

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Phospholipase C delta1 PH domain [1 – 179]

Protein description:- PLC delta1 PH domain [1 – 179]

Clone number:- DU 3871

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 10 mg/L

Calculated molecular mass:-

Monoisotopic 50, 101.58 daltons
Average Mass 50, 133.64 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.01

Purity:- 75 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 10 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- –20 °C

Assay:- To be determined

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CLONE DATA SHEET

PLC delta1 PH domain [1 - 179]

<u>Protein</u>	PLC delta1 PH domain [1 - 179]
<u>Clone number</u>	DU 3871
<u>Species</u>	Mouse
<u>Accession no</u>	BC025798
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIRYIADKHNML GGCPKERAIEISMLEGAVLDIRYGVSR IAYS KDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLVPRGSPGIRPSSPPPRAPTGMDSGR DFLT LHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKLQE DCKTIWQESRKVMRSPESQLFSIEDIQEVRMGHRTEGLEKF ARDI PEDRCFSIVFKDQRNTLDLIAPSPADVQHWVQGLRKI IDRSGSMDQRQKLQHWIHSCLRKADKNKDNKMNFKEVKDFL KELNVQVDDSKGEIPGSTRAAAS</p>
<u>Native sequence</u>	<p>Amino acids M1 – S179 (end) of mouse PLC delta1. Residue M242 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220</p> <p>The following sequence is present after the <i>Bam</i>H1 site of pGEX4T and before the start of the PLC delta 1 sequence, PGIRPSSPPPRAPTG, residues 227 – 241. And the following sequence is present after the PLC delta 1 sequence, KGEIPGSTRAAAS, residues 421 – 433.</p>
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Sal</i> 1 of pGEX4T

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**Nucleotide
sequence**

ggatccccaggaattcgcccttcgtcccctcctccgcgcgc
cccgaccggcATGGACTCCGGTCGGGACTTCCTGACCTTGC
ACGGGCTGCAGGATGACCCGGACCTTCAGGCCCTCCTGAAG
GGCAGCCAGCTTCTGAAGGTGAAATCCAGCTCGTGGCGTAG
AGAACGCTTCTACAAGCTACAGGAGGACTGCAAGACCATCT
GGCAGGAATCTCGCAAGGTCATGAGGTCCCCGGAGTCGCAG
CTGTTCTCCATCGAGGACATTCAGGAGGTGCGGATGGGACA
CCGCACAGAAGGCCTGGAGAAATTTGCCAGAGACATACCCG
AGGACCGATGCTTCTCCATCGTCTTCAAGGACCAGCGCAAT
ACCCTAGACCTCATCGCCCCGTCTCCAGCTGACGTCCAGCA
TTGGGTGCAGGGCCTGCGCAAGATCATCGACCGCTCGGGCT
CCATGGACCAGCGGCAGAAGCTGCAGCACTGGATTCACTCT
TGCTTGCGAAAGGCTGATAAAAACAAGGATAACAAGATGAA
CTTCAAGGAGGTGAAGGACTTCCTGAAGGAGCTCAACGTAC
AGGTGGATGACAGCaagggcgagattcccgggtcgac