

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Diacylglycerol Kinase gamma [1 -752]

<u>Enzyme description:-</u>	DGK gamma [1 - 752]
<u>Clone number:-</u>	DU 19538
<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	88, 804.77 daltons
Average Mass	88, 862.29 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.08
<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 gamma [1 - 752]

<u>Protein</u>	DGK gamma [1 - 752]
<u>Clone number</u>	DU 19538
<u>Species</u>	Human
<u>Accession number</u>	BC112363
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMGIRNSKAYVDMGEERWVSLTPEE FDQLQKYSEYSSKKIKDALTEFNEGGSLKQYDPHEPISYDVFKLFMRAY LEVDPQLPLSTHLFLAFSOKPRHETSDHPTEGASNSEANSADTNIQNAD NATKADEACAPDTESNMAEKQAPAEDQVAASPLEPPVPRSSSSSESPVVY LKDVCYLSLLETGRPQDKLEFMFRLYDSDENGLLDQAEMDCIVNQLH IAQYLEWDPTELRPILKEMLQGM DYDRDGFVSLQEWVHGGMTTIPLLVL LGMDDSGSGDGRHAWTMKHFKKPTYCNFCHIMLMGVRKQGLCCTYCKY TVHERCVSKNIPGCVKTYSAKRSGEFHRKCELSTLCDGGELRDHILLP TSICPITRDRPGEKSDGCVSAKGELVMQYKIIPTPGTHPLLVLVNP KSG GRQGERILRK FHYLLNPKQVFNL DNGGPTPGLNFFRDTPDFRVLACGGD GTVGWILDCIDKANFAKHPPVAVLPLGTGNDLARCLRWGGGYEGGSLTK ILKDIEQSPLVMLDRWHLEVIPREEVENGDQVPYSIMNNYFSIGVDASI AHRFHVMREKHPEKFNSRMKNKLWYFEFGTSETFAATCKKLHDHIELEC DVGVDLSNIFLEGIAILNIPSMYGGTNLWGENKKNRAVIRESRKGVTD PKELKFCVQDLSDQLLEVVGLEGAMEMGQIYTGLKSAGRRLAQCASVTI RTNKLLPMQVDGEPWMQPCCTIKITHKNQAPMMMGPPQKSSFFSLRRKS RSKD
<u>Native sequence</u>	Amino acids M1 – D752 (end) of human DGK gamma. Residue M26 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 – 24
<u>Cloning sites</u>	<i>Sal</i> I and <i>Not</i> I sites in pFastBAC HTc

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Nucleotide sequence of insert

gtcgcacATGGGTGAAGAACGGTGGGTCTCCCTCACTCCAGAAGAATTTG
ACCAACTCCAGAAATATTGAGAATATTCCTCCAAGAAGATAAAAAGATGC
CTTGACTGAATTTAATGAGGGTGGGAGCCTCAAACAATATGACCCACAT
GAGCCGATTAGCTATGATGTCTTCAAGCTGTTTCATGAGGGCGTACCTAG
AGGTGGACCTTCCCCAGCCACTGAGCACTCACCTCTTCCTGGCCTTCAG
CCAGAAGCCCAGACACGAGACCTCTGACCACCCGACGGAGGGAGCCAGC
AACAGTGAGGCCAACAGCGCAGATACTAATATACAGAATGCAGATAATG
CCACCAAAGCAGACGAGGCCTGTGCCCCCTGATACTGAATCAAATATGGC
TGAGAAGCAAGCACCAGCTGAAGACCAAGTGGCTGCGAGCCCCCTGGAA
CCCCCGTCCCTCGGTCTTCAAGCTCGGAATCCCCAGTGGTGTACCTGA
AGGATGTTGTGTGCTACCTGTCCCTGCTGGAGACGGGGAGGCCTCAGGA
TAAGCTGGAGTTCATGTTTCGCCTCTATGATTCAGATGAGAACGGTCTC
CTGGACCAAGCGGAGATGGATTGCATTGTCAACCAAATGCTGCATATTG
CCCAGTACCTGGAGTGGGATCCACAGAGCTGAGGCCTATATTGAAGGA
GATGCTGCAAGGGATGGACTACGACCGGGACGGCTTTGTGTCTCTACAG
GAATGGGTCCATGGAGGGATGACCACCATCCCATTGCTGGTCCCTCCTGG
GGATGGATGACTCTGGCTCCAAGGGGGATGGGCGGCACGCCTGGACCAT
GAAGCACTTCAAGAAACCAACCTACTGCAACTTCTGCCATATCATGCTC
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CAAACAAGCTGCTGCCAATGCAAGTGGATGGAGAACCCTGGATGCAGCC
ATGTTGCACGATTAAAATTACTCACAAGAACCAAGCGCCCATGATGATG
GGGCTCCCCAGAAGAGCAGCTTCTCTCGTTGAGAAGGAAGAGCCGTT
CAAAAGACtaagcgccgc