

# *MRC PPU REAGENTS*

## Standard Operating Procedure

### Preparation of active Protein Kinase C theta [1 – 706]

<b><u>Enzyme description:-</u></b>	PRKCQ [1 - 706]
<b><u>Clone number:-</u></b>	DU 29920
<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>	Cobalt agarose

**Calculated molecular mass:-**

Monoisotopic      85, 180.09 daltons  
Average Mass      85, 236.37 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      7.14

**Purity:-**                                      85 %

**Activation protocol:-**                  Constitutively active

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,  
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-**                  -70 °C

**Assay buffer:-**

50 mM Tris-HCl pH 7.5, 0.1 mM CaCl<sub>2</sub>, 0.1 mg/ml PtdSerine, 10 uM DAG, 10 mM DTT,  
10 mM Magnesium Acetate

**Substrate:-**

ERM<sup>R</sup>PRKRQGSVRRV                      Final concentration: 300 uM

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## Clone Data Sheet

### PKC Theta [1 - 706]

**Protein** PRKCQ [1 - 706]

**Clone number** DU 29920

**Species** Human

**Accession number** NM\_001323265.1

**Tags** N-terminal His(6)

**Bacterially  
expressed protein**

MSYYHHHHHDYDIPTTENLYFQGGAMGSMSPFLRIGLSNFDGSCQSCQ  
GEAVNPYCAVLVKEYVESENGQMYIQKKPTMYPPWDSTFDAHINKGRVM  
QIIIVKGNVDLISETTVELYSLAERC RKNN GKTEIWLELKPQGRMLMNA  
RYFLEMSDTKDMNEFETEGFFALHQRRGAIKQAKVHHVKCHEFTATFFP  
QPTFCVSCHEFVWGLNKQGYQCRQCNAAIHKKCIDKVI AKCTGSAINSR  
ETMFHKERFKIDMPHRFKVYNYKSPTFCEHCGTLLWGLARQGLKCDACG  
MNVHHRCTKVANLCGINQKLMAEALAMIESTQQARCLRDTEQIFREGP  
VEIGLPCSIKNEARPPCLPTPGKREPQGISWESPLDEVDMCHLPEPEL  
NKERPSLQIKLKIEDFILHKMLGKGSFGKVFLAEFKKTNQFFAIKALKK  
DVVLMDDDDVECTMVEKRVLSLAWEH PFLTHMCTFQTKENLFFVMEYLN  
GGDLMYHIQSCHKFDLSRATFYAAEIIILGLQFLHSGKIVYRDLKLDNIL  
LDKDGHIKIADFGMCKENMLGDAKTNTFCGTPDYIAPEILLGQKYNHSV  
DWWSFGVLLYEMLIGQSPFHGQDEEELFHSIRMDNPFYPRWLEKEAKDL  
LVKLFVREPEKRLGVRGDIRQHPLFREINWEELERKEIDPPFRPKVKSP  
FDCSNFDKEFLNEKPRLSFADRALINSMDQNMFRNFSFMNPGMERLIS

**Native sequence** Amino acids M1 – S706 (end) of human PRKCQ.  
Residue M29 of the fusion protein is equivalent to M1 of the native  
enzyme. The His(6) tag is located at residues 5 – 10.

**Protease cleavage** rTEV (ENLYFQ) residues 18 - 23

**Cloning sites** *Bam*H1 and *Not*1 sites of pFastBac.Htb

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### Nucleotide Sequence Of Insert

