

# *MRC PPU REAGENTS*

## Standard Operating Procedure

### Preparation of active Protein Kinase C iota [1 – 596]

<b><u>Enzyme description:-</u></b>	PRKCI [1 - 596]
<b><u>Clone number:-</u></b>	DU 29919
<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      71, 587.76 daltons  
Average Mass      71, 633.99 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      5.60

**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 Iota [1 - 596]

**Protein** PRKCI [1 - 596]

**Clone number** DU 29919

**Species** Human

**Accession number** NM\_002740.5

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

**Bacterially  
expressed protein**

MSYYHHHHHDYDIPTTENLYFQAGAMGSMPTQRDSSTMSHTVAGGGSGD  
HSHQVRVKAYYRGDIMITHFEPISFEGLCNEVRDMCSFDNEQLFTMKW  
IDEEGDPCTVSSQLELEEAFLYELNKDSELLIHVFPCVPERPGMPCPG  
EDKSIYRRGARRWRKLYCANGHTFQAKRFNRRAHCAICTDRIWGLGRQG  
YKCINCKLLVHKKCHKLVTIECGRHSLPQEPVMPMDQSSMHSCHAQTVI  
PYNPSSHESLDQVGEEKEAMNTRESGKASSLGLQDFDLRLVIGRGSYA  
KVLVRLKKTDRITYAMKVVKKELVNDDEDIDWVQTEKHVFEQASNHPFL  
VGLHSCFQTESRLFFVIEYVNGGDLMFHMQRQRKLPPEEHARFYSAEISL  
ALNYLHERGIYRDLKLDNVLLDSEGHIKLTDYGMCKEGLRPGDTTSTF  
CGTPNYIAPEILRGEDYGFSDWWALGVLMFEMMAGRSPFDIVGSSDNP  
DQNTEDYLFQVILEKQIRIPRSLSVKAASVLKSFLNKDPKERLGCHPQT  
GFADIQGHPPFRNVDWDMMEQKQVPPFPKPNISGEFGLDNFDSQFTNEP  
VQLTPDDDDIVRKIDQSEFEGFEYINPLLMSAEECV

**Native sequence** Amino acids M1 – V596 (end) of human PRKCI.  
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

ggatccATGCCGACCCAGAGGGACAGCAGCACCATGTCCCACACGGTCCG  
AGGCGGCGGCAGCGGGGACCATTCCCACCAGGTCCGGGTGAAAGCCTACT  
ACCGCGGGGATATCATGATAACACATTTTGAACCTTCCATCTCCTTTGAG  
GGCCTTTGCAATGAGGTTCGAGACATGTGTTCTTTTGACAACGAACAGCT  
CTTCACCATGAAATGGATAGATGAGGAAGGAGACCCGTGTACAGTATCAT  
CTCAGTTGGAGTTAGAAGAAGCCTTTAGACTTTATGAGCTAAACAAGGAT  
TCTGAACTCTTGATTCATGTGTTCCCTTGTGTACCAGAACGTCCTGGGAT  
GCCTTGTCCAGGAGAAGATAAAATCCATCTACCGTAGAGGTGCACGCCGCT  
GGAGAAAGCTTTATTGTGCCAATGGCCACACTTTCCAAGCCAAGCGTTTC  
AACAGGCGTGCTCACTGTGCCATCTGCACAGACCGAATATGGGGACTTGG  
ACGCCAAGGATATAAGTGCATCAACTGCAAACCTTTGGTTCATAAGAAGT  
GCCATAAACTCGTCACAATTGAATGTGGGCGGCATTCTTTGCCACAGGAA  
CCAGTGATGCCCATGGATCAGTCATCCATGCATTCTGACCATGCACAGAC  
AGTAATTCCATATAATCCTTCAAGTCATGAGAGTTTGGATCAAGTTGGTG  
AAGAAAAAGAGGCAATGAACACCAGGGAAAGTGGCAAAGCTTCATCCAGT  
CTAGGTCTTCAGGATTTTGATTTGCTCCGGGTAATAGGAAGAGGAAGTTA  
TGCCAAAGTACTGTTGGTTCGATTAACAAAAACAGATCGTATTTATGCAA  
TGAAAGTTGTGAAAAAGAGCTTGTTAATGATGATGAGGATATTGATTGG  
GTACAGACAGAGAAGCATGTGTTGAGCAGGCATCCAATCATCCTTTCTCT  
TGTTGGGCTGCATTCTTGCTTTCAGACAGAAAGCAGATTGTTCTTTGTTA  
TAGAGTATGTAAATGGAGGAGACCTAATGTTTCATATGCAGCGACAAAGA  
AACTTCCCTGAAGAACATGCCAGATTTTACTCTGCAGAAATCAGTCTAGC  
ATTAATTTATCTTCATGAGCGAGGGATAATTTATAGAGATTTGAAACTGG  
ACAATGTATTACTGGACTCTGAAGGCCACATTAAACTCACTGACTACGGC  
ATGTGTAAGGAAGGATTACGGCCAGGAGATACAACCAGCACTTTCTGTGG  
TACTCCTAATTACATTGCTCCTGAAATTTTAAGAGGAGAAGATTATGGTT  
TCAGTGTGACTGGTGGGCTCTTGAGTGCTCATGTTTGAGATGATGGCA  
GGAAGGTCTCCATTTGATATTGTTGGGAGCTCCGATAACCCTGACCAGAA  
CACAGAGGATTATCTCTTCCAAGTTATTTTGGAAAAACAAATTCGCATAC  
CACGTTCTCTGTCTGTAAAAGCTGCAAGTGTCTGAAGAGTTTTCTTAAT  
AAGGACCCTAAGGAACGATTGGGTTGTCATCCTCAAACAGGATTTGCTGA  
TATTCAGGGACACCCGTTCTTCCGAAATGTTGATTGGGATATGATGGAGC  
AAAAACAGGTGGTACCTCCCTTTAAACCAAATATTTCTGGGGAATTTGGT  
TTGGACAACCTTTGATTCTCAGTTTACTAATGAACCTGTCCAGCTCACTCC  
AGATGACGATGACATTGTGAGGAAGATTGATCAGTCTGAATTTGAAGGTT  
TTGAGTATATCAATCCTCTTTTGTATGTCTGCAGAAGAATGTGTctgagcg  
gccgc

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