

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

Calculated molecular mass:-

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

Enzyme storage buffer:-

50mM Hepes/NaOH pH7.0, 150 mM NaCl, 5 mM DTT, 20 % glycerol

<u>Storage temperature:-</u>	-70 °C
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<u>Assay:-</u>	ADP Glo
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Assay buffer:-

12.5 mM glycine-NaOH pH 8.5, 50 mM KCl, 1 mM DTT, 0.05 % CHAPS, 2.5 mM MgCl₂

Substrate:-

PI(4,5)P₂ diC8 Final concentration: 0.05 mM

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Clone Data Sheet

PI3 kinase gamma [2 – 1102]

<u>Protein</u>	PI3 kinase gamma [2 – 1102]
<u>Clone Number</u>	DU 1747
<u>Species</u>	Human
<u>Accession number</u>	NM_002649
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMGSELENYKQPVVLRDNCRRRRR MKPRSAASLSSMELIPIEFVLPTSQRKCKSPETALLHVAGHGNVEQMK AQVWLRALETSVAADFYHRLGPHHFLLLYQKKGQWYEIYDKYQVVQTL CLRYWKATHRSPGQIHLVQRHPPSEESQAFQRQLTALIGYDVTDVSNVH DDELEFTRRGLVTPRMAEVASRDPKLYAMHPWVTSKPLPEYLWKKIANN CIFIVIHRSTTSQTIKVSPPDTPGAILQSFFTKMAKKKSLMDIPESQSE QDFVLRVCGRDEYLVGETPIKNFQWVRHCLKNGEEIHVVLDTPDPALD EVRKEEWPLVDDCTGVTGYHEQLTIHGKDHSVFTVSLWDCDRKFRVKI RGIDIPVLPRTDLTVFVEANIQHGGQVLCQRRTSPPKPFTEEVLWNVWL EFSIKIKDLPKGALLNLQIYCGKAPALSSKASAESPSSSESKGKVQLLYY VNLLIDHRFLLRRGEYVLHMWQISGKGEDQGSFNADKLTSATNPDKEN SMSISILLDNYCHPIALPKHQPTPDPEGDRVRAEMPNQLRKQLEAIIAT DPLNPLTAEDKELLWHFRYESLKHHPKAYPKLFSSVKWGQQEIVAKTYQL LARREVWDQSALDVGLTMQLLDCNFSDENVRAIAVQKLESLEDDDLHY LLQLVQAVKFEPYHDSALARFLLKRGLRNKRIGHFLFWFLRSEIAQSRH YQQRFAVILEAYLRGCGTAMLHDFEQVQVIEMLQKVTLDIKSLSAEKY DVSSQVISQLKQKLENLQNSQLPESFRVPYDPGLKAGALAIEKCKVMAS KKKPLWLEFKCADPTALSNETIGIIFKHGDDLQDMLILQILRIMESI WETESLDLCLLPYGCISTGDKIGMIEIVKDATTIAKIQQSTVGNTGAFKD EVLNHWLKEKSPTEEFQAVERFVYSCAGYCVATFVLGIGDRHNDNIM ITETGNLFDHDFGHILGNYKSFLGINKERVFPVLTPDFLFVMGTSGKKT SPHFQKFQDLCVKAYLALRHHTNLLIILFSMMLMTGMPQLTSKEDIEYI RDALTVGKNEEDAKKYFLDQIEVCRDKGWTVQFNWFLHLVLGKQGEKH SA
<u>Native sequence</u>	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
<u>Protease cleavage</u>	rTEV site (ENLYFQG) is residues 18 – 24
<u>Cloning sites</u>	<i>Bam</i> HI and <i>Not</i> I site of pFastBAC HTb

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Nucleotide sequence of insert

GGATCCGAGCTGGAGAACTATAAACAGCCCGTGGTGTGCTGAGAGAGGACA
ACTGCCGAAGGCGCCGGAGGATGAAGCCGCGCAGTGCTGCGGCCAGCCT
GTCCTCCATGGAGCTCATCCCCATCGAGTTCGTGCTGCCACCAGCCAG
CGCAAATGCAAGAGCCCCGAAACGGCGCTGCTGCACGTGGCCGGCCACG
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