ggatccATGTCGCCATTTCTTCGGATTGGCTTGTCCAACCTTGACTGCGG  
GTCTTCCAGTCTTGTTCAGGGCGAGGCTGTTAACCTTACTGTGCTGTGC  
TCGTCAAAGAGTATGTCTGAATCAGAGAACGGGCAGATGTATATCCAGAAA  
AAGCCTACCATGTACCCACCCTGGGACAGCACTTTTGTATGCCCATATCAA  
CAAGGGAAGAGTCATGCAGATCATTGTGAAAGGCAAAAACGTGGACCTCA  
TCTCTGAAACCACCGTGGAGCTCTACTCGCTGGCTGAGAGGTGCAGGAAG  
ACAACGGGAAGACAGAAATATGGTTAGAGCTGAAACCTCAAGGCCGAAT  
GCTAATGAATGCAAGATACTTTCTGGAAATGAGTGACACAAAGGACATGA  
ATGAATTTGAGACGGAAAGGCTTCTTTGCTTTGCATCAGCGCCGGGGTGCC  
ATCAAGCAGGCAAAGGTCCACCACGTCAAGTGCCACGAGTTCACTGCCAC  
CTTCTTCCCACAGCCCACATTTTGTCTGTCTGCCACGAGTTTGTCTGGG  
GCCTGAACAAACAGGGCTACCAGTGCCGACAATGCAATGCAGCAATTCAC  
AAGAAGTGTATTGATAAAGTTATAGCAAAGTGCACAGGATCAGCTATCAA  
TAGCCGAGAAACCATGTTCCACAAGGAGAGATTCAAAATTGACATGCCAC  
ACAGATTTAAAGTCTACAATTACAAGAGCCCGACCTTCTGTGAACACTGT  
GGGACCTGCTGTGGGGACTGGCACGGCAAGGACTCAAGTGTGATGCATG  
TGGCATGAATGTGCATCATAGATGCCAGACAAAGGTGGCCAACCTTTGTG  
GCATAAACCAGAAGCTAATGGCTGAAGCGCTGGCCATGATTGAGAGCACT  
CAACAGGCTCGCTGCTTAAGAGATACTGAACAGATCTTCAGAGAAGGTCC  
GGTTGAAATTGGTCTCCCATGCTCCATCAAAAATGAAGCAAGGCCGCCAT  
GTTTACCGACACCGGGAAAAAGAGAGCCTCAGGGCATTTCCTGGGAGTCT  
CCGTTGGATGAGGTGGATAAAATGTGCCATCTTCCAGAACCTGAACTGAA  
CAAAGAAAGACCATCTCTGCAGATTAACATAAAAATTGAGGATTTTATCT  
TGCACAAAATGTTGGGGAAAGGAAGTTTTGGCAAGGTCTTCTGGCAGAA  
TTCAAGAAAACCAATCAATTTTTTCGCAATAAAGGCCTTAAAGAAAGATGT  
GGTCTTGATGGACGATGATGTTGAGTGCACGATGGTAGAGAAGAGAGTTC  
TTTCTTGGCCTGGGAGCATCCGTTTTCTGACGCACATGTTTTGTACATTC  
CAGACCAAGGAAAACCTCTTTTTTGTGATGGAGTACCTCAACGGAGGGGA  
CTTAATGTACCACATCCAAAGCTGCCACAAGTTCGACCTTTCAGAGCGA  
CGTTTTATGCTGCTGAAATCATCTTGGTCTGCAGTTCCTTCATTCCAAA  
GGAATAGTCTACAGGGACCTGAAGCTAGATAACATCCTGTTAGACAAAGA  
TGGACATATCAAGATCGCGGATTTTGGAAATGTGCAAGGAGAACATGTTAG  
GAGATGCCAAGACGAATACCTTCTGTGGGACACCTGACTACATCGCCCCA  
GAGATCTTGTGGGTCAGAAATACAACCACTCTGTGGACTGGTGGTCCTT  
CGGGGTTCTCCTTTATGAAATGCTGATTGGTCAGTCGCCTTTCACGGGC  
AGGATGAGGAGGAGCTCTTCCACTCCATCCGCATGGACAATCCCTTTTAC  
CCACGGTGGCTGGAGAAGGAAGCAAAGGACCTTCTGGTGAAGCTCTTCGT  
GCGAGAACCTGAGAAGAGGCTGGGCGTGAGGGGAGACATCCGCCAGCACC  
CTTTGTTTCGGGAGATCAACTGGGAGGAACTTGAACGGAAGGAGATTGAC  
CCACCGTTCGGGCCGAAAGTGAAATCACCATTTGACTGCAGCAATTTCGA  
CAAAGAATTTCTAAACGAGAAGCCCCGGCTGTCATTTGCCGACAGAGCAC  
TGATCAACAGCATGGACCAGAATATGTTTCAGGAACTTTTCTTCATGAAC  
CCCGGGATGGAGCGGCTGATATCCtgagcggccgc