Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Diaglycerol Kinase Gamma [1 – 752]

Enzyme description:- DGK gamma [1 – 752]

Clone number:- DU 63057

Source:- Recombinant

Expression system:- Baculovirus Expression Vector System

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	112, 078.89 daltons
Average Mass	112, 151.66 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.97

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay: ADP Glo

Assay buffer:-
20 mM Tris-HCl, 67 mM KCl, 1 mM DTT, 0.05 mg/ml BSA, 10 mM $MgCl_2$

Substrate:-
Diacylglycerol Final concentration: 0.1 mM

Clone Data Sheet

DGK gamma [1 – 752]

Protein	DGK gamma [1 – 752]
Clone number	DU 63057
Species	Human
Accession number	BC112363
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEFPGRL**MGEERWVSLTPEEFDQLQKYSEYSSKKIKDALTEFNEGGSLKQYDPHEPISYDVFKLFMRAYLEVDLPQPLSTHLFLAFSQKPRHETSDHPTEGASNSEANSADTNIQNADNATKADEACAPDTESNMAEKQAPAEDQVAASPLEPPVPRSSSSESPVVYLKDVVCYLSLLETGRPQDKLEFMFRLYDSDENGLLDQAEMDCIVNQMLHIAQYLEWDPTELRPILKEMLQGMDYDRDGFVSLQEWVHGGMTTIPLLVLLGMDDSGSKGDGRHAWTMKHFKKPTYCNFCHIMLMGVRKQGLCCTYCKYTVHERCVSKNIPGCVKTYSKAKRSGEFHRKCELSTLCDGGELRDHILLPTSICPITRDRPGEKSDGCVSAKGELVMQYKIIPTPGTHPLLVLVNPKSGGRQGERILRKFHYLLNPKQVFNLDNGGPTPGLNFFRDTPDFRVLACGGDGTVGWILDCIDKANFAKHPPVAVLPLGTGNDLARCLRWGGGYEGGSLTKILKDIEQSPLVMLDRWHLEVIPREEVENGDQVPYSIMNNYFSIGVDASIAHRFHVMREKHPEKFNSRMKNKLWYFEFGTSETFAATCKKLHDHIELECDGVGVDLSNIFLEGIAILNIPSMYGGTNLWGENKKNRAVIRESRKGVTDPKELKFCVQDLSDQLLEVVGLEGAMEMGQIYTGLKSAGRRLAQCASVTIRTNKLLPMQVDGEPWMQPCCTIKITHKNQAPMMMGPPQKSSFFSLRRKSRSKD**
Native sequence	Amino acids M1 – D752 (end) of human DGK gamma. Residue M239 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Sal*1 + 2/*Hind*III sites into pFastBacDual GST

Nucleotide Sequence of Insert:

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gtcgactcATGGGTGAAGAACGGTGGGTCTCCCTCACTCCAGAAGAATTTGACCAACTCCAGAAAT
ATTCAGAATATTCCTCCAAGAAGATAAAAGATGCCTTGACTGAATTTAATGAGGGTGGGAGCCTCA
AACAATATGACCCACATGAGCCGATTAGCTATGATGTCTTCAAGCTGTTCATGAGGGCGTACCTAG
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ACAAGCTGCTGCCAATGCAAGTGGATGGAGAACCCTGGATGCAGCCATGTTGCACGATTAAAATTA
CTCACAAGAACCAAGCGCCCATGATGATGGGGCCTCCCCAGAAGAGCAGCTTCTTCTCGTTGAGAA
GGAAGAGCCGTTCAAAAGACtaagct
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