTCTGATGATTTTAC
TGCACACCTTTTCATGTCATTTAGCAACAAGTTTCTCATTCTAGTCCA
ATGGTAAAAAGTAAGCCTGCTCTCCTATCAGGCGGTCTGAGAATGAATA
AAGGTGCCATCACCCCTCCCGAACTACTTCTCCTGCAAATACGTGTTT
CCCAGAAGTAATCCATCTGAAGGACATTTGTCTGTTACCTGTCTCTGCTT
GAAAGAGGAAGACCTGAGGATAAGCTTGAGTTTATGTTTTCGCTTTTATG
ACACGGATGGGAATGGCTTCTGACAGCTCGGAGCTAGAAAATATCAT
CAGTCAGATGATGCATGTTGCAGAATACCTTGAGTGGGATGTCACTGAA
CTTAATCCAATCCTCCATGAAATGATGGAAGAAATTGACTATGATCATG
ATGGAACCGTGTCTCTGGAGGAATGGATTCAAGGAGGAATGACAACGAT
TCCACTTCTTGTGCTCCTGGGCTTAGAAAATAACGTGAAGGATGATGGA
CAGCACGTGTGGCGACTGAAGCACTTTAAACAAACCTGCCTATTGCAACC
TTTGCCTGAACATGCTGATTGGCGTGGGAAGCAGGGCCTCTGCTGTTT
CTTCTGCAAGTACACAGTCCATGAGCGCTGTGTGGCTCGAGCACCTCCC
TCTTGCATCAAGACCTATGTGAAGTCCAAAAGGAACACTGATGTCATGC
ACCATTACTGGGTTGAAGTAACTGCCCAACCAAGTGTGATAAGTGCCA
CAAACTGTTAAATGTTACCAGGGCCTGACAGGACTGCATTGTGTTTGG
TGTCAGATCACACTGCATAATAAATGTGCTTCTCATCTAAAACCTGAAT
GTGACTGTGGACCTTTGAAGGACCATATTTTACCACCACAACAATCTG
TCCAGTGGTACTGCAGACTCTGCCACTTCAGGAGTTTTCAGTTTCTGAG
GAAAGACAATCAACAGTGAAAAGGAAAAGAGTGGTTCCAGCAGCCAA
ACAAAGTGATTGACAAGAATAAAATGCAAAGAGCCAACCTCTGTTACTGT
AGATGGACAAGGCCTGCAGGTCCTCCTGTGCCTGGTACTCACCCACTT
TTAGTTTTTGTGAACCCCAAAAGTGGTGGAAAACAAGGAGAACGAATTT
ACAGAAAATTCAGTATCTATTAATCCTCGTCAGGTTTACAGTCTTTT
TGGAATGGACCAATGCCAGGGTTAAACTTTTTTCCGTGATGTTTCTGAC
TTCAGAGTGTAGCCTGTGGTGGAGATGGAACCGTGGGCTGGGTTTTGG
ATTGCATAGAAAAGGCCAATGTAGGCAAGCATCCTCCAGTTGCGATTCT
GCCTCTTGGGACTGGCAATGATCTAGCAAGATGCCTGCGATGGGGAGGA
GGTTACGAAGGTGAGAATCTGATGAAAATTTCTAAAAGACATTGAAAACA
GCACAGAAATCATGTTGGACAGGTGGAAGTTTGAAGTCATACCTAATGA
CAAAGATGAGAAAGGAGACCCAGTGCCTTACAGTATCATCAATAATTAC
TTTTCCATTGGCGTGGATGCCTCCATTGCACACAGATTCCACATCATGA
GAGAAAAACACCAGAGAAATTCACAGTAGAATGAAGAACAATTTTG
GTATTTTTGAGTTTGGCACATCTGAACTTTCTCAGCCACCTGCAAGAAG
CTACATGAATCTGTAGAAATAGAATGTGATGGAGTACAGATAGATTTAA
TAAACATCTCTCTGGAAGGAATTGCTATTTTGAATATAACCAAGCATGCA
TGGAGGATCCAATCTTTGGGGAGAGTCTAAGAAAAGACGAAGCCATCGA
CGAATAGAGAAAAAAGGGTCTGACAAAAGGACCACCGTCACAGATGCCA
AAGAGTTGAAGTTTGAAGTCAAGATCTCAGTGACCAGCTGCTGGAGGT
GGTCGGCTTGAAGGAGCCATGGAGATGGGGCAAATATACACAGGCCTG
AAAAGTGTGGCCGGCGGCTGGCTCAGTGTTCCTGCGTGGTTCATCAGGA
CGAGCAAGTCTCTGCCAATGCAAATTGATGGGGAGCCATGGATGCAGAC

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CCCATGCACAATAAAAATTACACACAAGAACCAAGCCCAATGCTGATG
GGCCCGCCTCCAAAACCGGTTTATTCTGCTCCCTCGTCAAAGGACAA
GAAACCGAAGCAAGGAAtaggtacc