

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PI3 kinase gamma [2 – 1102]

Enzyme description:-	PI3 kinase gamma [2 – 1102]
<u>Clone number:-</u>	DU 1747
<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>	3-5 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	129, 611.53 daltons
Average Mass	129, 694.07 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.93
<u>Purity:-</u>	80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50mM Hepes/NaOH pH7.0, 150 mM NaCl, 5 mM DTT, 20 % glycerol	
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	ADP Glo
<u>Assay buffer:-</u>	
12.5 mM glycine-NaOH pH 8.5, 50 mM KCl, 1 mM DTT, 0.05 % CHAPS, 2.5 mM MgCl ₂	
<u>Substrate:-</u>	
PI(4,5)P ₂ diC8	Final concentration: 0.05 mM

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Clone Data Sheet

PI3 kinase gamma [2 – 1102]

Protein PI3 kinase gamma [2 – 1102]

Clone Number DU 1747

Species Human

Accession number NM_002649

Tags N-terminal His(6)

Baculovirus expressed protein

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MSYYHHHHHDYDIPTTENLYFQGAMGSELENYKQPVVLRDNCRRRRR
MKPRSAASLSSMELIPIEFVLPTSQRKCKSPETALLHVAGHG NVEQMK
AQVWLRALETSVAADFYHRLGPHHFLLLYQKKGQWYEIYDKYQVVQTL
CLRYWKATHRSPGQIHLVQRHPPSEESQAFQRQLTALIGYDVTDVSNVH
DDELEFTRRGLVTPRMAEVASRDPKLYAMHPWVTSKPLPEYLWKKIANN
CIFIVIHRTTTSQTIKVSPPDDTPGAILQSFFTKMAKKKSLMDIPESQSE
QDFVLRVCGRDEYLVGETPIKNFQWVRHCLKNGEEIHVVLDTPDPALD
EVRKEEWPLVDDCTGVTGYHEQLTIHGKDHESVFTVSLWDCDRKFRVKI
RGIDIPVLP RNTDLTVFVEANIQHGGQVLCQRRTSPKPFTEEVLWNVWL
EFSIKIKDLPKGALLNLQIYCGKAPALSSKASAESPSSKSKGVQLLYY
VNLIDHRFLRRGEYVLHMWQISGKGEDQGSFNADKLT SATNPDKEN
SMSISILLDNYCHPIALPKHQPTDPEGDRVRAEMP NQLRKQLEAI IAT
DPLNPLTAEDKELLWHFRYESLKHPKAYPKLFSSVKWGQQEIVAKTYQL
LARREVWDQSALDVGLTMQLLDCNFSDENVRAIAVQKLESLEDDDLVHY
LLQLVQAVKFEPYHDSALARFLLKRGLRNKRIGHFLFWFLRSEIAQSRH
YQORFAVILEAYLRGCGTAMLHDFTOQVQVIEMLOKVTLDIKSLSAEKY
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KKKPLWLEFKCADPTALSNETIGIFKHGDDL RQDMLILQILRIMESI W
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EVLNHWLKEKSPTEEFQAVERFVYSCAGYCVATFVLGIGDRHNDNIM
ITETGNLFHIDFGHILGNYKSFLGINKERVFPVLT PDFL FVMGTSGKKT
SPHFQKFQD ICVKAYLALRHHTNLLIILFSMMLMTGMPQLTSKEDIEYI
RDALTVGKNEEDAKKYFLDQIEVCRDKGWTVQFNWFLHLVLGLIKQGEKH
SA
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Native sequence Amino acids E2 – A1102 (end) of human PI3 kinase gamma. Residue E29 of the fusion protein is equivalent to E2 of the native enzyme. The His(6) tag is located at residues 5 - 10

Protease cleavage rTEV site (ENLYFQG) is residues 18 – 24

Cloning sites *Bam*HI and *Not*I site of pFastBAC HTb

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Nucleotide sequence of insert

GGATCCGAGCTGGAGA ACTATAAACAGCCCGTGGTGCTGAGAGAGGACA
ACTGCCGAAGGCGCCGGAGGATGAAGCCGCGCAGTGCTGCCGCCAGCCT
GTCTCCATGGAGCTCATCCCCATCGAGTTCGTGCTGCCACCAGCCAG
CGCAAATGCAAGAGCCCCGAAACGGCGCTGCTGCACGTGGCCGGCCACG
GCAACGTGGAGCAGATGAAGGCCAGGTGTGGCTGCGAGCGCTGGAGAC
CAGCGTGGCGGCGACTTCTACCACCGGCTGGGACCGCATCACTTCCTC
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CTGACGTCAGCAACGTGCACGACGATGAGCTGGAGTTCACGCGCCGTGG
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AGAGTTCCATATGATCCTGGACTGAAAGCAGGAGCGCTGGCAATTGAAA
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