

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Diacylglycerol Kinase Alpha [1 – 735]

<u>Enzyme description:-</u>	DGK alpha [1 – 735]
<u>Clone number:-</u>	DU 39323
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus Expression Vector System
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 110, 387.83 daltons
Average Mass 110, 459.52 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.16

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 MgCl₂

Substrate:-

Diacylglycerol Final concentration: 0.1 mM

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Clone Data Sheet

DGK alpha [1 – 735]

<u>Protein</u>	DGK alpha [1 – 735]
<u>Clone number</u>	DU 39323
<u>Species</u>	Human
<u>Accession number</u>	BC031870.2
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSPGIPGSTRAAMAK ERGLISPSDFAQLQKYMEYSTKKVSDVLKLFEDGEMAKYVQGDAIGYEG FQQFLKIYLEVDNVPRHLSLALFQSFETGHCLNETNVTKDVVCLNDVSC YFSLLEGGRPEDKLEFTFKLYDTRNGILDSSEVDKIILQMMRVAEYLD WDVSELRPILQEMMKEIDYDGSGSVSQAEWVRAGATTVPLLVLLGLEMT LKDDGQHMWRPKRFPRPVYCNLCESSIGLGKQGLSCNLCKYTVHDQCAM KALPCEVSTYAKSRKDIGVQSHVWVRGGCESGRCDRCQKKIRIYHSLTG LHCVWCHLEIHDDCLQAVGHECDCGLLRDHILPPSSIYPSVLASGPDRK NSKTSQKTMDLNLSTSEALRIDVPNTHPLLVFNPKSGGKQQQRVLW KFQYILNPRQVFNLLKDGPEIGLRLFKDVPDSRILVCGGDGTVGWILET IDKANLPVLPVAVLPLGTGNDLARCLRWGGGYEGQNLAKILKDLEMSK VVHMDRWSVEVIPOQTEEKSDPVPFQIINNYFSIGVDASIAHRFYIMRE KYPEKFNSRMKNKLWYFEFATSESIFSTCKKLEESLTVEICGKPLDLSN LSLEGIAVLNIPSMHGGSNLWGDTRRPHGDIYGINQALGATAKVITDPD ILKTCVPDLSDKRLEVVGLEGAIEMQIYTKLKNAGRRLAKCSEITFHT TKTLPMQIDGEPWMQTPCTIKITHKNQMPMLMGPPRSTNFFGFLS</p>
<u>Native sequence</u>	Amino acids M1 – S735 (end) of human DGK alpha. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLVQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Not1</i> sites into pFastBacDual GST-2

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Nucleotide Sequence of Insert:

gcggccgcgATGGCCAAGGAGAGGGGCCTAATAAGCCCCAGTGATTTTGC
CCAGCTGCAAAAATACATGGAATACTCCACCAAAAAGGTCAGTGATGTCC
TAAAGCTCTTCGAGGATGGCGAGATGGCTAAATATGTCCAAGGAGATGCC
ATTGGGTACGAGGGATTCCAGCAATTCCTGAAAATCTATCTCGAAGTGGA
TAATGTTCCCAGACACCTAAGCCTGGCACTGTTTCAATCCTTTGAGACTG
GTCAGTGTAAATGAGACAAATGTGACAAAAGATGTGGTGTGTCTCAAT
GATGTTTCCCTGCTACTTTTCCCTTCTGGAGGGTGGTCGGCCAGAAGACAA
GTTAGAATTCACCTTCAAGCTGTACGACACGGACAGAAATGGGATCCTGG
ACAGCTCAGAAGTGGACAAAATATCCTACAGATGATGCGAGTGGCTGAA
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GAAAGAGATTGACTATGATGGCAGTGGCTCTGTCTCTCAAGCTGAGTGGG
TCCGGGCTGGGGCCACCACCGTGCCACTGCTAGTGCTGCTGGGTCTGGAG
ATGACTCTGAAGGACGACGGACAGCACATGTGGAGGCCCAAGAGGTTCCC
CAGACCAGTCTACTGCAATCTGTGCGAGTCAAGCATTGGTCTTGGCAAAC
AGGGACTGAGCTGTAACCTCTGTAAGTACACTGTTACGACCAGTGTGCC
ATGAAAGCCCTGCCTTGTGAAGTCAGCACCTATGCCAAGTCTCGGAAGGA
CATTGGTGTCCAATCACATGTGTGGGTGCGAGGAGGCTGTGAGTCCGGGC
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GGTGGGCCATGAGTGTGACTGTGGGCTGCTCCGGGATCACATCCTGCCTC
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TCAATCCTAAGAGTGGCGGGAAGCAGGGGCAAAGGGTGTCTGGAAGTTC
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TTCAACAGCAGAATGAAGAACAAGCTATGGTACTTCGAATTTGCCACATC
TGAATCCATCTTCTCAACATGCAAAAAGCTGGAGGAGTCTTTGACAGTTG
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GCAGTGCTAAACATCCCTAGCATGCATGGTGGCTCCAACCTCTGGGGTGA
TACCAGGAGACCCCATGGGGATATCTATGGGATCAACCAGGCCTTAGGTG
CTACAGCTAAAGTCATCACCGACCCTGATATCCTGAAAACCTGTGTACCA
GACCTAAGTGACAAGAGACTGGAAGTGGTTGGGCTGGAGGGTGAATTTGA
GATGGGCCAAATCTATAACCAAGCTCAAGAATGCTGGACGTCGGCTGGCCA
AGTGCTCTGAGATCACCTTCCACACCACAAAACCCCTTCCCATGCAAATT
GACGGAGAACCCCTGGATGCAGACGCCCTGTACAATCAAGATCACCCACAA
GAACCAGATGCCATGCTCATGGGCCACCCCCCGCTCCACCAATTTCT
TTGGCTTCTTGAGCtaagcggccgc

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