

# University of Dundee

## Standard Operating Procedure

### Preparation of active PKC zeta [2 - 592]

<b><u>Enzyme description:-</u></b>	PKC zeta [2 - 592]
<b><u>Clone number:-</u></b>	DU 1447
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6)
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	5 mg/L
<b><u>Calculated molecular mass:-</u></b>	71, 199 daltons
<b><u>Purity:-</u></b>	>80 %
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	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.
<b><u>Storage temperature:-</u></b>	-70 °C [Long term stability to be determined]

**Assay:-** Standard filter binding assay

**Assay buffer:-**  
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA,  
0.1 mM sodium vanadate, 10 mM magnesium acetate

**Substrate:-**  
ERM<sup>R</sup>PRKRQGSVRRV Final concentration: 30 μM

**Specific activity range:-** To be determined

# University of Dundee

## 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** MSY~~Y~~HHHHHHHDYDIPTT~~EN~~LYFQ~~G~~AMDPEF~~PS~~RTGPKMEGSGGRVRLKA  
HYGGDIFITSVDAATTFEELCEEVRDMCRLHQ~~QH~~PLTLKWVDSEGD~~P~~CT  
VSSQMELEEA~~F~~RLARQCRDEGLI~~I~~HVFPSTPEQPGLPCPGEDKSIYRRG  
ARRWRKLYRANGHLFQAKRFNRRAYCGQC~~S~~ERIWGLARQGYRCINCKLL  
VHKRCHGLVPLTCRKHMD~~S~~VMP~~S~~Q~~E~~PPVDDKNEDADLPSEETDGIAYIS  
SSRKHDSIKDDSEDLKPVIDGMDGIKISQGLGLQDFDLIRVIGRGSYAK  
VLLVRLKKN~~D~~QIYAMKVVKELVHDD~~E~~IDWVQTEKHVFEQASSNPFLV  
GLHSCFQ~~T~~SRLFLVIEYVNGGDLMFHMQRQRKLP~~E~~EHARFYAAEICIA  
LNFLHERGI~~I~~YRDLKLDNVL~~L~~DADGH~~I~~KLTDYGMCKEGLGPGDTTSTFC  
GTPNYIAPEILRGE~~E~~YGF~~S~~V~~D~~WWALGVLMFEMMAGRSPFDIITDNPDMN  
TEDYLFQVILEKPIRIPRFLSVKASHVLKGF~~L~~NKDPKERLGC~~R~~PQTGFS  
DIKSHAFFRSIDWDLLEKKQALPPFQ~~P~~QITDDYGLDNFDTQFTSEPVQL  
TPDDEDAIKRIDQSEFEGFEYINPLLLSTEE~~S~~V

**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

# University of Dundee

## Complete nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACCATTACGATATCCCAACGA  
CCGAAAACCTGTATTTTCAGGGCGCCATGGATCCGGAATTCCCCAGCAG  
GACCGGCCCAAGATGGAAGGGAGCGGCGGCCGCTCCGCCTCAAGGCG  
CATTACGGGGGGGACATCTTCATCACCAGCGTGGACGCCGCCACGACCT  
TCGAGGAGCTCTGTGAGGAAGTGAGAGACATGTGTCTGCTGCACCAGCA  
GCACCCGCTCACCTCAAGTGGGTGGACAGCGAAGGTGACCCTTGCACG  
GTGTCCTCCAGATGGAGCTGGAAGAGGCTTTCGCGCTGGCCCGTCAGT  
GCAGGGATGAAGGCCTCATCATTTCATGTTTTCCCGAGACCCCTGAGCA  
GCCTGGCCTGCCATGTCCGGGAGAAGACAAATCTATCTACCGCCGGGGA  
GCCAGAAGATGGAGGAAGCTGTACCGTGCCAACGGCCACCTCTTCCAAG  
CCAAGCGCTTTAACAGGAGAGCGTACTGCGGTCAGTGCAGCGAGAGGAT  
ATGGGGCCTCGCGAGGCAAGGCTACAGGTGCATCAACTGCAAACCTGCTG  
GTCCATAAGCGCTGCCACGGCCTCGTCCCGCTGACCTGCAGGAAGCATA  
TGGATTCTGTTCATGCCTTCCCAAGAGCCTCCAGTAGACGACAAGAACGA  
GGACGCCGACCTTCTTCCGAGGAGACAGATGGAATTGCTTACATTTCC  
TCATCCCGGAAGCATGACAGCATTAAGACGACTCGGAGGACCTTAAGC  
CAGTTATCGATGGGATGGATGGAATCAAATCTCTCAGGGGCTTGGGCT  
GCAGGACTTTGACCTAATCAGAGTCATCGGGCGCGGGAGCTACGCCAAG  
GTTCTCCTGGTGCGGTTGAAGAAGAATGACCAAATTTACGCCATGAAAG  
TGGTGAAGAAAGAGCTGGTGCATGATGACGAGGATATTGACTGGGTACA  
GACAGAGAAGCACGTGTTTGAGCAGGCATCCAGCAACCCCTTCTGGTC  
GGATTACACTCCTGCTTCCAGACGACAAGTCGGTTGTTCTGCTCATTG  
AGTACGTCAACGGCGGGGACCTGATGTTCCACATGCAGAGGCAGAGGAA  
GCTCCCTGAGGAGCACGCCAGGTTCTACGCGGCCGAGATCTGCATCGCC  
CTCAACTTCTGCACGAGAGGGGGATCATCTACAGGGACCTGAAGCTGG  
ACAACGTCCTCCTGGATGCGGACGGGCACATCAAGCTCACAGACTACGG  
CATGTGCAAGGAAGGCCTGGGCCCTGGTGACACAACGAGCACTTTCTGC  
GGAACCCCGAATTACATCGCCCCGAAATCCTGCGGGGAGAGGAGTACG  
GGTTCAGCGTGGACTGGTGGGCGCTGGGAGTCTCATGTTTGAGATGAT  
GGCCGGGCGCTCCCGTTTCGACATCATCACCGACAACCCGGACATGAAC  
ACAGAGGACTACCTTTTCCAAGTGATCCTGGAGAAGCCATCCGGATCC  
CCCGGTTCTGTCCGTCAAAGCCTCCCATGTTTTAAAAGGATTTTTAAA  
TAAGGACCCCAAAGAGAGGCTCGGCTGCCGGCCACAGACTGGATTTTCT  
GACATCAAGTCCCACGCTTCTTCCGCAGCATAGACTGGGACTTGCTGG  
AGAAGAAGCAGGCGCTCCCTCCATTCCAGCCACAGATCACAGACGACTA  
CGGTCTGGACAACCTTTGACACACAGTTCACCAGCGAGCCCGTGCAGCTG  
ACCCAGACGATGAGGATGCCATAAAGAGGATCGACCAGTCAGAGTTCG  
AAGGCTTTGAGTATATCAACCCATTATTGCTGTCCACCAGGAGTCGGT  
Gtga