

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Diacylglycerol Kinase zeta [1 -929]

<u>Enzyme description:-</u>	DGK zeta [1 - 929]
<u>Clone number:-</u>	DU 19618
<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>Calculated molecular mass:-</u>	
Monoisotopic	107, 412.86 daltons
Average Mass	107, 480.73 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.16
<u>Purity:-</u>	>80 %
<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

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Clone Data Sheet

Diacylglycerol Kinase zeta [1 - 929]

Protein DGK zeta [1 - 929]

Clone number DU 19618

Species Human

Accession number BC041770.1

Tags N-terminal His(6)

Baculovirus expressed protein

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MSYHHHHHHDYDIPTTENLYFQGAMGSMEPRDGSPEARSSDSESASAS
SSGSERDAGPEPDKAPRRLNKRFPGLRFLGHRKAITKSGLQHLAPPPP
TPGAPCSESERQIRSTVDWSESATYGEHIWFETNVSGDFCYVGEQYCVA
RMLQKSVSRRKCAACKIVVHTPCIEQLEKINFRCKPSFRESGSRNVREP
TFVRRHHVHRRRQDGKCRHCGKGFQOKFTFHSKEIVAISCSWCKQAYHS
KVSCFMLQQIEEPCSLGVHAAVVIPTWILRARRPQNTLKASKKKKRAS
FKRKSSKKGPEEGRWRPFIIRPTPSPLMKPLLVFVNPKSGGNQGAKIQ
SFLWYLNPRQVFDLSQGGPKEALEMYRKVHNLRLACGGDGTVGWILST
LDQLRLKPPPPVAILPLGTGNDLARTLNWGGGYTDEPVSILSHVEEGN
VVQLDRWDLHAEPNPEAGPEDRDEGATDRLPLDFVNNYFSLGFDHAVTL
EFHESREANPEKFNFRNKMFYAGTAFSDFLMGSSKDLAKHIRVVC DG
MDLTPKIQDLKPKCVVFLNIPRYCAGTMPWGHGPEHHDFEPQRHDDGYL
EVI GFTMTSLAALQVGGHGERLTQCREVLTTSKAI PVQVDGEPCKLAA
SRIRIALRNQATMVQKAKRRSAAPLHSDQQPVEQLRIQVSRVSMHDYE
ALHYDKEQLKEASVPLGTVVVPGSDLELCRAHIERLQOEPDGAGAKSP
TCQKLSPKWCFLDATTASRFYRIDRAQEHNLNYVTEIAQDEIYILDPELL
GASARPDLP TPTSPLPTSPCSPTPRSLQDAAPPQGEELIEAAKRNDFC
KLQELHRAGGDLHRDEQSRTLLHAVSTGSKDVVRYLLDHAPPEILDA
VEENGETCLHQAAALGQRTICHYIVEAGASLMKTDQOQDTPRQRAEKAQ
DTELAAYLENRQHYQMIQREDQETAV
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Native sequence Amino acids M1 – V929 (end) of human DGK zeta.
Residue M29 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 *Bgl*II - *Not*I to *Bam*H1 - *Not*I sites in pFastBAC HTb

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Nucleotide sequence of insert

agatctATGGAGCCGCGGGACGGTAGCCCCGAGGCCCGGAGCAGCGACT
CCGAGTCGGCTTCCGCCTCGTCCAGCGGCTCCGAGCGCGACGCCGGTCC
CGAGCCGGACAAGGCGCCGCGGGCGACTCAACAAGCGGGCGCTTCCCGGGG
CTGCGGCTCTTCGGGCACAGGAAAGCCATCACGAAGTCGGGCCTCCAGC
ACCTGGCCCCCCTCCGCCACCCCTGGGGCCCCGTGCAGCGAGTCAGA
GCGGCAGATCCGGAGTACAGTGGACTGGAGCGAGTCAGCGACATATGGG
GAGCACATCTGGTTCGAGACCAACGTGTCCGGGGACTTCTGCTACGTTG
GGGAGCAGTACTGTGTAGCCAGGATGCTGCAGAAGTCAGTGTCTCGAAG
AAAGTGCGCAGCCTGCAAGATTGTGGTGCACACGCCCTGCATCGAGCAG
CTGGAGAAGATAAAATTTCCGCTGTAAGCCGTCTTCCGTGAATCAGGCT
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CGCAAAGTGACAACCTGCGGATCCTGGCGTGCAGGGGGCGACGGCACGG
TGGGCTGGATCCTCTCCACCCTGGACCAGCTACGCCTGAAGCCGCCACC
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TCCGAGTGGTGTGTGATGGAATGGACTTGACTCCCAAGATCCAGGACCT
GAAACCAAGTGTGTTGTTTTCTGAACATCCCAGGTAAGTGTGCGGGC
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GAGGTGGTGTGCTCACCACATCCAAGGCCATCCCGGTGCAGGTGGATGGCG
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AGCGACCAGCAGCCGGTGCAGAGCAGTTGCGCATCCAGGTGAGTCGCG
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GGAGGCCCTCTGTGCCGCTGGGCACTGTGGTGGTCCCAGGAGACAGTGAC
CTAGAGCTCTGCCGTGCCACATGAGAGACTCCAGCAGGAGCCCGATG
GTGCTGGAGCCAAGTCCCCGACATGCCAGAACTGTCCCCAAGTGGTG
CTTCTGGACGCCACCCTGCCAGCCGCTTCTACAGGATCGACCGAGCC
CAGGAGCACCTCAACTATGTGACTGAGATCGCACAGGATGAGATTTATA
TCCTGGACCCTGAGCTGCTGGGGGCATCGGCCCGGCTGACCTCCAAC
CCCCACTTCCCCTCTCCCCACCTCACCTGCTCACCCACGCCCCGGTCA
CTGCAAGGGGATGCTGCACCCCTCAAGGTGAAGAGCTGATTGAGGCTG
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CGACCTCATGCACCGAGACGAGCAGAGTCGCACGCTCCTGCACCACGCA
GTCAGCACTGGCAGCAAGGATGTGGTCCGCTACCTGCTGGACCACGCC
CCCCAGAGATCCTTGATGCGGTGGAGGAAAACGGGGAGACCTGTTTGCA

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CCAAGCAGCGGCCCTGGGCCAGCGCACCATCTGCCACTACATCGTGGAG
GCCGGGGCCTCGCTCATGAAGACAGACCAGCAGGGCGACACTCCCCGGC
AGCGGGCTGAGAAGGCTCAGGACACCGAGCTGGCCGCCTACCTGGAGAA
CCGGCAGCACTACCAGATGATCCAGCGGGAGGACCAGGAGACGGCTGTG
taggcggccgc