

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PKC zeta [2 - 592]

<u>Enzyme description:-</u>	PKC zeta [2 - 592]
<u>Clone number:-</u>	DU 1447
<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>	5 mg/L
<u>Calculated molecular mass:-</u>	71, 199 daltons
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 0.1 mM sodium vanadate, 10 mM magnesium acetate
<u>Substrate:-</u>	ERM ₁ PRKRQGSVRRV Final concentration: 30 μM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

PKC zeta [2 - 592]

Protein PKC zeta [2 – 592]

Clone number DU 1447

Species Human

Accession number NM_002744

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMDPEFPSRTGPKMEGSGGRVRLKA
HYGGDIFITSVDAATTFEELCEEVRDMCRLHQHPLTLKWVDSEGDPC
VSSQMELEEAFLARQCRDEGLIIHVFPSTPEQPGLPCPGEDKSIYRRG
ARRWRKLYRANGHLFQAKRFNRRAYCGQCSEIRIWGLARQGYRCINCKLL
VHKRCHGLVPLTCRKHMSVMP SQEPPVDDKNEDADLPSEETDGIAYIS
SSRKHDSIKDSEDLKPVIDGMDGIKISQGLGLQDFDLIRVIGRGSYAK
VLLVRLKKNQIYAMKVVKELVHDEDEDIDWVQTEKHVFEQASSNPFLV
GLHSCFQTTSRLFLVIEYVNGGDLMFHMQRQRKLPPEEHARFYAAEICIA
LNFLHERGIIYRDLKLDNVLLDADGHIKLTDYGMCKEGLGPGDTTSTFC
GTPNYIAPEILRGEEYGF SVDWWALGVLMFEMMAGRSPFDIITDNPDMN
TEDYLFQVILEKPIRIPRFLSVKASHVLKGFLNKDPKERLGCRPQTGFS
DIKSHAFFRSIDWDLLEKKQALPPFPQITDDYGLDNFDTQFTSEPVQL
TPDDEDAIKRIDQSEFEGFEYINPLLLSTEEVS

Native sequence Amino acids P2 – V592 (end) of human PKC zeta.
Residue P31 of the fusion protein is equivalent to P2 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Eco*R1 and *Spe*1 sites of pFastBAC HTa

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Complete nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGATCCGGAATTCCCCAGCAG
GACCGGCCCAAGATGGAAGGGAGCGGCGGCCGTCCGCCTCAAGGCG
CATTACGGGGGGGACATCTTCATCACCAGCGTGGACGCCGCCACGACCT
TCGAGGAGCTCTGTGAGGAAGTGAGAGACATGTGTCTGTGCACCAGCA
GCACCCGCTCACCCCTCAAGTGGGTGGACAGCGAAGGTGACCCTTGCACG
GTGTCCTCCAGATGGAGCTGGAAGAGGCTTTCGCCTGGCCCGTCACT
GCAGGGATGAAGGCCTCATCATTGTTTTTCCCGAGCACCCCTGAGCA
GCCTGGCCTGCCATGTCCGGGAGAAGACAAATCTATCTACCGCCGGGGA
GCCAGAAGATGGAGGAAGCTGTACCGTGCCAACGGCCACCTCTTCCAAG
CCAAGCGCTTTAACAGGAGAGCGTACTGCGGTCACTGCAGCGAGAGGAT
ATGGGGCCTCGCGAGGCAAGGCTACAGGTGCATCAACTGCAAAGTCTG
GTCCATAAGCGCTGCCACGGCCTCGTCCCGCTGACCTGCAGGAAGCATA
TGGATTCTGTGATGCCTTCCCAAGAGCCTCCAGTAGACGACAAGAACGA
GGACGCCGACCTTCCTTCGAGGAGACAGATGGAATTGCTTACATTTCC
TCATCCCGGAAGCATGACAGCATTAAAGACGACTCGGAGGACCTTAAGC
CAGTTATCGATGGGATGGATGGAATCAAATCTCTCAGGGGCTTGGGCT
GCAGGACTTTGACCTAATCAGAGTCATCGGGCGCGGGAGCTACGCCAAG
GTTCTCCTGGTGCGGTTGAAGAAGAATGACCAAATTTACGCCATGAAAG
TGGTGAAGAAAGAGCTGGTGCATGATGACGAGGATATTGACTGGGTACA
GACAGAGAAGCACGTGTTTGAGCAGGCATCCAGCAACCCCTTCCTGGTC
GGATTACACTCCTGCTTCCAGACGACAAGTCGGTTGTTCTGTCATTG
AGTACGTCAACGGCGGGGACCTGATGTTCCACATGCAGAGGCAGAGGAA
GCTCCCTGAGGAGCACGCCAGGTTCTACGCGGCCGAGATCTGCATCGCC
CTCAACTTCCTGCACGAGAGGGGGATCATCTACAGGGACCTGAAGCTGG
ACAACGTCCTCCTGGATGCGGACGGGCACATCAAGCTCACAGACTACGG
CATGTGCAAGGAAGGCCTGGGCCCTGGTGACACAACGAGCACTTTCTGC
GGAACCCCGAATTACATCGCCCCGAAATCCTGCGGGGAGAGGAGTACG
GGTTCAGCGTGGACTGGTGGGCGCTGGGAGTCTCATGTTTGAGATGAT
GGCCGGGCGCTCCCCGTTGACATCATCACCGACAACCCGGACATGAAC
ACAGAGGACTACCTTTTCCAAGTGATCCTGGAGAAGCCCATCCGGATCC
CCCGTTTCTGTCCGTCAAAGCCTCCCATGTTTTAAAGGATTTTTTAA
TAAGGACCCCAAAGAGAGGCTCGGCTGCCGGCCACAGACTGGATTTTCT
GACATCAAGTCCCACGCGTTCTTCCGCAGCATAGACTGGGACTTGCTGG
AGAAGAAGCAGGCGCTCCCTCCATTCCAGCCACAGATCACAGACGACTA
CGGTCTGGACAACCTTTGACACACAGTTCACCAGCGAGCCCGTGCAGCTG
ACCCAGACGATGAGGATGCCATAAAGAGGATCGACCAGTCAGAGTTTCG
AAGGCTTTGAGTATATCAACCCATTATTGCTGTCCACCGAGGAGTCGGT
Gtga